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A mathematical study of the role of calcium in the regulation of saliva secretion

Laurence Palk

Abstract

Xerostomia is estimated to affect 30% of adults over the age of 65. The condition is characterised by a lack of saliva secretion, resulting in a range of health problems. A rise in the concentration of free cytosolic calcium (Ca$^{2+}$) is essential to initiate saliva secretion. We construct mathematical models of saliva secretion and Ca$^{2+}$ dynamics in salivary acinar and duct cells.

We investigate how the distribution of K$^+$ channels affects the rate of primary saliva secretion. Maximum saliva secretion is hypothesised to occur when a small amount of K$^+$ conductance is located in the apical membrane, with the majority in the basolateral membrane. Apical K$^+$ channels have since been experimentally located.

For a range of applied agonist, the concentration of Ca$^{2+}$ in salivary cells is seen experimentally to oscillate and to travel in waves across the cytosol. We construct a model of Ca$^{2+}$ oscillations in parotid acinar cells that requires paired oscillations of IP$_3$. This ODE model reproduces a number of experimentally observed phenomena. The model is later spatially extended. An inhomogeneous distribution of Ca$^{2+}$ channels is shown to produce apical to basal Ca$^{2+}$ waves, as seen experimentally.

We investigate how Ca$^{2+}$ wave properties affect the rate of saliva secretion. Mean Ca$^{2+}$ concentration is found to be the most significant property in regulating secretion. Wave speed was found to encode a range of secretion rates. Ca$^{2+}$ oscillation frequency and amplitude had little effect on the fluid secretion rate.

Recent experimental results show coupled oscillations of Ca$^{2+}$ and IP$_3$ in HSY cells, a duct cell line. We present a mathematical model of HSY cells in which IP$_3$ oscillations are not required for the generation of Ca$^{2+}$ oscillations. The inclusion of passive IP$_3$ oscillations is shown to increase the Ca$^{2+}$ oscillation frequency range and be consistent with the experimental data.

These single-cell models provide insight into the regulation of saliva secretion by themselves. They also suggest what is important to include, and what can be simplified in constructing a whole-organ model. With a greater understanding of the regulation of saliva secretion, and the role Ca$^{2+}$ plays, it is hoped that we might learn how salivary gland dysfunction occurs.
Acknowledgements

I thank my supervisors James Sneyd and Edmund Crampin for giving me the opportunity to study the interesting field of mathematical physiology. They have provided support and guidance when it was needed, whilst allowing my development as an independent researcher. For this I’m extremely grateful.

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I thank my fellow PhD students and members of the calcium and ‘spit’ research groups for making the University of Auckland an interesting and enjoyable place to study. In particular I thank Kate Patterson and Oliver Maclaren for their help on Section 6.9.2. Also, Katie Sharp for her diligent proofreading of this thesis.

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Glossary

**ATP** Adenosine triphosphate.

**cAMP** Cyclic adenosine monophosphate.

**CICR** Ca$^{2+}$ induced Ca$^{2+}$ release.

**DAG** Diacylglycerol.

**ER** Endoplasmic reticulum.

**IP$_3$** Inositol trisphosphate.

**IP$_3$R** Inositol trisphosphate receptor.

**NaK** Na$^+$- K$^+$- ATPase.

**NKCC** Na$^+$- K$^+$- 2Cl$^-$ cotransporter.

**PIP$_2$** Phosphatidylinositol 4,5-bisphosphate.

**PLC** Phospholipase C.

**RyR** Ryanodine receptor.

**SEM** Scanning electron microscope.

**SERCA** Sarco/endoplasmic reticulum Ca$^{2+}$-ATPase.
Chapter 1

Introduction

Adequate supply of saliva is important for speech, mastication and general health. Enzymes in saliva are the first mechanism by which food is digested. Salivary enzymes also provide protection to teeth with saliva gland dysfunction often leading to problems with dental cavities [35]. It is estimated that 30% of adults over the age of 65 in the U.S. will suffer from xerostomia, more commonly known as dry mouth [87], which greatly affects the sufferers’ quality of life. The condition is linked to Sjögren’s syndrome, where dry mouth is the hallmark symptom [39], and is an adverse effect of radiation therapy in the treatment of head and neck cancer [86].

Mathematics has a long history of aiding the understanding of physiological processes, dating back to Helmholtz and his mathematical analysis of hearing in the 1860s. Where variables are difficult, or impossible, to measure experimentally, mathematical modelling has been able to provide a quantitative picture. As experimental procedures and instrumentation improves so does our knowledge. Mathematical models are increasingly used, not only to explain experimental phenomena, but also to predict behaviour and even suggest future experimental direction. In this thesis we use mathematical modelling to explain, predict and direct the study of saliva secretion, in particular focusing on the role of Ca$^{2+}$ as an intracellular messenger. By improving our understanding of the regulation of saliva secretion we hope to learn how salivary gland dysfunction, such as xerostomia, may occur.

To begin, in Chapter 2 a background on the physiology of saliva secretion is presented. Next, in Chapter 3, the current mathematical models which relate to saliva secretion and Ca$^{2+}$ signalling are reviewed. In Chapter 4 we develop a mathematical model of saliva secretion in parotid acinar cells. Model components are based on experimental data and simulations agree well with experimental results. The model is used to investigate how the distribution of K$^+$ channels affects the efficiency of saliva secretion. Most models
place $K^+$ channels exclusively in the basolateral membrane with experimental localisation confirming $K^+$ channels here. Our model predicts saliva secretion is increased with a small $K^+$ conductance in the apical membrane, with the majority in the basolateral membrane. Following this work, experimental evidence has suggested that apical $K^+$ channels are indeed found in parotid acinar cells [1].

$Ca^{2+}$ is known to be a second messenger, linking neuronal stimulation with increased secretion of saliva. The release of agonist by neurons increases the production of the messenger inositol trisphosphate ($IP_3$), which in turn increases the concentration of $Ca^{2+}$ in the cytosol. With certain neuronal stimulation, the $Ca^{2+}$ concentration in salivary cells is shown to exhibit oscillations. Our model of the parotid acinar cell presented in Chapter 4 incorporates a model of the $Ca^{2+}$ dynamics. Oscillations of $Ca^{2+}$ are reliant on paired oscillations of $IP_3$.

Recent experimental evidence from parotid acinar cells shows not only oscillations in $Ca^{2+}$, but also travelling waves [122]. In Chapter 5 we present a spatial $Ca^{2+}$ model which uses the inhomogeneity of $Ca^{2+}$ release channels to reproduce these travelling waves. Interestingly, experimental observations show that $Ca^{2+}$ waves always originate at the apical pole of the cell before travelling to the basal pole. This sparked the following questions. Do these directed $Ca^{2+}$ waves have a significance in the regulation of saliva secretion? Is frequency encoding used in salivary cells? Are $Ca^{2+}$ oscillations important in salivary glands or an artefact of cell design? In Chapter 6 we investigate how the properties of $Ca^{2+}$ oscillations and waves, such as amplitude, frequency and wave speed, affect the regulation of saliva secretion.

The majority of previous work on salivary glands has focussed on acinar cells, which are responsible for regulating the secretion of primary saliva. Duct cells then modify the ionic concentration of the primary saliva as it travels to the mouth. HSY cells are a duct cell line. New experimental methodology has produced time-course measurements showing paired $Ca^{2+}$ and $IP_3$ oscillations in HSY cells [107]. There is a long-standing question around the role of $IP_3$, specifically whether oscillations of $IP_3$ are required to generate $Ca^{2+}$ oscillations. In Chapter 7 we develop a mathematical model of HSY cell $Ca^{2+}$ oscillations that predicts oscillations of $IP_3$ are passive reflections of the $Ca^{2+}$ oscillations. We postulate that oscillations of $IP_3$ facilitate long period oscillations of $Ca^{2+}$ and propose a series of experimental procedures that could confirm this hypothesis.

Finally, in Chapter 8 we discuss the major findings of this thesis and identify areas for further research. The work of Chapter 4 and Chapter 6 has been published in the Journal of Theoretical Biology with the corresponding references found in the bibliography [76, 77].
Chapter 2

Background physiology

2.1 The physiology of saliva secretion

Most mammals have three major salivary glands: parotid, submandibular and sublingual, as shown in Figure 2.1. In humans the parotid gland is the largest, sitting just in front of the ear and producing mostly a watery serous fluid. The parotid gland will be the focus of our modelling. The sublingual gland is the smallest, sitting underneath the tongue and secreting mostly mucous. Finally the submandibular gland, sitting beneath the lower jaw, produces a mixture of serous and mucous. On top of these major salivary glands it is estimated there are a further 600 minor salivary glands located in the mouth [73].

The first research on salivary glands dates back to the 17th Century. Niels Stensen is credited with the discovery of the parotid gland. The parotid duct is still sometimes referred to as Stensen’s duct. Until Stensen’s research it was believed that saliva was a cerebral fluid originating in the brain [50]. The structure of the salivary glands was first reported by Stensen’s Professor, Francois Dubois, writing that salivary glands consist of distinct lobules (or acini) separated by tissue [104]. The analogy commonly used is that each major salivary gland has a structure likened to a bunch of grapes. A tree-like network of ducts end at a cluster of acinar cells. Modern confocal and SEM imaging (Figures 2.2 and 2.3) show this structure agrees well with early drawings of Maziarski [68] published in 1900 and shown in Figure 2.3 inset.

The secretion of saliva is a two-part process, as first proposed by Thaysen et al. [108] and later confirmed by micropuncture studies [123]. Acinar cells secrete primary saliva into a shared lumen. This primary saliva then travels down the duct where the ionic content is modified by the duct cells.

Xerostomia is estimated to affect between 20-30% of the elderly [87, 39, 103] and
Figure 2.1: Figure showing the location of the three major salivary glands. Labelled (1) is the parotid gland, (2) the submandibular gland and (3) the sublingual gland. Source: Wikipedia Commons article on Salivary Glands [119].

is characterised by a reduced rate, or complete lack, of saliva secretion. Sufferers have problems eating and talking, and an increased risk of plaque, gum disease, dental cavities and tooth loss. Xerostomia is linked to Sjögren’s syndrome, where dry mouth is the hallmark symptom. The condition is also a side effect of many commonly used drugs [102]. Those who receive radiation therapy for the treatment of head and neck cancer are particularly at risk [87]. For most there is no cure and treatment of xerostomia generally involves using saliva substitutes in the form of sprays, gels and lozenges [114]. A new treatment, involving the gene-transfer of aquaporins to duct cells is current being clinically tested [6]. We shall discuss the significance of this in Chapter 8.

2.1.1 The salivation process in parotid acinar cells

Electrophysiology and the plasma membrane

The plasma, or cell, membrane separates the cytoplasm from the cell exterior, or interstitium. The cell membrane is selectively permeable to ions with embedded proteins regulating the movements of ions through ion channels, cotransporters and ATPases. Ionic transport across a membrane is classified as either passive or active. Passive transport of ions occurs down an electrochemical gradient. Here an ion will move across the membrane due to the electrical potential, established by a charge difference across the
2.1. The physiology of saliva secretion

Figure 2.2: Confocal image of the mouse parotid gland. The green signal (Alexa Fluor 488-conjugated phalloidin) shows duct and acinar structure with the blue signal (4,6-diamidino-2-phenylindol) showing the cell nuclei. The sympathetic nerves are shown by the red signal (tyrosine hydroxylase antisera in combination with Alexa Fluor 546-conjugated secondary antibody). The tree-like structure is clearly visible with the duct end surrounded by a cluster of acinar cells. Image reproduced from Warner et al. [116].

membrane, and the chemical potential, given by the concentration difference. The point at which the chemical potential is balanced by an opposing electrical potential is called the Nernst potential.

Active transport of ions occurs against an electrochemical gradient. Here the cell either uses free energy, from the hydrolysis of ATP, to pump ions across the membrane through an ATP-ase. Alternatively, an ion might couple with another ion to be transported across the cell membrane. Antiporters use the downhill electrochemical gradient of an ion moving in one direction to transport another ion in the opposite, against its electrochemical gradient. A cotransporter works by binding ions together and using the downhill gradient of one ion to bring other ions across the membrane.

In the process of saliva secretion cells use both active and passive ion transport to generate an osmotic gradient. Given differing solute concentration, water molecules will pass through a semi-permeable membrane from an area of low solute concentration to an area of high concentration. In saliva secretion a concentration gradient is established, with the concentration higher in the cytosol than the interstitium and higher again in the
duct. This concentration gradient osmotically drives water into the duct and eventually into the mouth. Whilst cell membranes are permeable to water, aquaporins greatly increase the water permeability. Aquaporins are water-selective transport proteins that rapidly transport water molecules whilst preventing the movement of other ions and solutes. Numerous aquaporin genes have been localised in salivary glands. Of concern to our work is the localisation of aquaporin-5 (Aqp5) in the apical membrane of acinar cells [62]. Ma et al. [62] find that saliva secretion is reduced by greater than 60% in Aqp5 knockout mice. This suggests that the majority of water transport into the lumen is transcellular and passes through the aquaporins in the apical membrane. A gene therapy introducing aquaporins to duct cells has recently been shown to be effective in
the treatment of hyposalivation [6]. This treatment not only shows the importance of aquaporins, but also the necessity for greater understanding of duct cells. In Chapter 7 we present a model of Ca\(^{2+}\) signalling in the HSY cells, a parotid duct cell line.

**Cl\(^{-}\) transport**

The transepithelial movement of Cl\(^{-}\) ions is the principal mechanism responsible for primary saliva secretion. This was first proposed by Silva et al. [91] and is now generally accepted [70]. A schematic of the Cl\(^{-}\)-driven secretion model is shown in Figure 2.4. In this model an osmotic gradient is established with water travelling both paracellularly and transcellularly. A Na\(^{+}\)-K\(^{+}\)-ATPase exchanger (NaK) creates an inward Na\(^{+}\) electrochemical gradient which a Na\(^{+}\)-K\(^{+}\)-2Cl\(^{-}\) cotransporter (NKCC) exploits to bring Cl\(^{-}\), Na\(^{+}\) and K\(^{+}\) into the cytosol. The electrochemical potential then allows Cl\(^{-}\) ions to move into the lumen through apical Cl\(^{-}\) channels. K\(^{+}\) ions are extruded through K\(^{+}\) channels which were traditionally only localised in the basolateral membrane [75, 78]. In Chapter 4 we predict that the presence of apical K\(^{+}\) channels could increase the rate of saliva secretion. Subsequent experimental work has found K\(^{+}\) channels in the apical membrane [1]. The Cl\(^{-}\)-driven saliva secretion model is supported by experimental evidence. Evans et al. [33] report that saliva secretion is reduced by 63% in NKCC knockout mice over 50 minutes of stimulation. The reduction is more pronounced over the first five minutes where secretion rates are reduced by 85% in NKCC knockout mice. The localisation of the channels responsible for this Cl\(^{-}\)-driven secretion can be found in Table 2.1.

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<td>Basolateral</td>
<td>[33]</td>
</tr>
<tr>
<td>Na(^{+})-K(^{+})-ATPase exchanger</td>
<td>Basolateral</td>
<td>[121]</td>
</tr>
<tr>
<td>cAMP-activated Cl(^{-}) channel</td>
<td>Apical</td>
<td>[2]</td>
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<tr>
<td>Intermediate and maxi K(^{+}) channels</td>
<td>Basolateral</td>
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<td>Apical</td>
<td>[1]</td>
</tr>
<tr>
<td>Na(^{+})/H(^{+}) exchanger</td>
<td>Basolateral</td>
<td>[15]</td>
</tr>
<tr>
<td>Cl(^{-})/HCO(_3)(^{-}) exchanger</td>
<td>Basolateral</td>
<td>[48] [125]</td>
</tr>
</tbody>
</table>

Table 2.1: Experimental localisation of ion channels and transporters

**HCO\(_3\)\(^{-}\) transport**

Saliva secretion in NKCC knockout mice is reduced by up to 85%, as shown by Evans et al. [33]. However, secretion is not prevented entirely. Cl\(^{-}\)-driven secretion relies
Figure 2.4: Schematic of saliva secretion in a model driven by Cl\(^-\) movement. A question mark is included next to the apical K\(^+\) and basal Cl\(^-\) channels. In Chapter 4 we investigate the effect of including these channels on the rate of saliva secretion. Recent work by Almassy et al. [1] has confirmed the existence of apical K\(^+\) channels. Figure adapted from Palk et al. [76].

entirely on the NKCC, which suggests that an alternative mechanism exists. Melvin et al. [70] propose a mechanism which relies on the secretion of HCO\(_3^-\) shown in Figure 2.5. Carbonic anhydrases catalyse the reversible reaction of CO\(_2\) and H\(_2\)O into HCO\(_3^-\) and H\(^+\). The protons from this reaction are expelled by a Na\(^+\)/H\(^+\) exchanger and secretion is driven by movement of HCO\(_3^-\) into the lumen via apical anion channels. Stimulation with an agonist increases cytosolic Ca\(^{2+}\) which increases the pH. The pH level can stay high for a sustained period of time, hence HCO\(_3^-\)-dependent secretion can continue after agonist has been removed [70]. The hypothesis of HCO\(_3^-\)-driven secretion is supported by evidence from Park et al. [79]. They show the saliva secretion rate in Na\(^+\)/H\(^+\) exchanger (Nhe1) knockout mice is reduced by 17% after 10 minutes of stimulation, increasing to a 35% reduction after 20 minutes. The localisation of the HCO\(_3^-\)-dependent secretion channels and exchangers can be found in Table 2.1.

It is hypothesised that saliva secretion happens in two phases. Following neurotransmitter stimulation, Cl\(^-\)-driven secretion occurs rapidly and is responsible for the
2.1. The physiology of saliva secretion

The majority of the saliva secretion. As time continues, and the cytosolic pH increases, the HCO$_3^-$-dependent secretion becomes more important. The work presented here focusses on Cl$^-$-dependent secretion, as this is the most dominant. In Chapter 8 we shall discuss the need to develop a HCO$_3^-$-driven saliva secretion model in the future.

![Figure 2.5: Schematic of saliva secretion in a model driven by HCO$_3^-$ movement. An apical anion channel allows HCO$_3^-$ extrusion into the lumen. This establishes a concentration gradient, driving water flow. Figure reproduced from Melvin et al. [70].](image)

2.1.2 Neuronal signalling

All secretion by salivary glands occurs as a response to neurotransmitter stimulation [5]. Autonomic nerves are found adjacent to the basal region of both acinar and duct cells [5]. Glands are stimulated by both parasympathetic and sympathetic nerves, with each giving a different response. Both nerve types release a neurotransmitter which binds to receptors at the basal membrane, activating an effector.

The sympathetic nerves, known for their fight or flight response, are responsible for protein secretion. The nerve endings release norepinephrine which binds to alpha and beta receptors. These increase the production of cAMP. The pathway by which protein secretion is increased is currently unknown [5].

The parasympathetic nerves, responsible for rest and digest processes, are considered responsible for fluid secretion. These nerves release acetylcholine which binds to muscarinic cholinergic receptors to increase the generation of phospholipase C (PLC). The increase in PLC increases the production of inositol trisphosphate (IP$_3$), which re-
leases Ca\(^{2+}\) from internal stores. By increasing the open probability of the Cl\(^{-}\) and K\(^{+}\) channels, the Ca\(^{2+}\) concentration then causes increased saliva secretion.

## 2.2 Ca\(^{2+}\) signalling

Ca\(^{2+}\) is essential for many cellular functions, including muscle contraction, cell growth, neuronal signalling and fertilisation. Douglas and Rubin [28] first showed the importance of Ca\(^{2+}\) in the saliva secretion process. They showed saliva secretion did not occur in the absence of cytosolic Ca\(^{2+}\). Ca\(^{2+}\) is now known to be a second messenger linking the neuronal stimulation of cells with secretion of saliva. Ca\(^{2+}\) concentration in the cytoplasm is highly regulated. If the concentration gets too high it can cause cell death. As a result the resting level of Ca\(^{2+}\) in the cytoplasm is low. In order to maintain this low resting concentration, whilst being able to react to stimulus, Ca\(^{2+}\) is stored in the endoplasmic reticulum (ER). The ER is divided into two types, rough and smooth. The smooth ER acts as the internal stores for Ca\(^{2+}\). Ca\(^{2+}\) is pumped into this store via the sarco/endoplasmic reticulum Ca\(^{2+}\)-ATPase (SERCA). The release of Ca\(^{2+}\) is due to fluxes through the IP\(_3\) receptor (IP\(_3\)R) and ryanodine receptor (RyR).

Upon stimulus with an agonist, the Ca\(^{2+}\) concentration in the cytosol is not only seen to increase, but also to exhibit oscillations and waves. De Koninck and Schulman [25], Tang and Othmer [106] and Berridge [9] present experimental and theoretical arguments that these Ca\(^{2+}\) oscillations enable signal encoding in the Ca\(^{2+}\) oscillation frequency of some cell types. In Chapter 6 we investigate the various Ca\(^{2+}\) wave properties such as frequency, amplitude and wave speed, and their effect on the regulation of saliva secretion. We now briefly introduce the most important components involved in Ca\(^{2+}\) signalling.

### 2.2.1 Inositol trisphosphate (IP\(_3\)) and the IP\(_3\) receptor (IP\(_3\)R)

Inositol trisphosphate (IP\(_3\)) is the messenger responsible for signalling the release of Ca\(^{2+}\) from internal stores in non-excitable cells. Upon stimulation with an agonist the production of PLC is increased. PLC production is both agonist and Ca\(^{2+}\) dependent [13]. PLC is an enzyme that cleaves phospholipids, in particular cleaving phosphatidylinositol 4,5-bisphosphate (PIP\(_2\)) into diacylglycerol (DAG) and IP\(_3\). A neuronal release of agonist will therefore increase the PLC concentration which subsequently causes an increase in the IP\(_3\) concentration. IP\(_3\) is removed via a Ca\(^{2+}\) dependent phosphorylation by IP\(_3\) 3-kinase and a dephosphorylation by IP\(_3\) 5-phosphatase [92].
After stimulation with a neurotransmitter and the subsequent production of IP$_3$, it is the IP$_3$Rs which control the release of Ca$^{2+}$ from the internal stores. IP$_3$ binds to the IP$_3$R, opening the receptor channels and allowing the release of Ca$^{2+}$ into the cytosol. The IP$_3$R has been studied extensively since its identification in 1988 [37]. Both Ca$^{2+}$ and IP$_3$ are essential for IP$_3$R activation. IP$_3$ increases the open probability of the IP$_3$R [41, 90]. Ca$^{2+}$ has a more complex relationship. At low Ca$^{2+}$ concentrations an increase in Ca$^{2+}$ opens the receptor to allow more Ca$^{2+}$ release, sometimes referred to as Ca$^{2+}$-induced Ca$^{2+}$ release (CICR). At high Ca$^{2+}$ concentrations the receptor is inactivated by Ca$^{2+}$ [41, 90].

There is much debate around the role of IP$_3$ in Ca$^{2+}$ signalling. It is clear it provides a link between neuronal stimulation and Ca$^{2+}$ release, but questions remain about its actual role in the generation of Ca$^{2+}$ oscillations. Experimental evidence shows Ca$^{2+}$ oscillations both with coupled IP$_3$ oscillations and with constant IP$_3$ [107]. Oscillations have also been demonstrated where the IP$_3$ has been clamped constant [115].

The generation of periodic Ca$^{2+}$ oscillations in non-excitatory cells is explained primarily by two hypotheses, with mathematical models categorised accordingly. In one, oscillations in Ca$^{2+}$ are paired with the opening and closing of the IP$_3$R, and can occur at constant IP$_3$ concentration. Models of this type are classified as Class I models. Low cytosolic Ca$^{2+}$ concentration increases due to CICR. When the Ca$^{2+}$ concentration gets high, the IP$_3$Rs shut. Re-uptake of Ca$^{2+}$ into the ER occurs, reducing the Ca$^{2+}$ concentration and allowing the IP$_3$Rs to open again. This negative feedback loop between the IP$_3$R and cytosolic Ca$^{2+}$ is hypothesised to be the generator of Class I model Ca$^{2+}$ oscillations. In Chapter 7 we present a Class I model of HSY cell Ca$^{2+}$ oscillations and investigate the role of passive IP$_3$ oscillations.

In Class II models, IP$_3$ is required to oscillate, with Ca$^{2+}$ feedback on the production and degradation of IP$_3$ the generator of oscillations. Class I and Class II models can be further classified by the dominant feedback mechanism between Ca$^{2+}$ and IP$_3$. Where Ca$^{2+}$ feedback on the IP$_3$ production is dominant, we shall call the model Class I/II Positive. Where Ca$^{2+}$ primarily causes the increased degradation of IP$_3$, we shall call the model Class I/II Negative. In Chapter 7 we argue Class II Positive models are incapable of producing stable periodic oscillations of Ca$^{2+}$. Class II Negative models can generate oscillations in Ca$^{2+}$. A Class II Negative model is used to explain the Ca$^{2+}$ oscillations in parotid acini seen in Chapter 4. In Section 3.2 we discuss mathematical Ca$^{2+}$ models in greater detail.
2.2.2 The ryanodine receptor (RyR)

Ryanodine is a natural alkaloid found in the stems and roots of the South American plant *Ryania speciosa*. It was first used as an insecticide, having a paralytic effect on skeletal and cardiac muscle. Ryanodine was found to exhibit a high binding affinity to a protein on the ER membrane. This protein is now known as the ryanodine receptor (RyR) [34]. The RyR is another Ca$^{2+}$ channel that allows the release of Ca$^{2+}$ from stores in the ER. Within a physiological range, the open-probability of the RyR continues to increase as the cytosolic Ca$^{2+}$ concentration increases [54]. This is unlike the IP$_3$R, where high Ca$^{2+}$ concentration inactivates the receptor.

CICR was first discovered in skeletal muscle in the 1970’s [32]. The RyRs, requiring only the action of Ca$^{2+}$ to activate, have been considered to play a major role in CICR since their discovery in the 1980’s [32]. CICR through the RyRs is crucial in the contraction of skeletal and cardiac muscle, with disregulation of the RyRs associated with life threatening diseases [17].

At low concentrations ryanodine locks the RyR in the open state, at higher concentration ryanodine causes the RyR to shut. The application of ryanodine is often used to determine the importance of the RyRs in Ca$^{2+}$ signalling. In Chapter 4 the RyRs are seen to play a significant role in the oscillations of Ca$^{2+}$ in parotid acinar cells.

2.2.3 The SERCA

Cytosolic Ca$^{2+}$ is maintained at very low resting concentrations. The ability of the cell to release large quantities of Ca$^{2+}$ from internal stores make Ca$^{2+}$ a very effective cellular messenger. The concentration of Ca$^{2+}$ in the ER is estimated to be between three to four orders of magnitude larger than the resting concentration in the cytosol [37]. In order to maintain this large gradient, the cells must use energy to pump Ca$^{2+}$ into the stores via the sarco/endoplasmic reticulum Ca$^{2+}$-ATPase (SERCA). The activity of the SERCA increases with cytoplasmic Ca$^{2+}$ concentration [61]. High cytosolic Ca$^{2+}$ is toxic and the activity of the SERCA is essential in preventing cell death.

2.2.4 Plasma membrane Ca$^{2+}$ channels

Changes in cytosolic Ca$^{2+}$ occur due to both Ca$^{2+}$ fluxes between the ER and cytosol and Ca$^{2+}$ influx and efflux across the plasma membrane. In electrically excitable cells such as neurons, plasma membrane Ca$^{2+}$ fluxes are of huge importance. The duct and acinar cells of the salivary glands are non-excitable. Plasma membrane fluxes are, however, still significant, as we will see in Chapter 4.
Influx of Ca\(^{2+}\) across the plasma membrane is proposed through both second messenger opened channels and store-operated Ca\(^{2+}\) channels (SOC) \[4\]. Camello et al. \[16\] investigate the efflux of Ca\(^{2+}\) and show the rate of Ca\(^{2+}\) extrusion increases as the cytosolic Ca\(^{2+}\) concentration increases.
Chapter 3

Mathematical modelling applied to saliva secretion and Ca\(^{2+}\) dynamics

3.1 Saliva secretion models

The current mathematical models of saliva secretion contain many of the components of the theoretical model of Silva et al. [91]. Here the excretion of salt by sharks was investigated. Active transport of Cl\(^{-}\) through the basolateral membrane allows an electrochemical potential to drive Cl\(^{-}\) across the apical membrane. Much of this model has remained in saliva secretion models, with a NaK still accepted as the mechanism by which Na\(^{+}\) is removed from the cytosol, in exchange for K\(^{+}\). A schematic of this model can be seen in Figure 3.1.

Petersen et al. [81] were the first to propose a model in which secretion of saliva could be controlled by stimulation. They localised a Ca\(^{2+}\) dependent K\(^{+}\) channel in the basolateral membrane. Increased cytosolic Ca\(^{2+}\) concentration would increase the cycling of K\(^{+}\) into the cell through the NaK and NKCC and out of the cell through the Ca\(^{2+}\) dependent K\(^{+}\) channel. As this cycling rate increases, the uptake of Cl\(^{-}\) is increased and the fluid secretion rate increases. A schematic of this model is given in Figure 3.2. Although the details of the secretion pathway at the apical membrane were not known, the authors hypothesised that a Cl\(^{-}\) channel is present and is responsible for driving the secretion of fluid.

Cook and Young [20] were the first to investigate saliva secretion mathematically. A steady-state electrical model was used to investigate the presence of apical K\(^{+}\) channels. Here a NaK establishes an inward Na\(^{+}\) gradient which a NKCC exploits to bring Cl\(^{-}\) and K\(^{+}\) into the cell. K\(^{+}\) and Cl\(^{-}\) channels in the apical and basal membranes allow the system to reach a steady-state. Several assumptions are made in this simple model. The
Figure 3.1: A schematic of Cl⁻ secretion in the shark rectal gland enabling the excretion of salt. A process which is important for all marine animals. Figure reproduced from Silva et al. [91].
3.1. Saliva secretion models

Figure 3.2: A schematic of fluid secretion presented by Petersen et al. [81]. The presence of Ca$^{2+}$ activated basolateral K$^+$ channels allows the regulation of saliva secretion by neuronal stimulation. Secretion is stimulated by the action of neurotransmitters acetylcholine, substance P or noradrenaline (abbreviated as Ach, S P and NA respectively).
Chapter 3. Mathematical modelling applied to saliva secretion and Ca\(^{2+}\) dynamics

secreted primary saliva is assumed isotonic, all ionic concentrations are constant, ion transport across epithelia is at steady-state and all resistances are constant. We know from experimental data that the cytosolic concentrations change substantially from un-stimulated to stimulated conditions [38, 100]. The primary saliva, although plasma-like, is not entirely isotonic [64]. Currents and fluxes across cell membranes are now well studied and are known to be a function of cytosolic and extracellular ionic concentrations, membrane potentials and the intracellular calcium concentration. In Chapter 4 we investigate the distribution of K\(^+\) channels using a dynamic secretion model which does not make these assumptions.

The first dynamic mathematical model of saliva secretion was presented by Gin et al. [40]. Here expressions for the fluxes through ion channels and transporters were based on experimental data. Differential equations for the cytosolic and lumenal concentrations, and the cell volume are solved numerically. The model also includes one equation for the cell membrane, in effect assuming the apical and basal membranes have the same electrical potential. Localisation studies demonstrate that saliva secretion is a directed process. Cl\(^-\) is first transported across the basolateral membrane before an electrochemical potential drives extrusion of ions across the apical membrane into the lumen. Not only are ion channels, cotransporters and exchangers localised to the distinct apical and basolateral membranes (Table 2.1), but also the apical and basolateral membrane potentials are shown experimentally to be different [11, 56, 60, 65]. The existence of distinct membrane potentials was shown by Cook and Young [20] to be important in the question of K\(^+\) channel location. In Chapter 4 we present a dynamic model of saliva secretion with distinct membrane potentials. We use this model to investigate the effect of apical K\(^+\) channels on saliva secretion rates.

The work of Gin et al. [40] was the first to incorporate a model of Ca\(^{2+}\) dynamics into a saliva secretion model. Simulations show the model reproduces a number of experimental Ca\(^{2+}\) traces. Ca\(^{2+}\) oscillations were considered by the authors to be due to Class II mechanisms (details follow in Section 3.2), with oscillations in the IP\(_3\) concentration required for the periodic oscillations in Ca\(^{2+}\). It is easy to show, however, that the Ca\(^{2+}\) oscillations presented by Gin et al. [40] are in fact due to fluxes across the plasma membrane. Closed cell simulations showed no oscillations in Ca\(^{2+}\). In Chapter 7 we present an argument that Class II Positive models are incapable of producing stable oscillations of Ca\(^{2+}\). In Chapter 4 we argue that a Class II Negative model is responsible for the Ca\(^{2+}\) oscillations in parotid acinar cells.
3.2 Calcium models

Mathematical models have been used to understand Ca\(^{2+}\) dynamics, such as waves and oscillations, for over 30 years. Where experimentalists could only accurately measure one variable, the cytosolic Ca\(^{2+}\) concentration, mathematical models provide quantitative insight into the others. Numerous approaches have been taken and here we shall provide a brief review.

Kuba and Takeshita [55] first proposed a Ca\(^{2+}\) model in 1981 to explain Ca\(^{2+}\) oscillations in sympathetic neurons. A compartmental, deterministic approach was taken, which the majority of Ca\(^{2+}\) models have continued to follow. Here Ca\(^{2+}\) is considered stored in the cytosol, the internal stores, or external to the cell. The periodic oscillations in Ca\(^{2+}\) are the result of the movement of Ca\(^{2+}\) between these three compartments.

In 1988 Meyer and Stryer [71] proposed a model of Ca\(^{2+}\) oscillations for non-excitable cells. Here the periodic release of Ca\(^{2+}\) from the ER was paired with oscillations in IP\(_3\). Two years later Goldbeter et al. [42] presented an alternative model which produced oscillations in Ca\(^{2+}\) at a constant IP\(_3\) concentration. Since these early publications, Ca\(^{2+}\) models have been considered in two classes. Class I models can produce oscillations at constant IP\(_3\) concentration, with oscillations the result of the periodic opening and closing of the IP\(_3\)R. Class II models require oscillations in IP\(_3\), where Ca\(^{2+}\) feedback on IP\(_3\) production and degradation is the generator of Ca\(^{2+}\) oscillations. The feedback mechanisms in Class I and Class II models can be seen in Figure 3.3. In Section 3.2.3 we introduce a third class of models which we call Open-Class. Sneyd et al. [99] proposed an experimental protocol for distinguishing Class I or Class II mechanisms. In subsequent work by Harvey et al. [47] the infallibility of this test was questioned. Recent experimental procedures have allowed the measurement of IP\(_3\) during Ca\(^{2+}\) oscillations. In Chapter 7 we make new hypotheses around the role of IP\(_3\) specifically applied to salivary duct cells.

3.2.1 Class I Ca\(^{2+}\) models

A well studied Class I model is that of Atri et al. [3]. An equation for the rate of change of Ca\(^{2+}\) is paired with an equation representing the fraction of open IP\(_3\)Rs. In its most minimal form, a Class I model can be written as follows,

\[
\frac{dC}{dt} = \text{release}(C, R) - \text{reuptake}(C),
\]

\[
\frac{dR}{dt} = \text{opening}(C) \cdot (1 - R) - \text{closing}(C) \cdot R. \tag{3.1}
\]
Chapter 3. Mathematical modelling applied to saliva secretion and Ca\(^{2+}\) dynamics

Figure 3.3: Class I and Class II Ca\(^{2+}\) model feedback mechanisms. Red arrows show positive feedback and blue, thin, arrows show negative feedback. Bold arrows show the feedback mechanisms that are strictly necessary for Ca\(^{2+}\) oscillations in a given model, with faded arrows showing feedback mechanisms that may exist, but are not responsible for the Ca\(^{2+}\) oscillations.

Here \(C\) represents the cytosolic Ca\(^{2+}\) concentration and \(R\) represents the fraction of open IP\(_3\)Rs. The release of Ca\(^{2+}\) from internal stores is dependent on the fraction of open receptors and the cytosolic Ca\(^{2+}\) concentration. The release term can incorporate CICR through the RyR plus IP\(_3\)-dependent release of Ca\(^{2+}\) through the IP\(_3\)R. The re-uptake of Ca\(^{2+}\) through the SERCA pump is Ca\(^{2+}\) dependent. The fraction of closed IP\(_3\)Rs, \(1 - R\), open at a Ca\(^{2+}\) dependent rate. Similarly, the open receptors, \(R\), close at a Ca\(^{2+}\) dependent rate. A negative feedback loop between Ca\(^{2+}\) and the IP\(_3\)Rs is the generator of periodic Ca\(^{2+}\) oscillations.

Various other variables are often added to the simple form of the model. De Young and Keizer [26] model the binding and unbinding of Ca\(^{2+}\) and IP\(_3\) to the IP\(_3\)Rs by representing the IP\(_3\)R in various sub-units. This replaces the variable \(R\) by a number of variables which together represent the IP\(_3\)R dynamics.

The system of equations (3.1) models the release and re-uptake of Ca\(^{2+}\) from the ER. We call this a closed-cell model. This model is often extended to be an open-cell model, including Ca\(^{2+}\) fluxes across the plasma membrane. Here a third variable is added representing the ER or total cell Ca\(^{2+}\) concentration. Examples of open-cell Class I models include the models of Sneyd et al. [99] and Li and Rinzel [58].

Another extension of the simple Class I model is the inclusion of passive IP\(_3\) oscillations. Here Ca\(^{2+}\) oscillations occur due to the feedback between Ca\(^{2+}\) and the IP\(_3\)R. The oscillating Ca\(^{2+}\) affects the production and/or degradation of IP\(_3\), and IP\(_3\) oscillations occur as passive reflections of Ca\(^{2+}\). In Chapter 7 we will discuss the importance of passive IP\(_3\) oscillations in the generation of long period Ca\(^{2+}\) oscillations in duct cells.
3.2. Calcium models

3.2.2 Class II Ca\(^{2+}\) models

In Class II Ca\(^{2+}\) models, oscillations in IP\(_3\) are required for oscillation in Ca\(^{2+}\). Kuba and Takeshita [55] first proposed a Class II oscillatory mechanism with another example from Cuthbertson and Chay [23]. A Class II model in its simplest form can be written as follows,

\[
\begin{align*}
\frac{dC}{dt} &= \text{release}(C, P) - \text{reuptake}(C), \\
\frac{dP}{dt} &= \text{production}(C) - \text{degradation}(C) \cdot P,
\end{align*}
\]

(3.2)

where \(C\) is the cytosolic Ca\(^{2+}\) concentration and \(P\) is the IP\(_3\) concentration. Release of Ca\(^{2+}\) from the ER increases with IP\(_3\) and is Ca\(^{2+}\) dependent. Re-uptake through the SERCA is Ca\(^{2+}\) dependent. Ca\(^{2+}\) affects the production and degradation rates of IP\(_3\). Given stimulation with an agonist the IP\(_3\) production increases. The increased IP\(_3\) concentration opens the IP\(_3\)Rs and releases Ca\(^{2+}\) into the cytosol. Higher cytosolic Ca\(^{2+}\) causes an increase in Ca\(^{2+}\) dependent IP\(_3\) degradation and a subsequent reduction in the IP\(_3\) concentration. This negative feedback loop produces stable periodic oscillations of Ca\(^{2+}\). In Chapter 4 we present a Class II model of Ca\(^{2+}\) oscillations in the parotid acinar cells.

In Chapter 7 we argue that Ca\(^{2+}\) feedback on IP\(_3\) degradation is necessary for Ca\(^{2+}\) oscillations in Class II models. Several Class II models are proposed which claim, erroneously, to generate Ca\(^{2+}\) oscillations from Ca\(^{2+}\) feedback on IP\(_3\) production [27, 40, 99]. However, it is simple to show that oscillations occur as a result of the periodic pumping of Ca\(^{2+}\) across the plasma membrane. We discuss these Open-Class models further in the following section 3.2.3.

As with Class I models, it is common to add additional variables to the simple Class II model. It is possible to include the IP\(_3\)R kinetics with Ca\(^{2+}\) oscillation still dependent on IP\(_3\) oscillations. The system of equations (3.2) represents a closed-cell model. The addition of Ca\(^{2+}\) influx and efflux across the plasma membrane is often added [76].

3.2.3 Ca\(^{2+}\) models dependent on plasma membrane fluxes

(Open-Class models)

Camello et al. [16] show that Ca\(^{2+}\) efflux across the plasma membrane is Ca\(^{2+}\) dependent. This Ca\(^{2+}\) dependent efflux is crucial in generating stable periodic orbits in a number of Ca\(^{2+}\) models. Ca\(^{2+}\) release from the ER increases the cytosolic Ca\(^{2+}\) concentration and thus increases Ca\(^{2+}\) efflux across the plasma membrane. With co-operative CICR and
Chapter 3. Mathematical modelling applied to saliva secretion and Ca\(^{2+}\) dynamics

Ca\(^{2+}\) efflux, the internal stores of Ca\(^{2+}\) can be greatly depleted. Once the ER Ca\(^{2+}\) is depleted, Ca\(^{2+}\) release and efflux stop. Ca\(^{2+}\) enters the cell through the influx channels and slowly refills the ER. Once the concentration in the ER reaches a critical level, CICR causes another rise in cytosolic Ca\(^{2+}\) and depletion of the ER. In this class of model, Ca\(^{2+}\) oscillations require paired oscillations in the total cellular Ca\(^{2+}\) concentration. We shall call these “Open-Class” models.

A simplified Open-Class model can be written as follows,

\[
\frac{dC}{dt} = \text{release}(C, C_{er}) - \text{reuptake}(C) + \text{influx} - \text{efflux}(C),
\]

\[
\gamma \frac{dC_{er}}{dt} = \text{reuptake}(C) - \text{release}(C, C_{er}),
\]

here \(C\) is cytosolic Ca\(^{2+}\) concentration and \(C_{er}\) is the concentration of Ca\(^{2+}\) in the ER. Release of Ca\(^{2+}\) from the ER is dependent on the cytosolic Ca\(^{2+}\) concentration and the concentration gradient between the ER and cytosol. Cytosolic Ca\(^{2+}\) changes as a result of ER release and re-uptake, plus influx and efflux across the plasma membrane. The parameter \(\gamma\) gives a measure of the relative size of the cytosol to the ER. The ER is contained in the cytosol and is predicted to be around five times smaller in volume [40]. A movement of ions between the ER and cytosol will therefore have a larger effect on the change of ER concentration that to the cytosolic concentration.

The total Ca\(^{2+}\) contained in the cell is a combination of the cytosolic and ER Ca\(^{2+}\),

\[C_t = C + C_{er}/\gamma.\]

It is possible to rearrange Equation (3.3) and rewrite it in terms of total cellular concentration \(C_t\),

\[
\frac{dC}{dt} = \text{release}(C, C_t) - \text{reuptake}(C) + \text{influx} - \text{efflux}(C),
\]

\[
\frac{dC_t}{dt} = \text{influx} - \text{efflux}(C).
\]

In this rearranged form it is possible to see the negative feedback responsible for generating Ca\(^{2+}\) oscillations. A rise in \(C_t\) increases the Ca\(^{2+}\) release from the ER. This causes an increase in the cytosolic Ca\(^{2+}\) concentration. As the cytosolic Ca\(^{2+}\) concentration increases, the efflux across the plasma membrane rises. This causes a reduction in the whole cell Ca\(^{2+}\) concentration.

Goldbeter et al. [42] and Dupont and Goldbeter [29] presented models where the oscillations of cytosolic Ca\(^{2+}\) are paired with oscillations in the total cellular Ca\(^{2+}\) concentration. As previously mentioned, there are several Ca\(^{2+}\) models [27, 40, 99] that claim to be
Class II models but will actually reduce to this Open-Class model. This can be achieved by observing the non-existence of Ca$^{2+}$ oscillations when influx = efflux($C$) = 0. It is also possible to observe stable periodic oscillations in Ca$^{2+}$ for a range of constant IP$_3$ concentration in these models.

Experimentally, determining the importance of the plasma membrane Ca$^{2+}$ fluxes is relatively straightforward. A common procedure when measuring Ca$^{2+}$ oscillations is to remove all Ca$^{2+}$ from the external medium surrounding the cell. These Ca$^{2+}$-free experiments remove the possibility of Ca$^{2+}$ influx across the plasma membrane. With zero Ca$^{2+}$ influx, periodic oscillations in the total cell Ca$^{2+}$ are impossible. In Chapter 4 and Chapter 7 we discuss the importance of plasma membrane fluxes in parotid acinar and duct cells.

### 3.2.4 Alternative approaches to Ca$^{2+}$ modelling

So far we have presented a range of deterministic, compartmental Ca$^{2+}$ models which have been used to aid the understanding Ca$^{2+}$ signalling in cellular processes. Recent experimental phenomena have led to new areas of Ca$^{2+}$ modelling. Spatial Ca$^{2+}$ models solved using partial differential equations, have recently been used to explain travelling and spiral waves such as those observed in hippocampal slice cultures shown in Figure 3.4 [85, 97, 96, 120]. In Chapter 5 we present a spatial model of Ca$^{2+}$ waves in parotid acinar cells. In Chapter 6 we then analyse the importance of Ca$^{2+}$ waves in the regulation of saliva secretion.

Improvements in the spatial resolution and sampling rate of microscopes have led to the study of highly localised releases of Ca$^{2+}$. These local releases, named sparks, quarks, puffs or blips, are thought to be the result of Ca$^{2+}$ release through individual or small clusters of Ca$^{2+}$ release receptors [31]. The release of Ca$^{2+}$ from an individual channel, a blip, is thought to increase the release of Ca$^{2+}$ from other receptors in the same cluster through CICR. If a cluster of receptors all release Ca$^{2+}$, a spark (if RyRs) or puff (if IP$_3$Rs) occurs. A large enough spark or puff is then able to trigger a global Ca$^{2+}$ wave or oscillation [10]. Complex stochastic models have been used to investigate these Ca$^{2+}$ puffs and sparks [21, 88, 89]. Experimental data from salivary glands show regular oscillations of Ca$^{2+}$ with dense clustering of IP$_3$Rs at the apical membrane and RyRs at the basal pole. For this reason we shall not take the stochastic approach in our modelling. In Chapter 8 we discuss the comparison of a deterministic and stochastic Class I Ca$^{2+}$ model as an area of future work.
Figure 3.4: A spiral Ca$^{2+}$ wave in Hippocampal slice cultures, figure reproduced from Harris-White et al. [46]. Scale 550 µm horizontally by 400 µm vertically.
Chapter 4

A dynamic model of saliva secretion

We present a mathematical model of the parotid acinar cell with the aim of investigating how the distribution of $K^+$ and $Cl^-$ channels affects saliva secretion. What follows in Sections 4.1-4.6.8 is a copy of the article as it appears in the Journal of Theoretical Biology, volume 266(4). The full reference can be found in the bibliography [76]. A few minor corrections have been made following publication, these will be shown in footnotes. A small number typographical errors have also been corrected following publication. An addendum can be found in Section 4.7 which reviews the impact of this work following its publication.
Chapter 4. A dynamic model of saliva secretion

4.1 Abstract

We construct a mathematical model of the parotid acinar cell with the aim of investigating how the distribution of K\(^+\) and Cl\(^-\) channels affects saliva production. Secretion of fluid is initiated by Ca\(^{2+}\) signals acting on the Ca\(^{2+}\) dependent K\(^+\) and Cl\(^-\) channels. The opening of these channels facilitates the movement of Cl\(^-\) ions into the lumen which water follows by osmosis. We use recent results into both the release of Ca\(^{2+}\) from internal stores via the inositol (1,4,5)-trisphosphate receptor (IP\(_3\)R) and IP\(_3\) dynamics to create a physiologically realistic Ca\(^{2+}\) model which is able to recreate important experimentally observed behaviours seen in parotid acinar cells. We formulate an equivalent electrical circuit diagram for the movement of ions responsible for water flow which enables us to calculate and include distinct apical and basal membrane potentials to the model. We show that maximum saliva production occurs when a small amount of K\(^+\) conductance is located at the apical membrane, with the majority in the basal membrane. The maximum fluid output is found to coincide with a minimum in the apical membrane potential. The traditional model whereby all Cl\(^-\) channels are located in the apical membrane is shown to be the most efficient Cl\(^-\) channel distribution.

4.2 Introduction

Saliva production is an important process for both digestion and oral health. Problems with salivation can cause issues with dental cavities, oral pain and infections. In mastication, an adequate supply of saliva is needed to provide lubrication to the mouth and facilitate swallowing. Humans and most mammals possess three major pairs of salivary glands (parotid, sublingual and submandibular). The parotid gland is the largest of these, and is also the only major salivary gland that produces purely serous fluid, with the other glands producing mucous. The structure of the parotid gland consists of bunches of acini connected by ducts. Each acinus comprises several epithelial parotid acinar cells secreting fluid into a shared lumenal cavity. The salivary fluid passes from the lumen of the acini into a branching network of ducts, where it is collected and travels to the mouth. Dysregulation of fluid secretion from the parotid acinar cells can lead to conditions such as xerostomia (dry mouth), where sufferers experience pain with eating and speech because of a lack of saliva.

Fluid is known to travel through the parotid cells by the process of osmosis. Given a membrane impermeable to ions but permeable to water, water will flow from the area of low ionic concentration to that of high concentration. The parotid gland must maintain
an ionic gradient, increasing from the interstitium, to the cytoplasm and then into the lumen, to facilitate water movement through the cell to the duct. Whilst maintaining this gradient the acinar cells must avoid accumulation of too much water in the cell, as the cell membrane is incapable of sustaining any significant pressure difference.

Existing models for fluid flow through the parotid acinar cell place K\(^+\) channels exclusively in the basolateral membrane [40, 74, 113, 112]. However, there is building evidence for the existence of K\(^+\) channels in the apical membrane (Sørensen et al. [101] find apical K\(^+\) channels in frog skin glands, Catalan and Melvin [Unpublished] in parotid acinar). Cook and Young [20] looked at adding apical K\(^+\) channels to a model with constant ionic concentrations and steady state currents. We aim to investigate how the distribution of K\(^+\) channels changes the saliva production of the parotid acinar cell in a dynamic model where salivation is initiated by Ca\(^{2+}\) signalling.

We use the currently accepted model for saliva production (see Nauntofte [74] for a review) where the movement of Cl\(^-\) into the cytoplasm and then into the lumen creates a concentration gradient which water follows by osmosis. In this model the basolateral membrane contains a Na\(^+\)-K\(^+\)-2Cl\(^-\) cotransporter (NKCC), a Na\(^+\)-K\(^+\)-ATPase (NaK) and a K\(^+\) ion channel. Cl\(^-\) moves into the cell via the NKCC and then moves through Ca\(^{2+}\) sensitive apical Cl\(^-\) channels into the lumen. The Na\(^+\) and K\(^+\) ions are removed from the cell by K\(^+\) channels and the NaK pump. See Figure 4.1 for a diagram of the model used.

Calcium (Ca\(^{2+}\)) plays a critical role in epithelial tissue as a second messenger activating fluid flow. Previous models have investigated Ca\(^{2+}\) signalling in a variety of epithelial tissues including airway epithelium [95, 118, 117]. As Ca\(^{2+}\) concentration increases in parotid cells the ionic channels at the basal and apical membrane open and fluid production is seen to increase. The salivation process is initiated in the otic ganglion parasympathetic nerve which runs from the brain to the parotid gland. When stimulated, for example when we smell food, these nerves release acetylcholine, which binds to receptors on the cell surface, leading to the production of inositol (1,4,5)-trisphosphate (IP\(_3\)) in the cytoplasm. The raised IP\(_3\) concentration releases Ca\(^{2+}\) from internal stores in the endoplasmic reticulum (ER). As Ca\(^{2+}\) concentration increases in parotid cells the ionic channels at the basal and apical membrane open and fluid production is seen to increase. We create a model for intracellular Ca\(^{2+}\) using recent results relating to the release of Ca\(^{2+}\) from internal stores via the inositol (1,4,5)-trisphosphate receptor (IP\(_3\)R) and the dynamics of IP\(_3\). Feedback of Ca\(^{2+}\) on the degradation of IP\(_3\) is the mechanism which our model uses to recreate Ca\(^{2+}\) oscillations.

Insight into the role of the K\(^+\) channel distribution in determining the rate of water
transport in the parotid cell is gained by observing the apical and basolateral membrane potentials during simulations with and without the addition of apical $K^+$ channels.

### 4.3 The model

![Diagram of ion movement in the parotid cell](image)

Figure 4.1: Mechanisms underlying fluid flow. The basal membrane separates the cytoplasm from the interstitium, with the apical membrane at the other pole separating the cytoplasm from the lumen. $Cl^-$ moves into the lumen through the apical membrane and water follows by osmosis. Paracellular movement of cations through the tight junctions balances the movement of negative $Cl^-$ ions into lumen. The model allows for the possibility of apical as well as basal $K^+$ channels. We also allow for possible $Cl^-$ efflux through the basal membrane.

#### 4.3.1 Model assumptions and notation

We introduce a subscript notation for ionic concentrations, with $[x]_i$, $[x]_l$, $[x]_e$, $[x]_{er}$ denoting, respectively, cytosolic, luminal, interstitial and ER concentrations of an ion $x$. We assume the ionic concentrations in the interstitium stay constant, while the concentrations in the cytoplasm and lumen change as a result of the ion movements seen
4.3. The model

in Figure 4.1. Stepwise increases in total ionic concentrations from the interstitium to the cytoplasm and then to the lumen result in fluid flow by osmosis into the lumen. Any difference between water flow into or out of the cell causes changes in cell volume and thus cytosolic volume, $w$, is also a variable of our model. Volumes of the ER, $w_{er}$, and the lumen $w_L$ are constant and given as fractions of the unstimulated steady-state cytosolic volume, $w_0$.

4.3.2 Calcium dynamics

In an earlier model of the parotid acinar cell by Gin et al. [40] Ca$^{2+}$ oscillations were found to depend on cell volume oscillations. It seems unlikely that parotid cells require oscillating volume to display Ca$^{2+}$ oscillations. Indeed, many cell types which do not transport fluid, and hence have a fixed volume, exhibit Ca$^{2+}$ oscillations. Therefore we aim to produce a model where Ca$^{2+}$ oscillations can be shown not to depend on oscillating cell volume.

We use a recent model of the inositol (1,4,5)-trisphosphate receptor (IP$_3$R) by Gin et al. [41] and a model of inositol (1,4,5)-trisphosphate (IP$_3$) dynamics by Politi et al. [82] with the aim of reproducing Ca$^{2+}$ behaviour seen experimentally. Ca$^{2+}$ is stored in the ER and is released via Ca$^{2+}$ and IP$_3$-dependent channels into the cytoplasm. Ca$^{2+}$ influxes and effluxes are also present from the interstitium to the cytoplasm. A schematic diagram of our Ca$^{2+}$ model is shown in Figure 4.2.

Two feedback mechanisms have been found capable of creating Ca$^{2+}$ oscillations. In one, Ca$^{2+}$ feeds back on the inositol (1,4,5)-trisphosphate receptor (IP$_3$R), and in the other Ca$^{2+}$ feeds back on IP$_3$ metabolism. This second feedback mechanism can be positive or negative in nature, with Ca$^{2+}$ increasing IP$_3$ production or increasing IP$_3$ degradation. Sneyd et al. [99] found that in pancreatic acinar cells Ca$^{2+}$ oscillations were dependent on IP$_3$ oscillations and thus the feedback on IP$_3$ metabolism was responsible for Ca$^{2+}$ oscillations. Given the similarity of the pancreatic acinar to the parotid acinar cell our model assumes that the Ca$^{2+}$ oscillations arise from feedback of Ca$^{2+}$ on IP$_3$ metabolism.

IP$_3$ dynamics

Our model of IP$_3$ dynamics is based on Politi et al. [82]. The IP$_3$ production rate, $\nu$ is proportional to the applied agonist concentration. IP$_3$ then degrades by Ca$^{2+}$-dependent phosphorylation up to a maximum rate $k_{3K}$ and constant dephosphorylation at a rate $k_{5p}$. High IP$_3$ concentrations cause the channels in the ER to open which causes an increase
Figure 4.2: Schematic of the calcium model. Ca\(^{2+}\) fluxes are shown with solid black arrows. IP\(_3\) and Ca\(^{2+}\) feedback on the IP\(_3\)R and IP\(_3\) degradation are shown with dashed arrows. Agonist concentration stimulates IP\(_3\) production via PLC. IP\(_3\) then degrades by dephosphorylation at rate \(k_{5p}\) and phosphorylation at a Ca\(^{2+}\)-dependent rate \(k_{3K}\) (grey arrows). Increases in Ca\(^{2+}\) and IP\(_3\) concentration raise the open probability of the IP\(_3\)R, releasing Ca\(^{2+}\) from the ER.

in the cytosolic Ca\(^{2+}\) concentration. As the Ca\(^{2+}\) concentration rises IP\(_3\) degradation is increased and IP\(_3\) concentration falls, causing a subsequent closing of the ER channels and a drop in Ca\(^{2+}\) concentration. As the Ca\(^{2+}\) concentration decreases IP\(_3\) is able to increase in concentration due to the lowered degradation rate. It is in this cycle that the Ca\(^{2+}\) and IP\(_3\) oscillations occur. The rate of change of IP\(_3\) can be expressed in the following differential equation

\[
\frac{d([IP_3]w)}{dt} = w_0 \nu - J_{IP_3deg},
\]

where \(w\) is the cell volume, \(w_0\) is the steady state cell volume, \(\nu\) is the agonist-dependent rate of IP\(_3\) production, which will be used as our control parameter for simulations, and

\[
J_{IP_3deg} = w_0 \left( k_{5p} + k_{3K} \frac{[Ca]_i^2}{[Ca]_i^2 + k_{deg}^2} \right) [IP_3],
\]

is the rate of degradation. Parameter values are given in Table 4.1.
4.3. The model

### IP₃ parameter values

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{3K}$</td>
<td>$40 \text{ s}^{-1}$</td>
</tr>
<tr>
<td>$k_{5p}$</td>
<td>$0.005 \text{ s}^{-1}$</td>
</tr>
<tr>
<td>$k_{\text{deg}}$</td>
<td>$400 \text{ nM}$</td>
</tr>
</tbody>
</table>

Table 4.1: Parameters modified slightly from those given for the negative feedback model in Politi et al. [82]

Sims and Allbritton [92] give the half life of IP₃ in the parotid acinar cell as approximately 1 second. The parameter value $k_{3K} = 40 \text{ s}^{-1}$ satisfies this half life at a intracellular Ca²⁺ concentration near the steady state value of 50 nM and using the half activation of $k_{\text{deg}}$ of 400 nM as given in Politi et al. [82]. The constant dephosphorylation rate $k_{5p}$ is a fitted parameter found to give Ca²⁺ oscillations seen experimentally.

In Politi et al. [82] parameter values are given for both positive and negative feedback relating to whether Ca²⁺ feeds back on IP₃ production or degradation, with positive feedback models being relevant to fast IP₃ behaviour. Given the IP₃ turnover in parotid cells is slow with a half life of 1 second our model includes only Ca²⁺ feedback on IP₃ degradation and has production of IP₃ linear with agonist.

### IP₃ receptor model

The IP₃ receptor model is based on the recent work of Gin et al. [41]. Where single channel data from the type-1 IP₃ receptor is used to determine rate constants and their dependence on both IP₃ and Ca²⁺.

![Figure 4.3: Four state model of the IP₃ receptor](image)

Following Gin et al. [41] we use a four-state model with one open state and three closed states, (Figure 4.3). The steady state open probability is given by

$$P_{IPR} = \frac{q_{12}q_{32}q_{24}}{q_{12}q_{32}q_{24} + q_{42}q_{23}q_{12} + q_{42}q_{32}q_{12} + q_{42}q_{32}q_{21}}.$$  

The rate constants between the states were investigated for their dependence on both
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the concentration of Ca\(^{2+}\) and IP\(_3\) and are given by,

\[
q_{12} = 0.74 \text{ ms}^{-1} \quad q_{21} = \Phi_{21}([\text{IP}_3]) \text{ ms}^{-1}, \\
q_{23} = \alpha_{23} \psi_{23}([\text{Ca}]) \phi_{23}([\text{IP}_3]) \text{ ms}^{-1}, \\
q_{32} = \alpha_{32} \psi_{32}([\text{Ca}]) \phi_{32}([\text{IP}_3]) \text{ ms}^{-1}, \\
q_{24} = 7.84 \text{ ms}^{-1} \quad q_{42} = 3.6 \text{ ms}^{-1},
\]

where

\[
\phi_{21}([\text{IP}_3]) = \frac{VP_{21}}{1 + kp_{21} [\text{IP}_3]^3} + bp_{21}, \\
\psi_{23}([\text{Ca}]) = a_{23} - \left( \frac{V_{23}}{k_{23}^2 + [\text{Ca}]_i^2} + b_{23} \right) \left( \frac{Vm_{23}[\text{Ca}]_i^5}{km_{23}^2 + [\text{Ca}]_i^5 + bm_{23}} \right), \\
\phi_{23}([\text{IP}_3]) = \frac{VP_{23}}{1 + kp_{23} [\text{IP}_3]^3} + bp_{23}, \\
\psi_{32}([\text{Ca}]) = \left( \frac{V_{32}}{k_{32}^3 + [\text{Ca}]_i^3} + b_{32} \right) \left( \frac{Vm_{32}[\text{Ca}]_i^7}{km_{32}^3 + [\text{Ca}]_i^7 + bm_{32}} \right), \\
\phi_{32}([\text{IP}_3]) = \frac{VP_{32}[\text{IP}_3]^3}{1 + kp_{32} [\text{IP}_3]^3} + bp_{32}.
\]

### IP\(_3\) receptor parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(bp_{21})</td>
<td>0.11 ms(^{-1})</td>
</tr>
<tr>
<td>(kp_{21})</td>
<td>(5 \times 10^{-10}) nM(^{-3})</td>
</tr>
<tr>
<td>(VP_{21})</td>
<td>0.0949 nM(^3) ms(^{-1})</td>
</tr>
<tr>
<td>(a_{23})</td>
<td>1/1.023 ms(^{-1})</td>
</tr>
<tr>
<td>(V_{23})</td>
<td>(1.08 \times 10^8) nM(^2) ms(^{-1})</td>
</tr>
<tr>
<td>(Vm_{23})</td>
<td>0.3545</td>
</tr>
<tr>
<td>(k_{32})</td>
<td>7 (\times 10^6) nM(^3) ms(^{-1})</td>
</tr>
<tr>
<td>(V_{32})</td>
<td>1.06</td>
</tr>
<tr>
<td>(kp_{32})</td>
<td>0.001 ms(^{-1})</td>
</tr>
<tr>
<td>(kp_{32})</td>
<td>1.5 (\times 10^{-10}) nM(^{-3})</td>
</tr>
<tr>
<td>(V_{32})</td>
<td>0.162 nM(^3) ms(^{-1})</td>
</tr>
<tr>
<td>(Vm_{32})</td>
<td>72 nM</td>
</tr>
<tr>
<td>(k_{32})</td>
<td>520 nM</td>
</tr>
<tr>
<td>(bm_{32})</td>
<td>0.005 ms(^{-1})</td>
</tr>
<tr>
<td>(bm_{32})</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Table 4.2: All parameters taken from Gin et al. [41] with the following exceptions, \# are scaling parameters, * parameters have been modified from original values in Gin et al. [41] to ensure correct scaled rates and a closed IPR in the absence of IP\(_3\).

The rate constants \(q_{21}\) and \(q_{32}\) depend on both Ca\(^{2+}\) and IP\(_3\). The dependence of the rate constants on Ca\(^{2+}\) was established at an IP\(_3\) concentration of 100 µM. Scaling factors ensure our model matches the experimental data at this IP\(_3\) concentration.

Parameter values can be found in Table 4.2 \(^1\). All values are those found in Gin et al. [41] with the following exception. \(bp_{21}\) is increased slightly from the paper value of 0.085 to the new value of 0.11. This is to ensure that when the IP\(_3\) concentration is 100 µM the rate \(q_{21}\) is equal to that found in the Ca\(^{2+}\) dependence experiments. The parameter \(bp_{32}\) is set to zero (as opposed to its value in Gin et al. [41] of 0.0007) this is to ensure

\(^1\) Parameter \(V_{23}\) is corrected from its incorrect value of \(1.08 \times 10^{-6}\) published in [76].
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that the IP₃ receptor is shut when the IP₃ concentration is zero.

Given the steady-state open probability, \( P_{IPR} \), the flux through the IP₃ is given by,

\[
J_{IPR} = w_0 k_{IPR} P_{IPR} ([Ca]_{er} - [Ca]_i).
\]

Here \( k_{IPR} = 0.04 \text{ s}^{-1} \) is the IP₃ receptor density, \([Ca]_{er}\) and \([Ca]_i\) are the \(Ca^{2+}\) concentrations in the ER and cytoplasm respectively.

**Calcium fluxes**

Experimental data shows the ryanodine receptor (RyR) is important for \(Ca^{2+}\) oscillations [14]. We use a RyR model developed by Keizer and Levine [54]. Here the flux through the RyR is given by

\[
J_{RyR} = w_0 k_{RyR} P_{RyR} ([Ca]_{er} - [Ca]_i),
\]

where \( k_{RyR} = 0.01 \text{ s}^{-1} \) is the receptor density and \( P_{RyR} \) is the steady state open probability given by

\[
P_{RyR} = \frac{w^\infty (1 + ([Ca]_i/K_b)^3)}{1 + (K_a/[Ca]_i)^4 + ([Ca]_i/K_b)^3},
\]

and

\[
w^\infty = \frac{1 + (K_a/[Ca]_i)^4 + ([Ca]_i/K_b)^3}{1 + 1/K_c + (K_a/[Ca]_i)^4 + ([Ca]_i/K_b)^3}.
\]

Here \( K_a^4 = k_a^-/k_a^+ \), \( K_b^3 = k_b^-/k_b^+ \) and \( K_c = k_c^-/k_c^+ \), (Table 4.3). We include a passive leak of \(Ca^{2+}\) from the ER into the cytoplasm,

\[
J_{er} = w_0 k_{er} ([Ca]_{er} - [Ca]_i),
\]

where \( k_{er} = 1.554 \times 10^{-4} \text{ s}^{-1} \).

\(Ca^{2+}\) is removed from the cytoplasm back to the ER by a SERCA pump. This is modelled with a Hill coefficient of 2 and a half activation, \(K_{SERCA}\), of 400nM [61],

\[
J_{SERCA} = \frac{V_{SERCA}[Ca]_i^2}{K_{SERCA}^2 + [Ca]_i^2}.
\]

\(Ca^{2+}\) enters and leaves the cytoplasm through the membrane via two fluxes, \(J_{in}\) and \(J_{pm}\). Efflux is approximated by a Hill function with Hill coefficient of 3 and half activa-
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Influx of $\text{Ca}^{2+}$ from the interstitium to the cytoplasm is modelled as by Gin et al. [40] with a constant leak, $\alpha_1$ and agonist dependent influx $\alpha_2\nu$.

$$J_{\text{in}} = \alpha_1 + \alpha_2\nu.$$  

**Calcium model equations**

Changes in $\text{Ca}^{2+}$ are the result of the fluxes into and out of the cell and ER. The system of equations for the $\text{Ca}^{2+}$ model is,

$$\frac{d([\text{Ca}]_w)}{dt} = J_{\text{IPR}} + J_{\text{RyR}} + J_{\text{er}} - J_{\text{SERCA}} + J_{\text{in}} - J_{\text{pm}},$$

$$\frac{d([\text{Ca}]_{\text{er}}w_{\text{er}})}{dt} = -(J_{\text{IPR}} + J_{\text{RyR}} + J_{\text{er}} - J_{\text{SERCA}}),$$

together with

$$\frac{d([\text{IP}_3]w)}{dt} = w_0\nu - J_{\text{IP}_3\text{deg}}.$$

Here, $w$ is the volume of the cytoplasm and $w_{\text{er}}$ is the volume of the ER.

### $\text{Ca}^{2+}$ parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{\text{IPR}}$</td>
<td>$0.04 \text{ s}^{-1}$</td>
</tr>
<tr>
<td>$k_{\text{RyR}}$</td>
<td>$0.01 \text{ s}^{-1}$</td>
</tr>
<tr>
<td>$k_{\text{er}}$</td>
<td>$1.554 \times 10^{-4} \text{ s}^{-1}$</td>
</tr>
<tr>
<td>$V_{\text{SERCA}}$</td>
<td>$1 \times 10^{-9} \text{ nmol s}^{-1}$</td>
</tr>
<tr>
<td>$K_{\text{SERCA}}$</td>
<td>$400 \text{ nM}$</td>
</tr>
<tr>
<td>$V_{\text{pm}}$</td>
<td>$15 \times 10^{-12} \text{ nmol s}^{-1}$</td>
</tr>
<tr>
<td>$K_{\text{pm}}$</td>
<td>$200 \text{ nM}$</td>
</tr>
<tr>
<td>$\alpha_1$</td>
<td>$2.31 \times 10^{-13} \text{ nmol s}^{-1}$</td>
</tr>
<tr>
<td>$\alpha_2$</td>
<td>$3.5 \times 10^{-4} \text{ s}^{-1}$</td>
</tr>
<tr>
<td>$w_0/w_{\text{er}}$</td>
<td>5.405</td>
</tr>
</tbody>
</table>

### RyR parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_a^+$</td>
<td>$1500 (\mu M)^{-4} \text{ s}^{-1}$</td>
</tr>
<tr>
<td>$k_a^-$</td>
<td>$28.8 \text{ s}^{-1}$</td>
</tr>
<tr>
<td>$k_b^+$</td>
<td>$1500 (\mu M)^{-3} \text{ s}^{-1}$</td>
</tr>
<tr>
<td>$k_b^-$</td>
<td>$385.9 \text{ s}^{-1}$</td>
</tr>
<tr>
<td>$k_c^+$</td>
<td>$1.75 \text{ s}^{-1}$</td>
</tr>
<tr>
<td>$k_c^-$</td>
<td>$0.1 \text{ s}^{-1}$</td>
</tr>
<tr>
<td>$K_a$</td>
<td>$372 \text{ nM}$</td>
</tr>
<tr>
<td>$K_b$</td>
<td>$636 \text{ nM}$</td>
</tr>
<tr>
<td>$K_c$</td>
<td>$0.0057$</td>
</tr>
</tbody>
</table>

Table 4.3: # parameters from Keizer and Levine [54], * from Lytton et al. [61], ** taken from Camello et al. [16], all remaining receptor densities and parameters are fitted to recreate experimental $\text{Ca}^{2+}$ oscillations

\[2\] No formal fitting procedure was used. Parameters values were chosen in order to best reproduce experimental results.
4.3.3 Ion channels and fluxes

The osmotic gradient across the apical membrane, which drives the fluid flow, is maintained primarily by movement of Cl\(^{-}\) ions through the Cl\(^{-}\) channels located in the apical membrane. We use a model developed by Arreola et al. [2], where the Cl\(^{-}\) channel open probability is a function of Ca\(^{2+}\). Details can be seen in Section 4.6.1.

Our model allows for K\(^{+}\) channels in both the apical and basal membrane, with the currents denoted by \(I_{K,a}\) and \(I_{K,b}\) respectively. In Section 4.4.4 we investigate the effect the distribution of the K\(^{+}\) channels has on saliva secretion. We use a K\(^{+}\) channel model developed by Takahata et al. [105] where the open probability of the channel increases as Ca\(^{2+}\) increases (Section 4.6.2). The maximum whole cell conductance \(g_{K}\) is distributed in either the apical or basal membrane with the parameter \(\alpha_{K}\). As \(\alpha_{K}\) increases from zero to one the whole cell K\(^{+}\) conductance is distributed from entirely in the apical membrane to entirely in the basal membrane. It is with this parameter we investigate how apical K\(^{+}\) channels affect secretion.

At the basal membrane the NKCC brings Cl\(^{-}\) into the cell along with Na\(^{+}\) and K\(^{+}\). The basal membrane also contains the NaK which exchanges 3 Na\(^{+}\) ions for 2 K\(^{+}\) ions. Previous models of the parotid acinar cell by Gin et al. [40] used complicated models for these fluxes with a large number of parameters (7 for the NKCC and 19 for the NaK). We simplify the NKCC model of Benjamin and Johnson [8] to a two-state model (details can be found in Section 4.6.5). Similarly we simplify the NaK model of Smith and Crampin [93] to a two-state model with only 2 parameters, a great reduction from the original 19 parameter model (Section 4.6.6). A comparison between simulations run with our simplified models and those used in previous studies can be found in Section 4.6.7. We see no qualitative difference in results using the simplified NKCC and NaK and only a 3% change in average fluid over a 300 second simulation compared to results computed with the original flux transporter models.

4.3.4 Intracellular ionic differential equations

Cl\(^{-}\) influx into the cytoplasm is via the NKCC. The apical Cl\(^{-}\) channel releases this Cl\(^{-}\) into the lumen.

\[
\frac{d([\text{Cl}]+w)}{dt} = - \frac{I_{\text{Cl}}}{z_{\text{Cl}}F} + 2J_{\text{NKCC}}.
\]

The factor of 2 in the cotransport term is present because for every K\(^{+}\) and Na\(^{+}\) that enter the cell, two Cl\(^{-}\) are transported into the cell. \(z_{\text{Cl}} = -1\) is the valence of Cl\(^{-}\) and \(F = 96490\) C mol\(^{-1}\) is Faraday’s constant.
Na$^+$ enters the cytoplasm via the NKCC and is extruded by the NaK;

$$\frac{d([\text{Na}]_{iw})}{dt} = -3J_{\text{NaK}} + J_{\text{NKCC}}.$$  

The rate of change of intracellular K$^+$ is due to the K$^+$ channels, the NKCC and the NaK;

$$\frac{d([\text{K}]_{iw})}{dt} = 2J_{\text{NaK}} + J_{\text{NKCC}} - \frac{I_{K,\text{Ap}}}{z_KF} - \frac{I_{K,\text{Ba}}}{z_KF}.$$  

Here $z_K = +1$ is the valence of K$^+$, $I_{K,\text{Ba}}$ and $I_{K,\text{Ap}}$ are the basal and apical K$^+$ currents respectively.

### Parameter values for ion movement

<table>
<thead>
<tr>
<th>Physical constants</th>
<th>Value</th>
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</thead>
<tbody>
<tr>
<td>$R$</td>
<td>8.315 J mol$^{-1}$ K$^{-1}$</td>
</tr>
<tr>
<td>$T$</td>
<td>310 K</td>
</tr>
<tr>
<td>$F$</td>
<td>96490 C mol$^{-1}$</td>
</tr>
<tr>
<td>Whole cell conductance’s</td>
<td></td>
</tr>
<tr>
<td>$g_{\text{Cl}}$</td>
<td>31.4 nS $^*$</td>
</tr>
<tr>
<td>$g_{\text{K}}$</td>
<td>14 nS $^{**}$</td>
</tr>
<tr>
<td>Pump Densities</td>
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</tr>
<tr>
<td>$\alpha_{\text{NaK}}$</td>
<td>$2.236 \times 10^{-17}$ mol</td>
</tr>
<tr>
<td>$\alpha_{\text{NKCC}}$</td>
<td>$3.2 \times 10^{-17}$ mol</td>
</tr>
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<td>Volumes</td>
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</tr>
<tr>
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<td>$10^{-12}$ L</td>
</tr>
<tr>
<td>$w_L/w_0$</td>
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<tr>
<td>Water permeabilities</td>
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<tr>
<td>$L_{\text{Pa}}$</td>
<td>$1.68 \times 10^{-15}$ L$^2$ J$^{-1}$ s$^{-1}$</td>
</tr>
<tr>
<td>$L_{\text{Pt}}$</td>
<td>$8.4 \times 10^{-17}$ L$^2$ J$^{-1}$ s$^{-1}$</td>
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<td>$C_m$</td>
<td>$10^{-11}$ F</td>
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<tr>
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</tr>
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</tr>
<tr>
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</tr>
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</tr>
<tr>
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</tr>
<tr>
<td>$z_{\text{K}}$</td>
<td>+1</td>
</tr>
<tr>
<td>$z_{\text{Na}}$</td>
<td>+1</td>
</tr>
</tbody>
</table>

Table 4.4: $^*$ from Arreola et al. [2], $^{**}$ from Thompson and Begenisich [109], other parameters are physical constants or model fits chosen to give the correct steady state concentrations and membrane potentials (No formal fitting procedure was used, parameters values were chosen in order to best reproduce experimental results).

### 4.3.5 A two membrane model and the role of the tight junction

Previous modelling of the parotid cell by Gin et al. [40] assumed that because the resistance in the tight junction is small the electrical properties at the apical and basal...
membranes will be the same. However it is seen in experiments that the lumen is far from electroneutral with respect to the interstitium [124]. Thus, here we include both an apical and basolateral membrane. The membrane potential at a given membrane is determined by the movement of ions through the membrane and any current applied to the membrane.

With a closed circuit model as seen in Figure 4.4 we include a current of positive \( K^+ \) and \( Na^+ \) ions through the tight junction, \( I_{\text{tight}} \). This movement of cations through the tight junction balances the movement of negative \( Cl^- \) ions to stop the lumen becoming too negatively charged. Membrane potentials, currents and fluxes are measured from the exterior of the cell to the cytoplasm. Thus,

\[
C_m \frac{dV_b}{dt} = -I_{K,Ba} - F J_{NaK} - 2F J_{pm} + 2F J_{in} + I_{tight},
\]

\[
C_m \frac{dV_a}{dt} = -I_{Cl} - I_{K,Ap} - I_{tight},
\]

where \( J_{in} \) and \( J_{pm} \) are the \( Ca^{2+} \) fluxes into and out of the cell. The factor of 2 is due to \( Ca^{2+} \) having a valence of +2.
In Figure 4.4 the tight junction current is shown to be moving anticlockwise around the circuit. Here $I_{\text{tight}}$ is the movement of negative ions and is equal and opposite to the movement of $K^+$ and $Na^+$ ions. We assume the resistances to ions moving through the tight junction and the cell, $R_{\text{tight}}$ and $R_{\text{cell}}$ respectively, are constant and independent of cell activity. Given that the sum of voltage drops around the circuit must be zero we have

$$V_b + V_{\text{cell}} - V_a + V_{\text{tight}} = 0.$$ 

Note that $V_a$ is negative as we measure its potential from the lumen to the cytoplasm. By substituting $V_{\text{tight}} = I_{\text{tight}} R_{\text{tight}}$ and $V_{\text{cell}} = I_{\text{tight}} R_{\text{cell}}$ we can then calculate the current through the tight junction

$$I_{\text{tight}} = \frac{V_a - V_b}{R_{\text{cell}} + R_{\text{tight}}}.$$ 

The current through the tight junction is the sum of the $K^+$ and $Na^+$ currents through the tight junctions. We denote the respective ionic currents as,

$$I_{t,Na} = g_{t,Na} I_{\text{tight}}$$

$$I_{t,K} = (1 - g_{t,Na}) I_{\text{tight}}$$

Here $g_{t,Na}$ is the relative conductance of $Na^+$ ions through the tight junction with respect to $K^+$ ions. As such it gives a measure for what fraction of the tight junctional current is due to $Na^+$ movement. Although we allow for resistance of ionic flow in both the cell and the tight junction, no significant potential changes are seen throughout the interior of the cell [11] and hence we set $R_{\text{cell}} = 0$.

**Luminal ionic concentrations and water transport**

We model the rate of water flow based on the difference between external and internal concentrations and a water permeability term. We assume cell volume changes purely based on the difference between apical and basal fluid flow,

$$\frac{dw}{dt} = q_b - q_a$$

where the rate of water flow through the apical and basal membranes is given by,

$$q_a = RT L_a \left( [Cl]_t + [Na]_t + [K]_t - \left( [Cl]_i + [Na]_i + [K]_i + [Ca]_i + \frac{x}{w} \right) \right),$$

$$q_b = RT L_b \left( [Cl]_i + [Na]_i + [K]_i + [Ca]_i + \frac{x}{w} - ( [Cl]_e + [Na]_e + [K]_e) \right).$$
4.3. The model

Here $x$ is the number of impermeable ions and other solutes in the cytoplasm, $L_{Pa}$ and $L_{Pb}$ are the permeability of the apical and basal membranes respectively to water. Interstitial ionic concentrations, with notation $[^{\text{e}}]$, are assumed constant with the values shown in Table 4.5.

Ma et al. [62] find in aquaporin-5 (APQ5) knockout mice saliva secretion is reduced by more than 60% compared to wild-type mice. This suggests that the majority of water flow is through the cell. We also allow for water flow through the tight junction to the lumen, paracellular water flow, and denote this $q_{\text{tight}}$,

$$q_{\text{tight}} = RTL_{Pt} ([\text{Cl}]_l + [\text{Na}]_l + [\text{K}]_l - ([\text{Cl}]_e + [\text{Na}]_e + [\text{K}]_e)),$$

with the permeability of the tight junction given by $L_{Pt}$. Water flow into the lumen will then be the sum of the apical fluid flow and the tight junctional fluid flow, $q_{\text{tot}} = q_a + q_{\text{tight}}$. We assume the flow of water into the lumen, $q_{\text{tot}}$, must be matched by a water flow out of the lumen and into the parotid duct. Any ions in the lumen will be removed by this water flow.

Sodium enters the lumen via the tight junctional current and so

$$w_L \frac{d([\text{Na}]_l)}{dt} = \frac{g_{t, \text{Na}}I_{\text{tight}}}{z_{\text{Na}}F} - q_{\text{tot}}[\text{Na}]_l.$$

Here $w_L$ is the luminal volume and is modelled as a constant. Luminal K$^+$ concentration changes due to the tight junction K$^+$ current, any current through the apical K$^+$ channel and removal due to fluid flow,

$$w_L \frac{d([\text{K}]_l)}{dt} = \frac{(1 - g_{t, \text{Na}})I_{\text{tight}}}{z_{\text{K}}F} + \frac{I_{\text{K,Ap}}}{z_{\text{K}}F} - q_{\text{tot}}[\text{K}]_l.$$

Cl$^-$ enters the lumen through the chloride channel and is removed at the rate of apical water flow,

$$w_L \frac{d([\text{Cl}]_l)}{dt} = \frac{I_{\text{Cl}}}{z_{\text{Cl}}F} - q_{\text{tot}}[\text{Cl}]_l.$$

4.3.6 Summary of the model

$$\frac{d([\text{Ca}]_i w)}{dt} = J_{\text{IPR}} + J_{\text{RyR}} + J_{\text{er}} - J_{\text{SERCA}} + J_{\text{in}} - J_{\text{pm}}, \quad (4.1)$$

$$\frac{d([\text{Ca}]_{\text{er}} w_{\text{er}})}{dt} = -(J_{\text{IPR}} + J_{\text{RyR}} + J_{\text{er}} - J_{\text{SERCA}}), \quad (4.2)$$
\[ \frac{d([\text{IP}_3]_w)}{dt} = w_0 - J_{\text{IP}_3\text{deg}}, \] 
(4.3)

\[ \frac{d([\text{Cl}]_w)}{dt} = - \frac{I_{\text{Cl}}}{z_{\text{Cl}}F} + 2J_{\text{NKCC}}, \] 
(4.4)

\[ \frac{d([\text{Na}]_w)}{dt} = -3J_{\text{NaK}} + J_{\text{NKCC}}, \] 
(4.5)

\[ \frac{d([\text{K}]_w)}{dt} = 2J_{\text{NaK}} + J_{\text{NKCC}} - \frac{I_{K,a}}{z_{\text{K}}F} - \frac{I_{K,b}}{z_{\text{K}}F}, \] 
(4.6)

\[ I_{\text{tight}} = \frac{V_a - V_b}{R_{\text{cell}} + R_{\text{tight}}}, \]

\[ w_L\frac{d([\text{Na}]_l)}{dt} = \frac{I_{\text{tight}}}{z_{\text{Na}}F} - q_{\text{tot}}[\text{Na}]_l, \] 
(4.7)

\[ w_L\frac{d([\text{K}]_l)}{dt} = \frac{(1 - g_{\text{t,Na}})I_{\text{tight}}}{z_{\text{K}}F} + \frac{I_{K,Ap}}{z_{\text{K}}F} - q_{\text{tot}}[\text{K}]_l, \] 
(4.8)

\[ w_L\frac{d([\text{Cl}]_l)}{dt} = \frac{I_{\text{Cl}}}{z_{\text{Cl}}F} - q_{\text{tot}}[\text{Cl}]_l, \] 
(4.9)

\[ C_m \frac{dV_a}{dt} = -I_{K,\text{Ba}} - FJ_{\text{NaK}} - 2FJ_{\text{pm}} + 2FJ_{\text{in}} + I_{\text{tight}}, \] 
(4.10)

\[ C_m \frac{dV_b}{dt} = -I_{\text{Cl}} - I_{K,Ap} - I_{\text{tight}}, \] 
(4.11)

\[ q_a = RTL_{P_a} \left( [\text{Cl}]_l + [\text{Na}]_l + [\text{K}]_l - \left( [\text{Cl}]_i + [\text{Na}]_i + [\text{K}]_i + [\text{Ca}]_i + \frac{x}{w} \right) \right), \]

\[ q_b = RTL_{P_b} \left( [\text{Cl}]_i + [\text{Na}]_i + [\text{K}]_i + [\text{Ca}]_i + \frac{x}{w} - ([\text{Cl}]_e + [\text{Na}]_e + [\text{K}]_e) \right), \]

\[ q_{\text{tight}} = RTL_{P_t} \left( ([\text{Cl}]_i + [\text{Na}]_i + [\text{K}]_i - ([\text{Cl}]_e + [\text{Na}]_e + [\text{K}]_e)) \right), \]

\[ q_{\text{tot}} = q_a + q_{\text{tight}}, \]

\[ \frac{dw}{dt} = q_b - q_a. \] 
(4.12)
4.3.7 Numerical simulations

In solving the system of equations the small membrane capacitance $C_m$ produces a highly stiff system of equations, which gives rise to difficulties during numerical solution. We ignore the fast dynamics of the membrane potentials and find solutions to the differential equations using a quasi-steady state approximation to the membrane potential $V_b$ and $V_a$. The system of differential equations is solved using the Matlab code `ode15s`. At each time step we solve $dV_b/dt = 0$ and $dV_a/dt = 0$ to find the quasi-steady state potentials using a simple Newton solver.

As such we solve the following at each time step,

$$
-I_{K, Ba}(V_b^{t_i}, C_i^{t_i-1}, K_i^{t_i-1}) - FJ_{NaK}(V_b^{t_i}, N_i^{t_i-1}, K_i^{t_i-1}) - 2FJ_{pm}(C_i^{t_i-1})
+ 2FJ_{in} + I_{tight}(V_b^t, V_a^t) = 0
$$

$$
-I_{Cl}(V_a^{t_i}, C_i^{t_i-1}, L_i^{t_i-1}, L_i^{t_i-1}) - I_{K, Ap}(V_a^{t_i}, C_i^{t_i-1}, K_i^{t_i-1}, K_i^{t_i-1}) - I_{tight}(V_b^t, V_a^t) = 0.
$$

Here the superscript notation denotes the time step. We use a simple Newton solver in the implementation of the above solution which finds the present membrane potentials using concentrations from the previous time step.

4.4 Results

4.4.1 Calcium model results

We aim to reproduce experimental traces found by Bruce et al. [14] using the unconstrained Ca$^{2+}$ flux density parameters, $k_{IPR}$, $k_{RyR}$, $V_{SERCA}$ and the constant IP$_3$ dephosphorylation rate $k_{5p}$ to fit the model to the data. In simulating the experimental procedures we hold the cell volume constant, this demonstrates that the model Ca$^{2+}$ oscillations are not dependent on cell volume oscillations.

In experimental recreation of the salivation process carbachol (CCh) is added to the parotid cells. CCh is known to stimulate the muscarinic acetylcholine receptors which results in the activation of phospholipase C which in turn induces the breakdown of phosphatidylinositol 4,5-bisphosphate (PIP2) in the cell membrane to release IP$_3$ into the cytosol. We model CCh application by increasing the IP$_3$ production rate, $\nu$, assuming a greater CCh concentration leads to a higher IP$_3$ production rate. The Ca$^{2+}$ concentration is measured using fura-2 dye.
Chapter 4. A dynamic model of saliva secretion

With the parameters found our model is able to recreate several experimentally observed Ca\textsuperscript{2+} behaviours. Firstly our model is able to recreate experimental results of Ca\textsuperscript{2+} oscillations at three different agonist concentrations, (Figure 4.5). We simulate different agonist concentrations by changing the IP\textsubscript{3} production rate, $\nu$. Ca\textsuperscript{2+} oscillations are seen for $\nu = 5100$ nM/s. As the IP\textsubscript{3} production rate is increased or decreased from this value to $\nu = 4800$ nM/s or $\nu = 5400$ nM/s the Ca\textsuperscript{2+} oscillations are damped. Any further increases or decreases in the IP\textsubscript{3} production rate would result in no Ca\textsuperscript{2+} oscillations being found. Unlike Gin et al. [40], who similarly recreated this result, our model is able to reproduce the experimental traces when cell volume is fixed.

![Figure 4.5: Ca\textsuperscript{2+} traces for three agonist concentrations with the model result in (a) and the experimental trace (b) reproduced from original figure in Gin et al. [40] with permission from the authors. Parameter $\nu$ is zero except when indicated by a horizontal bar with its non-zero value written above. In the experimental trace, F340/F380 represents the fluorescence of fura-2 and gives a measure of Ca\textsuperscript{2+} concentration.](image)

Ca\textsuperscript{2+} release from the ER is due to the IP\textsubscript{3} receptor, ryanodine receptor (RyR) and a leak term. Experimentally when a high concentration of ryanodine is added to the
cell, with the effect of blocking the RyR, Ca\(^{2+}\) oscillations are damped. As can be seen in Figure 4.6 our model recreates the observed Ca\(^{2+}\) traces and oscillations are damped when the ryanodine receptor is blocked.

Figure 4.6: Effect of blocking the Ryanodine receptor in the model (a) by setting \(k_{\text{RyR}} = 0\) and (b) experimentally by applying a large (500 \(\mu\)M) Ryanodine concentration. Ca\(^{2+}\) oscillations are initiated in the model by setting \(\nu = 5100\) nM/s, and experimentally by adding 300 nM carbachol at the times shown with the horizontal bars. With the exception of the period when \(k_{\text{RyR}} = 0\) all the model parameters are those found in Table 4.3. Experimental ‘ratio unit’ represents the fluorescence of fura-2 and gives a measure for Ca\(^{2+}\) concentration. Experimental trace reproduced from Bruce et al. [14] with permission from the authors.

Experimental traces show Ca\(^{2+}\) oscillations continue when extracellular Ca\(^{2+}\) is set
to zero. We recreate this experiment by setting $J_{in} = 0$. The parameter $V_{pm}$ is chosen to give the right time scale of Ca$^{2+}$ efflux from the cell. Parameters $\alpha_1$ and $\alpha_2$ are then subsequently found in order to have balanced influx and efflux in both stimulated and un-stimulated conditions. Parameter values can be found in Table 4.3.

The results from this simulation can be seen in Figure 4.7. Upon stimulation with 5100 nM agonist Ca$^{2+}$ oscillations are seen. As $J_{in}$ is set to zero the total Ca$^{2+}$ in the cell decreases due to the leak $J_{pm}$ which slowly damps the oscillations until eventually the total intracellular Ca$^{2+}$ decreases sufficiently to stop oscillations entirely. When $J_{in}$ is restored to its previous non-zero value Ca$^{2+}$ oscillations are again observed.

Figure 4.7: The effect of stopping Ca$^{2+}$ entry from the interstitium which is seen to damp oscillations, experimental result shown in (b), experiment reproduced in the model by setting $\nu = 5100$ nM/s, and experimentally by adding 100 nM carbachol at the times shown with the horizontal bars. Experimental ‘ratio unit’ represents the fluorescence of fura-2 and gives a measure for Ca$^{2+}$ concentration. Experimental trace reproduced from Bruce et al. [14] with permission from the authors.

### 4.4.2 Steady state results

We simulate the un-stimulated parotid cell by setting $\nu = 0$. We then use experimentally observed steady-state ionic concentrations to fit model parameters to the pump densities $\alpha_{NaK}$ and $\alpha_{NKCC}$. Water permeabilities $L_{Pa}$, $L_{Pb}$ and tight junctional conductance $g_{t,Na}$ are fitted to give correct steady state luminal concentrations and cell volume. Finally the resistance of the tight junction, $R_{tight}$ is fitted to give the correct steady state membrane potentials. A comparison between model steady state values and those observed experimentally can be seen in Table 4.5.
4.4. Results

With the parameter values found at zero agonist we have a Ca$^{2+}$ concentration of 50 nM. This similar to the 59 nM observed by Foskett and Melvin [36].

In the cytoplasm Cl$^{-}$ has a steady state of 61.2 mM, which agrees well with the 63 mM found by Foskett [38]. The K$^+$ steady state is found to be 142 mM in the cytoplasm, slightly less than that found by Izutsu and Johnson [52] of 152 mM. The steady state sodium in the cytoplasm is 14.1 mM which is between the values of 13 mM found by Izutsu and Johnson [52] and the 18.6 mM found by Soltoff et al. [100].

We have luminal steady-state values of 124 mM, 118 mM and 5.6 mM for Cl$^{-}$, Na$^+$ and K$^+$ respectively. Mangos et al. [64] found experimental values of 112.6 mM, 141.6 mM and 5.6 mM for ionic concentrations of Cl$^{-}$, Na$^+$ and K$^+$ in the primary fluid respectively. Our model results are similar to those by Mangos et al. [64] with a slightly lower luminal Na$^+$ concentration than found experimentally.

The steady-state membrane potentials are found as -50.4 mV for the basal membrane and -39.4 mV for the apical membrane. The basal membrane potential is between the measurements of -44.2 mV and -65 mV obtained by Berridge and Prince [11] and Lang and Walz [56] respectively. Measurements by Lang and Walz [56] and Martin et al. [65] find the lumen to be around 13 mV negative with respect to the interstitium which agrees nicely with the 11 mV negative found in our model.

<table>
<thead>
<tr>
<th>Cytosolic ion concentrations</th>
<th>Experimental Result</th>
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<tbody>
<tr>
<td>[Cl]$^i_i$</td>
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<tr>
<td>[K]$^i_i$</td>
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<tr>
<td>[Na]$^i_i$</td>
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<table>
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<th>Luminal ion concentrations</th>
<th>Experimental Result</th>
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</thead>
<tbody>
<tr>
<td>[Cl]$^l_l$</td>
<td>124.3 mM</td>
</tr>
<tr>
<td>[K]$^l_l$</td>
<td>5.6 mM</td>
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<tr>
<td>[Na]$^l_l$</td>
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</table>

<table>
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<th>Interstitial concentrations (constants)</th>
<th>Experimental Result</th>
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<tbody>
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<td>[Cl]$^e_e$</td>
<td>102.6 mM</td>
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<tr>
<td>[K]$^e_e$</td>
<td>5.3 mM</td>
</tr>
<tr>
<td>[Na]$^e_e$</td>
<td>140.2 mM</td>
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</table>

<table>
<thead>
<tr>
<th>Membrane potentials</th>
<th>Experimental Result</th>
</tr>
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<tbody>
<tr>
<td>V$_b$</td>
<td>-50.7 mM</td>
</tr>
<tr>
<td>V$_{tight}$</td>
<td>-11 mV</td>
</tr>
</tbody>
</table>

Table 4.5: Steady state results and comparison to experimental results
4.4.3 Fluid transport results

We next simulate fluid secretion in the presence of an agonist by setting the parameter $\nu = 5100$ nM/s. With this stimulation we see Ca$^{2+}$ oscillations around 140 nM (Figure 4.9a). Once stimulated the intracellular Cl$^{-}$ is seen to drop from 61.2 mM to 43 mM (see Figure 4.8a). Foskett [38] similarly observes a fall in cytosolic Cl$^{-}$ concentration, albeit slightly larger with a 50% reduction in Cl$^{-}$ upon stimulation. The cell volume is seen to shrink by 22% after stimulation with agonist (Figure 4.9a). Experimentally Foskett and Melvin [36] see an almost identical 15% cell volume reduction upon stimulation. Cytosolic Na$^{+}$ increases from 11.2 mM to 15.7 mM (Figure 4.8b), increases in Na$^{+}$ upon stimulation are found experimentally by Soltoff et al. [100], where Na$^{+}$ doubles in concentration after stimulation. No experimental data was found as to the change in cytosolic K$^{+}$ upon stimulation. Our model shows only a small increase (Figure 4.8c) which may explain the lack of data. As with experimental results seen by Mangos et al. [64] the luminal concentrations change very little when stimulated. The luminal Cl$^{-}$ and Na$^{+}$ increase slightly (Figures 4.8g and 4.8h) with no significant change in luminal K$^{+}$ as seen by Mangos et al. [64].

In Figure 4.9b the relationship between cell volume and Ca$^{2+}$ is shown. Foskett and Melvin [36] found cell shrinkage corresponded with Ca$^{2+}$ increases, and that the cell would continue to shrink even after Ca$^{2+}$ levels started to subside. This behaviour is observed in our model where a peak in the Ca$^{2+}$ is seen to precede a trough in the cell volume.

When stimulated, the basal membrane hyperpolarises from -51 mV to around -75 mV (Figure 4.8e) whilst the apical membrane oscillates around its steady-state value of -43 mV (Figure 4.8f). Experimentally Lundberg [60] finds the basal membrane to hyperpolarise by -22 mV which agrees well with our model. With this hyperpolarisation of the basal membrane stimulation is found to make the lumen more negative with respect to the interstitium than steady state values. Lundberg [60] observe a 10-22 mV hyperpolarisation of the lumen with respect to the interstitium. Our model shows a similar hyperpolarisation from -11 mV to -31 mV with respect to the interstitium.

Upon stimulation the average normalised water flow is found to be 0.0058 s$^{-1}$, 3 times its resting rate, see Figure 4.8d. Evans et al. [33] found a volume of 400 $\mu$l saliva secreted per 100 g of parotid gland in 50 minutes. One cell in our model produces $1.77 \times 10^{-5} \mu$l in 50 minutes. We therefore require $2.26 \times 10^7$ cells in 100 g to produce the experimentally result. This agrees well with the $10^8$ parotid cells Gin et al. [40] estimated to be found in 100 g of parotid gland.
Figure 4.8: Model ionic and potential changes with simulated saliva production. Parameter $\nu = 5100$ nM/s for the duration to represent continued stimulation with agonist.
Figure 4.9: Model volume (red trace) change with Ca\(^{2+}\) oscillations (black trace) upon stimulation with agonist. 4.9b shows the final 50 seconds of 4.9a and shows clearly the simultaneous Ca\(^{2+}\) and volume oscillations.

### 4.4.4 Location of K\(^{+}\) channels

In Figure 4.10a it can be seen that as the whole cell K\(^{+}\) conductance goes from being entirely in the basal membrane to being entirely in the apical membrane we see an initial increase in fluid output before the saliva secretion then diminishes. Maximum fluid production occurs for 5100 nM agonist when 20\% of the whole cell conductance is located in the apical membrane and the remaining 80\% is in the basal membrane. Figure 4.10b shows the apical membrane potential as the distribution of the K\(^{+}\) channels is changed. The distribution of K\(^{+}\) channels for which maximum fluid production occurs coincides with the distribution where the minimum membrane potential is seen. Similar results were observed by Cook and Young [20] in their steady-state model.

Figure 4.10: Dependence of fluid flow and apical membrane potential on location of K\(^{+}\) conductance at three different IP\(_3\) productions rates.
4.4. Results

4.4.5 Location of Cl\(^-\) channels

Existing models of the parotid acinar cell place Cl\(^-\) channels exclusively in the apical membrane. However, Marty et al. \[66\] reported basal Cl\(^-\) channels in the rat lacrimal glands which similarly transport fluid from the interstitium to the lumen via osmosis.

Using the same method of distributing the whole cell conductance between apical and basolateral membranes, as used above in Section 4.4.4, we can investigate the effect of the location of the Cl\(^-\) channels on average fluid flow. We find that maximum fluid secretion occurs when all the Cl\(^-\) conductance is located in the apical membrane, supporting the traditional models. As Cl\(^-\) is distributed into the basal membrane the average fluid flow is seen to decrease almost linearly, see Section 4.6.8.

4.4.6 Effect of tight junctional resistance

It can be seen in Figure 4.11a that as the resistance of the tight junction increases the fluid output from the model decreases. As the tight junctional resistance increases the flow of cations through the tight junction is reduced. This causes the apical membrane to depolarise (Figure 4.11b), which inhibits the flow of Cl\(^-\) through the apical Cl\(^-\) channel and thus reduces the water flow.

![Image of graphs showing water flow and apical membrane potential](image)

Figure 4.11: Change in normalised water flow and apical membrane potential with tight junctional resistance

4.4.7 The effect of water permeabilities on the model

The water permeabilities were chosen such that the majority of fluid would flow through the transcellular pathway, with only a small amount travelling through the tight junction.
In Table 4.6 the effect of varying the water permeabilities are calculated, showing that the model is robust, with large changes in the parameters having very little effect on the average fluid flow and steady-state ionic concentrations.

| Water permeabilities | Average Fluid flow $q_{\text{flow}}/w_0$ (s$^{-1}$) | Steady state concentrations (mM) | | |
|----------------------|-----------------------------|----------------------------------|---|---|---|
| $\{L_{Pa}, L_{Pb}, L_{Pt}\}$ | $6.10948 \times 10^{-3}$ | $[\text{Cl}]_I$ | $5.59$ | $118.68$ |
| $\{10 \times L_{Pa}, L_{Pb}, L_{Pt}\}$ | $6.16029 \times 10^{-3}$ | $[\text{K}]_I$ | $5.58$ | $118.49$ |
| $\{0.1 \times L_{Pa}, L_{Pb}, L_{Pt}\}$ | $5.14813 \times 10^{-3}$ | $[\text{Na}]_I$ | $124.27$ | $5.74$ | $119.85$ |
| $\{L_{Pa}, 10 \times L_{Pb}, L_{Pt}\}$ | $6.08307 \times 10^{-3}$ | $[\text{Cl}]_I$ | $5.59$ | $118.69$ |
| $\{L_{Pa}, 0.1 \times L_{Pb}, L_{Pt}\}$ | $5.99480 \times 10^{-3}$ | $[\text{K}]_I$ | $5.58$ | $118.49$ |
| $\{L_{Pa}, L_{Pb}, 10 \times L_{Pt}\}$ | $6.12486 \times 10^{-3}$ | $[\text{Na}]_I$ | $124.07$ | $5.64$ | $119.77$ |
| $\{L_{Pa}, L_{Pb}, 0.1 \times L_{Pt}\}$ | $6.06686 \times 10^{-3}$ | $[\text{Cl}]_I$ | $5.59$ | $118.70$ |

Table 4.6: The effect of water permeability on the model

### 4.5 Discussion

The model we have developed here with intracellular Ca$^{2+}$ oscillations driving secretion via secondary chloride transport we have shown to be consistent with a range of experimental data and extends previous parotid acinar cell models by Gin et al. [40] and Cook and Young [20]. Ca$^{2+}$ oscillations that previously depended on volume oscillations [40] or were altogether absent [20] are now based on physiological models of the IPR and IP$_3$ dynamics. Independent apical and basal membrane potentials allow for the inclusion of an apical K$^+$ channel and an understanding of how potentials change during stimulation.

There remain some differences between model predictions and experimental observation that warrant further comment. In Figure 4.7 Ca$^{2+}$ oscillations are seen instantaneously with the reintroduction of $J_{in}$. Experimentally, Ca$^{2+}$ oscillations are not seen immediately but instead return after a delay where the cytosolic Ca$^{2+}$ slowly increases. This delay in oscillations experimentally can be explained by the gradual increase in the Ca$^{2+}$ influx as the external Ca$^{2+}$-free solution is replaced by a solution containing Ca$^{2+}$. In our model, however, the term $J_{in}$ has no dependence on extracellular Ca$^{2+}$ so is applied suddenly causing instantaneous oscillations.

It should also be noted that experimentally when external Ca$^{2+}$ is reintroduced the cytosolic Ca$^{2+}$ increases to a peak slightly above the value seen when first stimulated. This is not seen in our model. This difference can be explained by the absence of Ca$^{2+}$ release-activated Ca$^{2+}$ channels (CRAC) or store-operated channels (SOC) in our model.
CRAC and SOC are situated on the plasma membrane and bring Ca\(^{2+}\) into the cytoplasm at varying rates dependent on ER Ca\(^{2+}\) levels, increasing when the internal stores are depleted.

Upon stimulation with agonist, concentrations of ions behave in the model as seen experimentally. Some slight inconsistencies are found with the magnitude of concentration and volume changes upon stimulation. Foskett [38] saw a 50% reduction in cytosolic Cl\(^{-}\) upon stimulation, whereas only a 30% reduction is seen in our model. Similarly Soltoff et al. [100] found Na\(^{+}\) to double in the cytoplasm upon stimulation, a much larger increase than the 40% found in the model results. Experimentally Foskett and Melvin [36] observed volume oscillations between 0.85 and 1 times the resting cell volume with agonist induced Ca\(^{2+}\) oscillations. However our model results show only small amplitude oscillations of cell volume. The above discrepancies could be due to the amplitude of the Ca\(^{2+}\) oscillations in our model. Upon stimulation with agonist Ca\(^{2+}\) increases from its steady state of 50 nM to oscillate around 140 nM. However Foskett and Melvin [36] see Ca\(^{2+}\) increase from 60 nM to 474 nM upon stimulation, 3 times the Ca\(^{2+}\) concentration in our model results.

Poulsen and Bundgaard [83] measured the apical membrane of the parotid acinar cell to be more than 12 times smaller in surface area than the basal membrane. However it is the apical membrane through which Cl\(^{-}\) exits to the lumen and is therefore essential for saliva production. Extending the work of Gin et al. [40] we include both an apical and basal membrane with distinct properties in our model. Several attempts have been made to measure the potentials of these two membranes. There is agreement that at the basal membrane the cytoplasm is negative with respect to the lumen. Values found experimentally range between -33 mV and -65 mV. Where there is disagreement is in whether, at steady-state, the lumen is negative or positive with respect to the interstitium. Berridge and Prince [11] and Lundberg [60] claim the lumen is positive with respect to the interstitium whilst Young [124], Martin et al. [65] and Lang and Walz [56] find negative readings. A feature of our model is the movement of Na\(^{+}\) and K\(^{+}\) ions through the tight junction into the lumen which requires a lumen negative driving force we therefore lumen negative with respect to the interstitium at steady-state. Our model agrees with experimental findings that the lumen becomes more negative when stimulated with agonist [11, 56, 60]. The reader should be aware that we are cautious relying entirely on readings by Berridge and Prince [11] and Lang and Walz [56] which are taken from insects, where the salivation process is thought to be very different to
mammals. However these readings are reconfirmed by similar recording in mammals.

The current through the tight junction is determined by the voltages of the apical and basal membranes and the resistance of the tight junction. There is very little experimental data as to the size of ionic currents through the tight junction or what the resistance to ionic flow must be. In our model the tight junctional resistance is fit to ensure a correct difference between apical and basal membrane potentials. The parameter $g_{t,Na}$ then assigns the fraction of tight junctional current that results from $Na^+$ and $K^+$ movement.

Simulations were run allowing for $Cl^-$ movement through the tight junction but the addition of large tight junctional $Cl^-$ only decreased the fluid production slightly. As the resistance of the tight junction to cations increases, fluid flow was seen to decrease due to a depolarisation of the apical membrane and a subsequent reduction in $Cl^-$ movement into the lumen.

Mangos et al. [64] measured the ionic concentrations of the primary fluid. We expect these to be consistent with the luminal concentrations found by our model. Mangos et al. [64] found the $Na^+$ concentration in the primary fluid to be 141.6 mM, higher than the $Cl^-$ concentration found, 112.6 mM. Our model is created such that $Na^+$ moves through the tight junction to the lumen to balance the movement of negatively charged $Cl^-$ into the lumen through the $Cl^-$ channels. It is a constraint of our model that the luminal $Na^+$ can never be greater than the luminal $Cl^-$ concentration. In order to recreate experimental concentrations of $Na^+$ in the lumen the model would require the addition of a mechanism bringing other negatively charged ions into the lumen. Evans et al. [33] find that impairing the $Na^+-K^+-2Cl^-$ cotransporter reduces saliva secretion by 60%. However this impairment does not stop saliva production entirely and a significant fluid flow is still observed. A second mechanism for $Cl^-$ uptake into the cell is thought to exist in the form of a $Cl^--HCO_3^-$ paired with a $Na^+-H^+$ exchanger. This mechanism using $HCO_3^-$ allows saliva production to continue even when the $Na^+-K^+-2Cl^-$ cotransporter is removed. The presence of these $Cl^--HCO_3^-$ exchangers has been found in rat sublingual gland. Future work will include modelling of $HCO_3^-$ in the cell. The addition of $HCO_3^-$ could provide the necessary negative ion movement into the lumen required to find experimentally observed luminal $Na^+$ concentrations that our current model is unable to reproduce.

Our model uses fitted parameters for water permeability. Poulsen and Bundgaard [83] found the basolateral membrane surface area to be 12.32 times larger than the apical
membrane surface area. In our model we assume permeability per area is the same for both the membranes and therefore \( L_{pb} = 12.32 \times L_{pa} \). The permeability per unit area of the tight junction is seen by Berry [12] to be higher than that of the apical or basal membrane. However, the tight junction is assumed to have a very small area in comparison to the two membrane and therefore \( L_{pt} \) is small. Ma et al. [62] found that knocking out APQ5 reduced saliva secretion by greater than 60%. Experimentally, if we were to effectively block all aquaporins we could make a prediction of the relative paracellular pathway of fluid flow to the lumen, giving us an idea of the size of \( L_{pt} \) in relation to \( L_{pa} \) and \( L_{pb} \). Changes to the model water permeabilities were found to make very little effect to the volume of saliva produced or the the steady-state concentrations.

We investigated the effect the distribution of K\(^+\) channels had on fluid output. Our results agree with those found by Cook and Young [20] where a small conductance through an apical K\(^+\) was found to increase fluid flow. The additional movement of positive ions through the apical membrane was initially able to reduce the apical membrane potential and facilitate the flow of Cl\(^-\) through the Cl\(^-\) channel. However as K\(^+\) conductance is distributed away from the basal membrane a depolorisation of the basal membrane is observed, this inhibits the effectiveness of the Na\(^+\)-K\(^+\)-ATPase, which in turn causes a decrease in the flux of the Na\(^+\)-K\(^+\)-2Cl\(^-\). The movement of Cl\(^-\) into the cytoplasm is then reduced and fluid flow is similarly reduced. This accounts for the initial increase in fluid output followed by a decrease as the conductance is moved to the apical membrane.

As agonist concentration increases both the mean and frequency of the Ca\(^{2+}\) oscillations increases and an increase in saliva production is seen. However if we increase agonist but scale the mean Ca\(^{2+}\) to some base concentration no significant change in saliva production is found. We therefore conclude that Ca\(^{2+}\) oscillation frequency has no effect on saliva production. Presently our model is non spatial and Ca\(^{2+}\) concentration is the only direct factor in fluid production. However the entry or exit of ions in the cell is distinctly distributed in either the apical or basal membrane. Ca\(^+\) waves have been seen in parotid acinar cells by Won et al. [122] and may be important in the signalling process required to initiate saliva production. We plan to build a spatial model of the parotid cell and investigate how Ca\(^+\) waves and specifically frequency affect saliva production\(^3\). Currently our model produces Ca\(^+\) oscillations with a smaller amplitude than seen experimentally. The addition of spatial Ca\(^+\) to the model could solve this problem with

\(^3\)A spatial model investigating Ca\(^{2+}\) waves can be found in Chapter 5. The effect of the different properties of Ca\(^{2+}\) waves on the rate of saliva secretion can be found in Chapter 6.
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high micro-domain Ca\(^{+}\) concentrations around the ER receptors causing an increased release of Ca\(^{+}\) from the ER and subsequently larger amplitude Ca\(^{2+}\) oscillations.

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4.6 Appendix

4.6.1 Cl\(^{-}\) channel

The main driving force of the fluid flow is the chloride channels located in the apical membrane. We use a model developed by Arreola et al. [2]. The steady state open probability of the chloride channels is given by,

\[
P_{\text{Cl}} = \frac{1}{1 + K_2(K_1^2/[\text{Ca}]_i^2 + K_1/[\text{Ca}]_i + 1)}
\]

where

\[
K_1 = 214 \exp\left(-0.13\frac{FV_a}{RT}\right) \text{ nM}
\]

\[
K_2 = 0.58 \exp\left(-0.24\frac{FV_a}{RT}\right)
\]

Here \(V_a\) is the membrane potential of the apical membrane. Total current through the Cl\(^{-}\) channels is then given by,

\[
I_{\text{Cl}} = g_{\text{Cl}}P_{\text{Cl}}(V_a - V_{\text{Cl}})
\]

\(g_{\text{Cl}}\) is the maximum whole cell conductance, 31.4 nS found by Arreola et al. [2]. \(V_{\text{Cl}}\) is the Nernst potential given by,

\[
V_{\text{Cl}} = \frac{RT}{z_{\text{Cl}}F}\log\left(\frac{[\text{Cl}]_l}{[\text{Cl}]_i}\right)
\]

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$z_{\text{Cl}} = -1$ is the valence of $\text{Cl}^-$, $R = 8.315 \text{ J mol}^{-1} \text{ K}^{-1}$, $T = 310 \text{ K}$ and $F = 96490 \text{ C mol}^{-1}$.

4.6.2 $\text{K}^+$ channels

We use the model of Takahata et al. [105], here the open probability of the $\text{K}^+$ channel is,

$$P_K = \frac{1}{1 + (K_d/[\text{Ca}]_i)^nH}$$

where $nH = 2.54$ and $K_d = 0.182 \mu M$. $K_d$ is modified from the value found by Takahata et al. [105] of $K_d = 0.43 \mu M$ to give a small open probability at steady state $\text{Ca}^{2+}$ concentrations.

The current through the $\text{K}^+$ channel at the basolateral membrane, $I_{K,\text{Ba}}$, and the apical membrane, $I_{K,\text{Ap}}$, are given by

$$I_{K,\text{Ba}} = \alpha_K g_K P_K (V_b - V_{K,\text{Ba}})$$

$$I_{K,\text{Ap}} = (1 - \alpha_K) g_K P_K (V_a - V_{K,\text{Ap}})$$

respectively, where $g_K$ is the maximum whole cell conductance of 14 nS, the value found by Thompson and Begenisich [109]. The factor $\alpha_K$ denotes the fraction of whole cell conductance in the basal membrane leaving $1 - \alpha_K$ in the apical membrane. $V_{K,\text{Ba}}$ and $V_{K,\text{Ap}}$ are the Nernst potentials at both the basolateral and apical membranes,

$$V_{K,\text{Ba}} = \frac{RT}{z_K F} \log \left( \frac{[\text{K}]_{\text{e}}}{[\text{K}]_{\text{i}}} \right)$$

$$V_{K,\text{Ap}} = \frac{RT}{z_K F} \log \left( \frac{[\text{K}]_{\text{l}}}{[\text{K}]_{\text{i}}} \right)$$

here $z_K = +1$ is the valence of $\text{K}^+$.

4.6.3 $\text{Na}^+ - \text{K}^+ - 2\text{Cl}^-$ cotransporter

We present a model by Benjamin and Johnson [8] which was used in a previous model of the parotid acinar cell by Gin et al. [40] for comparison with the simplified model shown in Section 4.6.5. The whole cell flux is then given by,

$$J_{\text{NKCC}} = \alpha_{\text{NKCC}} \nu_{\text{NKCC}}$$
where $\alpha_{\text{NKCC}}$ is the density of the cotransporter.

$$\nu_{\text{NKCC}} = -k_b^{\text{full}}k_b^{\text{empty}}[\text{Cl}]_i^2[\text{K}]_i[\text{Na}]_e + k_f^{\text{full}}k_f^{\text{empty}}[\text{Cl}]_e^2[\text{K}]_e[\text{Na}]_e$$

where

$$DJ_{\text{NKCC}} = Z_{\text{nkcc1}} + Z_{\text{nkcc2}} + Z_{\text{nkcc3}} + Z_{\text{nkcc4}} + Z_{\text{nkcc5}} + Z_{\text{nkcc6}}$$

and

$$Z_{\text{nkcc1}} = Z_1[\text{Cl}]_i, \ Z_{\text{nkcc2}} = Z_2[\text{Na}]_e, \ Z_{\text{nkcc3}} = Z_3[\text{Cl}]_i[\text{K}]_i, \ Z_{\text{nkcc4}} = Z_4[\text{Cl}]_e[\text{K}]_e,$$
$$Z_{\text{nkcc5}} = Z_5[\text{Cl}]_i^2[\text{K}]_i, \ Z_{\text{nkcc6}} = Z_6[\text{Cl}]_i[\text{K}]_i[\text{Na}]_e, \ Z_{\text{nkcc7}} = Z_7[\text{Cl}]_i^2[\text{K}]_i[\text{Na}]_e,$$
$$Z_{\text{nkcc8}} = Z_8[\text{Cl}]_e^2[\text{K}]_e[\text{Na}]_e, \ Z_{\text{nkcc9}} = Z_9[\text{Cl}]_i^2[\text{K}]_i[\text{Na}]_i[\text{Na}]_e,$$
$$Z_{\text{nkcc10}} = Z_{10}[\text{Cl}]_i^2[\text{Cl}]_i[\text{K}]_i[\text{Na}]_e, \ Z_{\text{nkcc11}} = Z_{11}[\text{Cl}]_i^2[\text{K}]_i[\text{Na}]_i[\text{Cl}]_e[\text{Na}]_e,$$
$$Z_{\text{nkcc12}} = Z_{12}[\text{Cl}]_i[\text{K}]_i[\text{Cl}]_i^2[\text{K}]_i[\text{Na}]_e, \ Z_{\text{nkcc13}} = Z_{13}[\text{Cl}]_i^2[\text{K}]_i[\text{Cl}]_i^2[\text{K}]_i[\text{Na}]_e,$$
$$Z_{\text{nkcc14}} = Z_{14}[\text{Cl}]_i^2[\text{K}]_i[\text{Na}]_i[\text{Cl}]_i[\text{K}]_i[\text{Na}]_e, \ Z_{\text{nkcc15}} = Z_{15}[\text{Cl}]_i^2[\text{K}]_i[\text{Na}]_i[\text{Cl}]_i^2[\text{K}]_i[\text{Na}]_e,$$
$$Z_{\text{nkcc16}} = K^2_{\text{Cl}}K_{\text{Na}}K_{\text{K}}(k_b^{\text{empty}} + k_f^{\text{empty}})$$

with

$$Z_1 = K_{\text{Cl}}K_{\text{K}}K_{\text{Na}}k_b^{\text{empty}}, \ Z_2 = K^2_{\text{Cl}}K_{\text{K}}k_f^{\text{empty}}, \ Z_3 = K_{\text{Cl}}K_{\text{Na}}k_b^{\text{empty}},$$
$$Z_4 = K_{\text{Cl}}K_{\text{K}}k_f^{\text{empty}}, \ Z_5 = K_{\text{Na}}k_b^{\text{empty}}, \ Z_6 = K_{\text{Cl}}k_f^{\text{empty}},$$
$$Z_7 = k_b^{\text{empty}} + k_b^{\text{full}}, \ Z_8 = k_f^{\text{full}} + k_f^{\text{empty}}, \ Z_9 = L_{\text{Na}}k_b^{\text{full}},$$
$$Z_{10} = L_{\text{Cl}}k_f^{\text{full}}, \ Z_{11} = L_{\text{Cl}}L_{\text{Na}}k_b^{\text{full}}, \ Z_{12} = L_{\text{Cl}}L_{\text{K}}k_f^{\text{full}},$$
$$Z_{13} = L^2_{\text{Cl}}L_{\text{K}}k_b^{\text{full}}, \ Z_{14} = L_{\text{Cl}}L_{\text{K}}L_{\text{Na}}k_b^{\text{full}}, \ Z_{15} = L^2_{\text{Cl}}L_{\text{K}}L_{\text{Na}}(k_b^{\text{full}} + k_f^{\text{full}}).$$

Where $L_{\text{ion}}$ is the reciprocal of $K_{\text{ion}}$. Finally

$$k_b^{\text{empty}} = \frac{K^2_{\text{Cl}}K_{\text{K}}K_{\text{Na}}k_f^{\text{full}}k_b^{\text{empty}}}{K^2_{\text{Cl}}K_{\text{K}}K_{\text{Na}}k_b^{\text{full}}}$$

### 4.6.4 Na$^+$ - K$^+$ - ATPase

Here we present the original four-state model of the NaK used by Gin et al. [40] in their model of the parotid acinar, for comparison to the two-state simplification shown in Section 4.6.6. Details of the original four-state simplification can be found in Smith and Crampin [93]. The flux through the NaK exchanger is given by,

$$J_{\text{NaK}} = \alpha_{\text{NaK}}\nu_{\text{NaK}}$$
Parameter values for Na\textsuperscript{+} - K\textsuperscript{+} - 2Cl\textsuperscript{−} cotransporter

<table>
<thead>
<tr>
<th>Parameter values</th>
<th>Value</th>
</tr>
</thead>
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</tr>
<tr>
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</tr>
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<td></td>
</tr>
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</tr>
<tr>
<td>( K_{\text{K}} )</td>
<td>234.74 mM</td>
</tr>
<tr>
<td>( K_{\text{Na}} )</td>
<td>22.38 mM</td>
</tr>
</tbody>
</table>

Table 4.7: Values from Benjamin and Johnson [8]

where \( \alpha_{\text{NaK}} \) is the density of the Na\textsuperscript{+} - K\textsuperscript{+} - ATPase.

Steady state flux is given by,

\[
\nu_{\text{NaK}} = \frac{\alpha_1^+ \alpha_2^+ \alpha_3^+ \alpha_4^+ - \alpha_1^- \alpha_2^- \alpha_3^- \alpha_4^-}{\Sigma}
\]

where,

\[
\alpha_1^+ = \frac{k_1^+ \tilde{\text{Na}}_i^3}{(1 + \tilde{\text{Na}}_i)^3 + (1 + \tilde{\text{K}}_i)^2 - 1}, \quad \alpha_2^+ = k_2^+
\]

\[
\alpha_3^+ = \frac{k_3^+ \tilde{\text{K}}_e^2}{(1 + \tilde{\text{Na}}_e)^3 + (1 + \tilde{\text{K}}_e)^2 - 1}, \quad \alpha_4^+ = \frac{k_4^+ \tilde{\text{MgATP}}}{1 + \text{MgATP}}
\]

\[
\alpha_1^- = k_1^- [\text{MgADP}], \quad \alpha_2^- = \frac{k_2^- \tilde{\text{Na}}_e^3}{(1 + \tilde{\text{Na}}_e)^3 + (1 + \tilde{\text{K}}_e)^2 - 1}
\]

\[
\alpha_3^- = \frac{k_3^- [\text{Pi}] [\text{H}^+]}{1 + \text{MgATP}}, \quad \alpha_4^- = \frac{k_4^- \tilde{\text{K}}_i^2}{(1 + \tilde{\text{Na}}_i)^3 + (1 + \tilde{\text{K}}_i)^2 - 1}
\]

where

\[
\tilde{\text{Na}}_i = \frac{[\text{Na}]}{K_{d,\text{Na}_i}}, \quad \tilde{\text{K}}_i = \frac{[\text{K}]}{K_{d,\text{K}_i}}
\]

\[
\tilde{\text{Na}}_e = \frac{[\text{Na}]}{K_{d,\text{Na}_e}}, \quad \tilde{\text{K}}_e = \frac{[\text{K}]}{K_{d,\text{K}_e}}
\]

\[
\tilde{\text{MgATP}} = \frac{[\text{MgATP}]}{K_{d,\text{MgATP}}}
\]

\[
K_{d,\text{Na}_i} = K_{d,\text{Na}_i}^0 \exp ((1 + \Delta)FV/3RT)
\]

\[
K_{d,\text{Na}_e} = K_{d,\text{Na}_e}^0 \exp (\Delta FV/3RT)
\]
Finally

\[ \Sigma = \alpha_1 \alpha_2 \alpha_3 + \alpha_1 \alpha_2 \alpha_4 + \alpha_1 \alpha_3 \alpha_4 + \alpha_2 \alpha_3 \alpha_4 \\
+ \alpha_2 \alpha_3 \alpha_4 + \alpha_2 \alpha_3 \alpha_4 + \alpha_2 \alpha_3 \alpha_4 + \alpha_3 \alpha_4 + \alpha_3 \alpha_4 + \alpha_3 \alpha_4 + \alpha_4 + \alpha_4 + \alpha_4 \]

Parameter values for Na\(^+\) - K\(^+\) - ATPase

| Rate constants | \( k_1^+ \) | 1050 s\(^{-1}\) | \( k_1^- \) | 172.1 s\(^{-1}\) M\(^{-1}\) |
| Dissociation constants | \( K_{d,Na}^0 \) | 15.5 mM | \( K_{d,K}^0 \) | 2.49 mM |
| \( K_{d,K}^0 \) | 0.213 mM | \( K_{d,MgATP} \) | 2.51 mM |
| Other parameters | [MgATP] | 4.99 mM | [MgADP] | 0.06 mM |
| [Pi] | 4.95 mM | \([H^+]\) | \(1000 \times 10^{-\triangle pH}\) mM |
| \( \triangle pH \) | 7.09 | \( \triangle \) | -0.031 |

Table 4.8: Values from Smith and Crampin [93] with the exception of [MgATP], [MgADP] and [Pi]

4.6.5 Na\(^+\) - K\(^+\) - 2Cl\(^-\) cotransporter simplification

We construct a two-state simplification of the model by Benjamin and Johnson [8], the original model can be seen in Section 4.6.3. Given an outside state, \( O \) and an inside state \( I \) we assume simultaneous binding and unbinding of Cl\(^-\), Na\(^+\) and K\(^+\). Denoting \( S_e = [K]_e[Na]_e[Cl]_e^2 \) as the ions outside the cell and \( S_i = [K]_i[Na]_i[Cl]_i^2 \) as ions inside the cell, the model can be written as,

\[ O + S_e \overset{k_1^+}{\underset{k_1^-}{\rightleftharpoons}} I \overset{k_2^+}{\underset{k_2^-}{\rightleftharpoons}} O + S_i \quad (4.13) \]

By setting the resulting scheme of differential equations to steady state and assuming the outside ions are supplied at a constant rate \( J \), the same rate at which the inside ions are removed, we find the steady-state flux:
4.6 Appendix

\[ J = \frac{k_1^+ k_2^+ S_e - k_1^- k_2^- S_i}{k_1^+ S_e + k_2^+ + k_1^- + k_2^- S_i}. \]  

(4.14)

The steady-state flux through the cotransporter, \( \nu_{NKCC} \) is equal to the rate at which ions are supplied on the cells exterior and removed on the cells interior. Therefore we have an expression for the whole cell flux which depends on four constants (reduced from seven constants in the original model),

\[ \nu_{NKCC} = \frac{\alpha_1 - \alpha_2 S_i}{\alpha_3 + \alpha_4 S_i}. \]  

(4.15)

We now complete a parameter search to best approximate the original model by Benjamin and Johnson [8] over a physiologically reasonable range of ionic concentrations. Parameters are found to be the following \(^5\), \( \alpha_1 = 157.55 \text{ s}^{-2} \), \( \alpha_2 = 2.0096 \times 10^7 \text{ M}^{-4} \text{ s}^{-2} \), \( \alpha_3 = 1.0306 \text{ s}^{-1} \), \( \alpha_4 = 1.3852 \times 10^6 \text{ M}^{-4} \text{ s}^{-1} \).

Then \( J_{NKCC} = \nu_{NKCC} \alpha_{NKCC} \) where \( \alpha_{NKCC} \) is the membrane density of the cotransporter.

4.6.6 \( \text{Na}^+ - \text{K}^+ - \text{ATPase simplification} \)

The \( \text{Na}^+ - \text{K}^+ - \text{ATPase} \) exchanges two external \( \text{K}^+ \) ions for three internal \( \text{Na}^+ \) at the expense of energy. We write a two state scheme simplification of the original model by Smith and Crampin [93], the original model can be seen in Section 4.6.4.

\[ O + 2[K]_e \xrightleftharpoons[k_1^-]{k_1^+} I + 3[\text{Na}]_e \]  

(4.16)

\[ I + 3[\text{Na}]_i \xrightleftharpoons[k_2^-]{k_2^+} O + 2[K]_i \]  

(4.17)

By setting the resulting system of differential equations to steady state, and assuming external \( \text{K}^+ \) and internal \( \text{Na}^+ \) get supplied at a constant rate, \( J \) we get

\[ J = \frac{k_1^+ k_2^+ [K]_e^2 [\text{Na}]_i^3 - k_1^- k_2^- [\text{Na}]_e^3 [K]_i^2}{k_1^+ [K]_e^2 + k_2^+ [\text{Na}]_i^3 + k_1^- [\text{Na}]_e^3 + k_2^- [K]_i^2} \]  

(4.18)

If we assume that the forward reaction rates are higher than the reverse rates, \( k_1^+ \gg k_1^- \) and \( k_2^+ \gg k_2^- \), and that the steady-state flux through the \( \text{Na}^+ - \text{K}^+ - \text{ATPase} \), \( \nu_{\text{NaK}} \), is equal to the constant rate \( J \), then

\(^4\)Parameters are fit using the Matlab least-squares routine \textit{lsqlinfit} 

\(^5\)The parameter values here have been corrected from how they appeared in [76].
\[ \nu_{\text{NaK}} = r \frac{[K]^2[\text{Na}]^3_i}{[K]^2_e + \alpha[\text{Na}]^3_i} \]  

(4.19)

We match our simplified model to the original by Smith and Crampin [93] to find the two model constants (a great reduction from the 18 in the original model)\(^6\). We find our constants to be \( r = 1.305 \times 10^6 \text{ M}^{-3} \text{ s}^{-1} \) and \( \alpha = 0.647 \text{ M}^{-1} \).

Then \( J_{\text{NaK}} = \alpha_{\text{NaK}} \nu_{\text{NaK}} \), where \( \alpha_{\text{NaK}} \) is the density of the \( \text{Na}^+ - \text{K}^+ - \text{ATPase} \) exchanger.

### 4.6.7 Comparing simulations run with simplified NKCC and NaK models versus the more complex models

We compare simulations run with the simplified NKCC and NaK seen in Section 4.6.5 and Section 4.6.6 to those run with the more complicated models in Section 4.6.3 and Section 4.6.4. In Figure 4.12 we can see that there is no qualitative difference between the fluid flow predicted by the two simulations.

![Figure 4.12: A comparison between a model simulation run with (a) the simplified NKCC and NaK fluxes found in Section 4.6.5, Section 4.6.6 and (b) run with more complicated 4-state models found in Section 4.6.3 and Section 4.6.4. \( \nu = 5100 \text{ nM/s} \) for duration to simulate continuous stimulation with agonist.](image)

All parameters are the same between the two simulations, and are those given in the main body of this manuscript, with the exception of \( \alpha_{\text{NKCC}} = 2.3 \times 10^{-16} \) for the simulation with complicated transporters (Figure 4.12b). If we compare the average fluid flow over a 300 second simulation (Table 4.9) we see that simplifying the parameters only affects the result by 3%.

\(^6\)Parameters are fit using the Matlab least-squares routine \texttt{lsqcurvefit}

\(^7\)Units have been added here where they were missing in original publication [76].
4.6.8 Location of Cl\(^{-}\) channels.

Similar to the apical and basal K\(^{+}\) currents in Section 4.6.2 we define two Cl\(^{-}\) currents, one in the apical and one in the basal membrane.

\[
I_{\text{Cl,Ap}} = \alpha_{\text{Cl}} g_{\text{Cl}} P_{\text{Cl}} (V_a - V_{\text{Cl,Ap}})
\]

\[
I_{\text{Cl,Ba}} = (1 - \alpha_{\text{Cl}}) g_{\text{Cl}} P_{\text{Cl}} (V_b - V_{\text{Cl,Ba}})
\]

where

\[
V_{\text{Cl,Ap}} = \frac{RT}{z_{\text{Cl}} F} \log \left( \frac{[\text{Cl}]}{[\text{Cl}]} \right)
\]

and

\[
V_{\text{Cl,Ba}} = \frac{RT}{z_{\text{Cl}} F} \log \left( \frac{[\text{Cl}]}{[\text{Cl}]} \right)
\]

Given the possibility of a basal Cl\(^{-}\) channel we must alter the equations for membrane potential seen in Section 4.3.5, we now have,

\[
C_m \frac{dV_b}{dt} = -I_{K,Ba} - I_{\text{Cl,Ba}} - F J_{\text{NaK}} - 2F J_{\text{pm}} + 2F J_{\text{in}} + I_{\text{tight}},
\]

\[
C_m \frac{dV_a}{dt} = -I_{\text{Cl,Ap}} - I_{K,Ap} - I_{\text{tight}},
\]

Then using the parameters \(\alpha_{\text{Cl}}\) we can distribute the whole cell Cl\(^{-}\) conductance between the apical and basal membranes.

In Figure 4.13 it can be seen that regardless of IP\(_3\) production rate, \(\nu\), or the distribution of K\(^{+}\) channels, the maximum fluid flow is always seen to occur when all of the Cl\(^{-}\) channels are located in the apical membrane.
Figure 4.13: Change in normalised water flow with distribution of Cl\(^-\) channels, in (a) with varying IP\(_3\) production rates and in (b) with varying K\(^+\) distributions. Figure (a) is produced with \(\alpha_k = 1\), Figure (b) with \(\nu = 5100\) nM/s.
4.7 Addendum

Following publication of this work the results were presented to the Yule lab at the University of Rochester Medical Center who expressed interest in experimentally testing for the existence of apical K+ channels. In their later published results, Almassy et al. [1] release Ca2+ locally at the apical pole using focal laser photolysis. A Ca2+ buffer was present to ensure the release of Ca2+ was confined to the apical pole. The local Ca2+ release at the apical pole resulted in increased K+ and Cl− currents across the plasma membrane. The use of large conductance (BK) and intermediate conductance (IK) K+ channel blockers established that both IK and BK channels were present in the apical membrane. Almassy et al. [1] state that the density of IK and BK channels is higher in the apical membrane than the basolateral membrane. Our model predicts maximum secretion rates when around 20% of the total cell K+ conductance is located in the apical membrane, with the majority in the basal membrane. This result appears to contradict the densities found experimentally, with the apical density found to be higher. However, we must consider the area of the basal membrane which is found to be 12 times larger than the apical membrane [83].

Several of the model components presented in this chapter have been used in other mathematical studies. Our steady-state model of the IP3R, seen in Section 4.3.2, interpolated the results of Gin et al. [41] giving a receptor open probability for physiologically realistic IP3 and Ca2+ concentrations. Means and Sneyd [69] use this IP3R model in their spatio-temporal model of Ca2+ dynamics in the interstitial cells of Cajal (ICC). The ICC cells are considered the pacemaker of rhythmic contractions of the gastro-intestinal tract. Maclaren et al. [63] use the Class II Ca2+ model from Section 4.3.2 to investigate the efficiency of primary saliva secretion. They hypothesise that secretion is maximised when the primary saliva is isosmotic. A small difference in solute concentration between the interstitium and lumen paired with a large membrane water permeability is considered to be most efficient at secreting saliva. This is in contrast to a situation with a large solute concentration difference and small membrane water permeability. The effect of multiple cells secreting into a shared lumen is also numerically investigated. It is found that coupling of cells through the shared lumen has a negligible effect of the rate of primary saliva secretion.
Chapter 5

A spatial model of Ca\(^{2+}\) waves in parotid acini

5.1 Introduction

In Chapter 4 we have seen a model which recreates experimentally observed Ca\(^{2+}\) oscillations in the parotid acinar. With improved spatial resolution of experimental equipment, Ca\(^{2+}\) waves have been observed in increasingly many cell types. These include heart myocytes, lung smooth muscle, pancreatic acinar cells, neurons and parotid acinar cells [53, 122].

Mathematical models have been used to explain the generation of Ca\(^{2+}\) waves. Dawson et al. [24] propose a model where Ca\(^{2+}\) is the only variable and CICR is incorporated using Heaviside functions. Several spatial models have used constant or diffusing IP\(_3\) concentrations to initiate Ca\(^{2+}\) waves through the cytosol, or even between cells [3, 94, 97]. In these models, Ca\(^{2+}\) oscillations arise through Class I mechanisms, where Ca\(^{2+}\) feedback on the IP\(_3\)R is responsible for Ca\(^{2+}\) oscillations. IP\(_3\) is assumed to be initially constant and then to diffuse and decay. No Ca\(^{2+}\) dependence is included in the IP\(_3\) dynamics.

In our model of Ca\(^{2+}\) oscillations in the parotid acinar cell, seen in Chapter 4, IP\(_3\) oscillations are required for oscillations of Ca\(^{2+}\). Here the production of IP\(_3\) was constant with Ca\(^{2+}\) dependent IP\(_3\) degradation. In this chapter we develop a spatial model of Ca\(^{2+}\) waves in the parotid acinar cell. IP\(_3\) production is agonist dependent and will occur at the cell membrane. IP\(_3\) will both diffuse throughout the cell and oscillate. We believe this to be the first Class II spatial model of Ca\(^{2+}\) waves.

Won et al. [122] find that Ca\(^{2+}\) waves travel from the apical to basal membrane, regardless of where the wave is initiated using IP\(_3\) photolysis. The Ca\(^{2+}\) wave is seen
to arrive at the basal membrane approximately 1 second following the peak in Ca\(^{2+}\) at the apical membrane. The authors hypothesise that the localisation of IP\(_3\)Rs at the apical pole is responsible for these directed waves. We shall test this hypothesis. We shall also check to ensure that our spatial model is able to recreate the experimental tests seen in Chapter 4. Here the application of ryanodine was seen to stop the oscillations in Ca\(^{2+}\). The removal of Ca\(^{2+}\) from the external medium caused the oscillations in the cytosolic Ca\(^{2+}\) concentration to damp.

5.2 A spatial model of Ca\(^{2+}\) waves in parotid acini

Zhang et al. [126] use fluorescence imaging to localise the RyRs and IP\(_3\)Rs. They find RyR throughout the cytosol, with the greatest density at the basal pole. The IP\(_3\)Rs were located at the apical pole. In Figure 5.1 we show a rectangular representation of the parotid acinar cell. We consider the cytosol and ER to be present at every point in the 2D space, with spatially inhomogeneous Ca\(^{2+}\) receptors densities. The inhomogeneity is only in the \(x\) direction. If we assume symmetry in the \(y\) direction, we can reduce the 2D problem to that of a line from the apical to basal membrane.

![Figure 5.1: A 2D rectangular representation of the parotid acinar cell, showing approximate locations of Ca\(^{2+}\) receptors. The coordinate \(x\) gives the distance from the apical membrane. If we assume symmetry in the \(y\) direction, we can solve the system on a line from the apical to basal membrane.](image)

We shall initially consider three variables in the generation of Ca\(^{2+}\) waves, all of which are allowed to diffuse. We give effective diffusion coefficients for Ca\(^{2+}\) and IP\(_3\) in the cytosol and Ca\(^{2+}\) in the ER. These are given by \(D_c\), \(D_p\) and \(D_s\) respectively. By giving an effective diffusion rate we can implicitly include the effect of Ca\(^{2+}\) and IP\(_3\) buffering. We assume Ca\(^{2+}\) is heavily buffered in the cytosol and ER. IP\(_3\) is unbuffered and has a high effective diffusion coefficient.
Figure 5.2: The location of the Ca\(^{2+}\) receptors and IP\(_3\) production in our 1D spatial Ca\(^{2+}\) model. The blue line shows the IP\(_3\)R density with the red line showing the RyR density. The shaded region at the basal pole indicates where the production of IP\(_3\) occurs following stimulation.

The autonomic nerves are located close to the basal membrane [5]. We assume that any external agonist will diffuse across the basal membrane stimulating IP\(_3\) production here. In our model agonist stimulation results in a non-zero IP\(_3\) production rate at the first few spatial nodes closest to the basal membrane.

Figure 5.2 shows the distribution of the Ca\(^{2+}\) receptors along our 1D line model, with the shaded region close to the basal pole showing the area where IP\(_3\) production occurs upon stimulation with an agonist. The IP\(_3\)Rs are located at the apical pole. For simplicity, we assume a homogeneous distribution of RyRs.

### 5.3 A 1D spatial Ca\(^{2+}\) model

We model Ca\(^{2+}\) in the cytosol, \(C\), Ca\(^{2+}\) in the ER (store Ca\(^{2+}\)), \(S\), and IP\(_3\), \(P\), with the following partial differential equations,

\[
\frac{\partial C}{\partial t} = D_c \frac{\partial^2 C}{\partial x^2} + (J_{IPR} + J_{ER} + J_{RyR} - J_{SERCA} + J_{in} - J_{pm}) ,
\]

\[
\frac{\partial S}{\partial t} = D_s \frac{\partial^2 S}{\partial x^2} - \frac{1}{\gamma} (J_{IPR} + J_{ER} + J_{RyR} - J_{SERCA}) ,
\]

\[
\frac{\partial P}{\partial t} = D_p \frac{\partial^2 P}{\partial x^2} + (J_{IP3prod} - J_{IP3deg}) ,
\]

(5.1)
where $D_c$, $D_s$ and $D_p$ are the effective diffusion coefficients and $\gamma = 5.405$ is the ratio of the cytosolic to ER volume. When we discretise the equations, the reaction terms at a given node are evaluated with the $C$, $S$ and $P$ values at that node. The $\mathrm{Ca}^{2+}$ and IP$_3$ fluxes have the same equations and parameters as found in Chapter 4 with the following exceptions due to the spatially inhomogeneous distribution of the IP$_3$R density, $\mathrm{Ca}^{2+}$ influx and IP$_3$ production.

The flux through the IP$_3$R at a given spatial node, $k$, is given by,

$$J^k_{\text{IPR}} = k^k_{\text{IPR}} P^k_{\text{IPR}} (S^k - C^k),$$

where,

$$k^k_{\text{IPR}} = 0.003 + 0.077 (1 - k/N)^4$$

and $N$ is the number of spatial nodes. The open probability at spatial node $k$ is the open probability function given in Section 4.3.2 evaluated with the $\mathrm{Ca}^{2+}$ and IP$_3$ concentration at node $k$, $P^k_{\text{IPR}} = P_{\text{IPR}}(C^k, P^k)$.

$\mathrm{Ca}^{2+}$ release through the RyR, $\mathrm{Ca}^{2+}$ re-uptake through the SERCA and ER leak is assumed to occur at every grid point. Function expressions and parameter values for $J_{\text{RyR}}$, $J_{\text{ER}}$ and $J_{\text{SERCA}}$ can be found in Section 4.3.2.

IP$_3$ production is assumed to occur at the basal pole, which we consider to be the nodes within 2% cell distance of the basal membrane. The IP$_3$ production rate at a given spatial node $k$ is therefore given by,

$$J^k_{\text{IP3prod}} = \nu \text{ nM/s \quad if \quad } k/(N-1) \geq 0.98,$$

$$J^k_{\text{IP3prod}} = 0 \text{ nM/s \quad if \quad } k/(N-1) < 0.98.$$

where $\nu$ is a parameter relating to the applied agonist concentration. IP$_3$ degradation is given by the expression and parameter values found in Section 4.3.2.

We assume any fluxes across the plasma membrane occur perpendicular to the spatial axis, $x$, across the lateral membrane. Homann et al. [51] find evidence the plasma-membrane $\mathrm{Ca}^{2+}$ ATPases are located on the apical membrane. This assumption is therefore not well justified. However, plasma membrane $\mathrm{Ca}^{2+}$ fluxes are small and results are not greatly altered using this assumption. The PDEs in equation 5.1 are then subject to the following no-flux boundary conditions on apical and basal membranes at $x = 0$.
and $x = L$ respectively,

$$\left. \frac{\partial C}{\partial x} \right|_{x=0,L} = 0,$$

$$\left. \frac{\partial S}{\partial x} \right|_{x=0,L} = 0,$$

$$\left. \frac{\partial P}{\partial x} \right|_{x=0,L} = 0,$$

where $L$ is the distance from the apical to basal membrane.

The plasma membrane fluxes, $J_{\text{in}}$, $J_{\text{pm}}$ at node, $k$, are given by,

$$J_{\text{in}}^k = \alpha_1 + \alpha_2 J_{\text{IP3prod}}^k,$$

and

$$J_{\text{pm}}^k = V_{\text{pm}} \left( \frac{C_k^3}{K_{\text{pm}}^3 + (C_k^3)} \right),$$

where $\alpha_1 = 2.1538 \text{nM/s}$, $\alpha_2 = 2 \times 10^{-5}$, $V_{\text{pm}} = 140 \text{s}^{-1}$ and $K_{\text{pm}} = 200 \text{nM}$.

### 5.3.1 Model initialisation

For the simulations presented here we use $N = 126$ spatial grid points and a time step of $\Delta t = 0.01$. The distance from the apical to basal membrane is assumed to be 25 $\mu$m, in close agreement to that found by Won et al. [122]. This gives a grid size of $\Delta x = 0.2$ $\mu$m. Diffusion coefficients are estimated as $D_c = 15 \mu$m$^2$/s, $D_s = 7.5 \mu$m$^2$/s and $D_p = 300 \mu$m$^2$/s. We initialise the model with homogeneous initial conditions,

$$C_{j=1..N}^{(0)} = 50 \text{nM},$$

$$S_{j=1..N}^{(0)} = 9.7 \times 10^4 \text{nM},$$

$$P_{j=1..N}^{(0)} = 0 \text{nM},$$

where the superscript denotes the time step and subscript, $j$, the spatial grid point. Simulations are implemented using a semi-implicit finite difference scheme, details can be seen in Appendix A. The results are presented below.

### 5.4 Spatial results

When we simulate the application of agonist, by setting the IP$_3$ production rate $\nu = 270$ mM/s at the basal pole, we see an increase in the IP$_3$ concentration at the basal membrane (Figure 5.3). The IP$_3$ rapidly diffuses through the cell to the apical membrane.
Here the increased IP$_3$ concentration opens the IP$_3$Rs and allows the release of Ca$^{2+}$ from the internal stores. The cytosolic Ca$^{2+}$ increases and diffuses back toward the basal membrane aided by CICR (Figure 5.4). As the Ca$^{2+}$ wave travels across the cell, the IP$_3$ degradation rate increases and the IP$_3$ concentration drops. IP$_3$ production at the basal membrane continues and another diffusive wave of IP$_3$ occurs from the basal to apical membrane, initiating an apical to basal Ca$^{2+}$ wave in return. This process repeats with a period of around 7 seconds. The peak in apical Ca$^{2+}$ concentration occurs approximately 1 second before the peak in basal Ca$^{2+}$ concentration, which agrees well with the experimental data of Won et al. [122].

Figure 5.3: IP$_3$ concentration in the cytosol in a simulation with the IP$_3$ production rate, $\nu = 270$ mM/s at the basal pole. IP$_3$ increases first at the basal membrane in response to the agonist applied here. The IP$_3$ then diffuses rapidly across the cell. A returning wave of Ca$^{2+}$, seen in Figure 5.4, then increases the IP$_3$ degradation as it travels from the apical to basal membrane. Waves repeat with a period of around 7 seconds.

As observed by Bruce et al. [14], when ryanodine is applied, parotid acini Ca$^{2+}$ oscillations are seen to damp and then disappear (see Chapter 4, Figure 4.6b). We test
Figure 5.4: Ca\textsuperscript{2+} concentration in the cytosol in a simulation with the IP\textsubscript{3} production rate, \( \nu = 270 \text{ mM/s} \) at the basal pole. Regular apical to basal Ca\textsuperscript{2+} waves occur with a period of around 7 seconds. The amplitude of the Ca\textsuperscript{2+} waves is reduced as they travel across the cell.

Our spatial Ca\textsuperscript{2+} model with the same conditions. Ca\textsuperscript{2+} oscillations are initiated with a non-zero IP\textsubscript{3} production rate at the basal membrane. Between 50 < \( t < 100 \) s, the application of ryanodine is simulated by setting \( J_{\text{RyR}} = 0 \). During this period oscillations in Ca\textsuperscript{2+} disappear, see Figure 5.5.

We tested our model for the ability to recreate the results of Bruce et al. [14] where Ca\textsuperscript{2+} oscillations were slowly damped when Ca\textsuperscript{2+} was removed from the cell exterior (Chapter 4, Figure 4.7b). We simulated this in the spatial model by adding a non-zero IP\textsubscript{3} production rate causing the onset of Ca\textsuperscript{2+} oscillations whilst blocking any external Ca\textsuperscript{2+} influx, result shown in Figure 5.6. Under Ca\textsuperscript{2+}-free conditions, model Ca\textsuperscript{2+} oscillations damp and disappear.
Figure 5.5: Ca\textsuperscript{2+} waves initiated in the apical pole and travelling to the basal pole with \( \nu = 270 \text{ mM/s} \) at the basal membrane. Waves disappear when the Ryanodine flux is set to zero for the period between 50 and 100 seconds. The model result can be compared to the experimental result seen in Chapter 4, Figure 4.6b.

### 5.5 Extension to a spatial secretion model

If we look to develop a full spatial secretion model, we must extend the three variable model above to include, the cytosolic and luminal ionic concentration of Cl\textsuperscript{-}, K\textsuperscript{+} and Na\textsuperscript{+}, the apical and basal membrane potentials and the cell volume. Being unbuffered and having a small molecular size, Cl\textsuperscript{-}, K\textsuperscript{+} and Na\textsuperscript{+} diffuse quickly throughout the cytosol. If we assume this diffusion is very rapid, we can take the limit and consider the concentration of these ions homogeneous throughout the cytosol. With this assumption we can solve PDEs for Ca\textsuperscript{2+} and IP\textsubscript{3} in the cytosol, and Ca\textsuperscript{2+} in the ER at every spatial grid point, paired with ODEs for the remaining nine variables.

The plasma membrane ionic fluxes depend on the local Ca\textsuperscript{2+} concentrations. For example, the apical Cl\textsuperscript{-} channel open probability depends only on the apical Ca\textsuperscript{2+} con-
5.5. Extension to a spatial secretion model

Figure 5.6: Ca\(^{2+}\) waves intitated under Ca\(^{2+}\) free conditions are seen to damp and then disappear. Agonist simulated with IP\(_3\) production rate \(\nu = 270\) mM/s at the basal membrane. \(J_{ia} = 0\) for the duration of the simulation to simulate external Ca\(^{2+}\)-free conditions. The model result can be compared to the experimental result in Chapter 4, Figure 4.7b.

The concentration. The flux through the Cl\(^-\) channel at a given time is therefore given by,

\[
I_{Cl} = g_{Cl} P_{Cl} (C(x = 0)) \cdot (V_a - V_{Cl}),
\]

where the open-probability of the Cl\(^-\) channel is evaluated at the Ca\(^{2+}\) concentration of the apical membrane, at \(x = 0\). For fluxes across the basal membrane, the evaluation of the open-probability will be using the Ca\(^{2+}\) concentration at the basal membrane, \(C(x = L)\).

The change in cell volume depends on the change in the total solute concentration in the cell. The concentration of Ca\(^{2+}\) in the cytosol is very small compared with the concentrations of K\(^+\), Cl\(^-\) and Na\(^+\). A change in the cytosolic Ca\(^{2+}\) concentration will result in a negligible change in the total solute concentration. Similarly, the small Ca\(^{2+}\)
influx and efflux has a negligible effect on changes in the membrane potentials. If we ignore the effect of Ca$^{2+}$ on changes in the cell volume and membrane potentials, then the ODEs solved for the water flow are only paired with Ca$^{2+}$ via the K$^+$ and Cl$^-$ channels at the apical and basal membranes. We discuss the implications of this in Section 5.6.

The spatial secretion model is tested by running simulations with a homogeneous distribution of Ca$^{2+}$ channels. The fluid flow results can then be compared to those in Chapter 4. Small quantitative differences were present due to the reduced order of our numerical scheme, but no qualitative difference in results was found (result not shown here).

**5.6 Discussion**

Our spatial Ca$^{2+}$ model shows periodic Ca$^{2+}$ waves travelling from the apical membrane to the basal membrane. As the wave travels towards the basal membrane the amplitude of the Ca$^{2+}$ peak is reduced. Won et al. [122] show a single Ca$^{2+}$ wave which travels from the apical to basal membrane increasing in amplitude as it travels. It is possible to increase the size of the Ca$^{2+}$ release at the basal membrane by increasing the density of the RyRs. The model presented here has a steady-state RyR model. If the receptor density is increased beyond a certain threshold value then CICR causes a large continued depletion of the ER and the cytosol becomes saturated with Ca$^{2+}$. When this occurs, persistent apical to basal Ca$^{2+}$ waves are not seen, instead the cytosol reaches a steady-state with a very high Ca$^{2+}$ concentration. Keizer and Levine [54] show that the RyR is both Ca$^{2+}$ and time dependent, reaching a peak release, determined by the Ca$^{2+}$ concentration and then reducing to a Ca$^{2+}$ dependent plateau. The time-dependency is not included in the model presented here. Adding the time-dependency to the model might allow a larger density of RyRs to be added at the basal membrane, enabling the Ca$^{2+}$ waves to grow in amplitude as they travel from the apical to basal membrane.

When the application of ryanodine is simulated in the spatial model, we see oscillations in Ca$^{2+}$ stop immediately. In comparison, experimental results show Ca$^{2+}$ oscillations damping over several seconds before reaching an equilibrium [14]. It is expected the addition of a dynamic RyR model with altered receptor distribution would also correct this model discrepancy.

We use a rectangle to represent the parotid acinar cell geometry. This is a poor approximation. A spherical coordinate system could provide a better approximation. Alternatively, a full 3D simulation, run on a realistic cell geometry, could be completed. In the absence of interesting geometric structure, such as the pacemaker micro domain...
5.6. Discussion

of the interstitial cells of Cajal [69], it is not clear that a more realistic geometry would greatly alter the results presented here.

We implement a semi-implicit finite difference scheme. The numerical scheme approximates the derivatives in space and time by a centred difference and backwards Euler’s method respectively. This numerical scheme proved sufficient in its role of testing whether the Class II Ca"^{2+}" model could produce the apical to basal Ca"^{2+}" waves seen experimentally. If the spatial model was to be used for a more thorough analysis, using a higher order method would be desirable. The addition of adaptive time-stepping would, also, greatly improve the efficiency of the numerical method.

An inhomogeneous distribution of Ca"^{2+}" channels, as found experimentally by Zhang et al. [126], with diffusing Ca"^{2+}" and IP"_{3}" is shown to produce apical to basal Ca"^{2+}" waves. These have qualitative similarities with those seen experimentally by Won et al. [122]. The questions remains, not how Ca"^{2+}" waves are generated, but why Ca"^{2+}" waves exist? We have seen that Cl"^{-}" channels are localised exclusively in the apical membrane, with the Na"^{+}"- K"^{+}"- 2Cl"^{-}" co-transporter and Na"^{+}"- K"^{+}"- ATPase found in the basal membrane. A directed Ca"^{2+}" wave, from the apical to basal membrane, will first open ions channels located in the apical membrane. After diffusing through the cell the Ca"^{2+}" wave will then open ion channels at the basal membrane. Does the timing between apical to basal Ca"^{2+}" peak encode some signalling information or increase the efficiency of saliva secretion?

The spatial model presented here has a large number of free parameters, including the effective diffusion coefficients and the receptor densities and profile. A given parameter set will determine the characteristics of the Ca"^{2+}" wave that exists, if a Ca"^{2+}" wave does indeed exist. If one parameter is changed, for example the IP"_{3}R" density at the apical pole, the wave speed, amplitude at both the apical and basal membrane, and the period of the waves would most likely all be altered. If a Ca"^{2+}" wave is simulated with two different parameter sets one could compare the secretion rates in the full spatial fluid flow model. If the secretion rates differed it would not be clear which Ca"^{2+}" wave property was responsible for the change. Taking an alternative approach whereby a search was made for the parameter set which only altered one property, such as the wave speed, would come with severe numerical cost.

The only significant coupling between the spatial Ca"^{2+}" model and the fluid secretion model is via the Ca"^{2+}" dependent K"^{+}" and Cl"^{-}" channels at the apical and basal membranes. The majority of the spatial data that is generated is, therefore, discarded. In Chapter 6 we approximate Ca"^{2+}" waves with time-dependent functions of Ca"^{2+}" at the apical and basal membrane. These ‘fake’ Ca"^{2+}" waves allow exactly one wave property to be altered and are used to investigate how Ca"^{2+}" wave properties affect the regulation of saliva secretion.
saliva secretion. Using this approach we hope to understand if Ca\textsuperscript{2+} waves may encode signalling information that homogeneous oscillations in Ca\textsuperscript{2+} could not.
Chapter 6

Modelling the effects of calcium waves and oscillations on saliva secretion

In the following chapter we investigate how individual Ca$^{2+}$ wave properties affect the regulation of saliva secretion. What follows is a copy of the article as it appears in the Journal of Theoretical Biology, Volume 305. The full reference is given in the bibliography [77].
Chapter 6. Modelling the effects of calcium waves and oscillations on saliva secretion

6.1 Abstract

An understanding of Ca$^{2+}$ signalling in saliva-secreting acinar cells is important, as Ca$^{2+}$ is the second messenger linking stimulation of cells to production of saliva. Ca$^{2+}$ signals effect secretion via the ion channels located both apically and basolaterally in the cell. By approximating Ca$^{2+}$ waves with periodic functions on the apical and basolateral membranes, we isolate individual wave properties and investigate them for their effect on fluid secretion in a mathematical model of the acinar cell. Mean Ca$^{2+}$ concentration is found to be the most significant property in signalling secretion. Wave speed was found to encode a range of secretion rates. Ca$^{2+}$ oscillation frequency and amplitude had little effect on fluid secretion.

6.2 Introduction

A problem commonly encountered in quantitative analysis of physiological processes is to determine which experimentally observed behaviours are important to the system and which can be approximated to produce a simple model capable of making predictions and increasing understanding. The salivation process is initiated with an electrical signal from the brain which releases an agonist, ACh, around the acinar cells. This agonist causes production of IP$_3$ which releases Ca$^{2+}$ from internal stores in the endoplasmic reticulum (ER). Ca$^{2+}$ feedback on IP$_3$ dynamics can cause periodic oscillations of Ca$^{2+}$ throughout the cytoplasm at a raised baseline. The raised Ca$^{2+}$ concentration opens K$^+$ and Cl$^-$ channels, causing a change in intracellular and luminal concentrations of Cl$^-$, Na$^+$ and K$^+$. This concentration change creates an osmotic gradient which leads to increasing fluid secretion from the acinar cells. This process is completed in a great many acinar cells simultaneously and is accompanied by a shrinking of cell volume. Once the fluid is secreted from the acinar cells into the lumen as primary saliva it travels down the parotid ducts where duct cells modify the ionic content before finally being secreted in the mouth.

One mechanism that is particularly well studied is that of the Ca$^{2+}$ dynamics. Ca$^{2+}$ is well known to have an important role as a second messenger in a vast array of cell types. Current models of saliva secretion in the parotid acinar cells by Palk et al. [76] and Gin et al. [40] use compartmental models of Ca$^{2+}$ to reproduce experimental results and incorporate into models for saliva secretion. These models assume homogeneous oscillations in Ca$^{2+}$ throughout the cytosol and hence can be modelled using ordinary differential equations. Experimentally, however, Ca$^{2+}$ is not only observed to oscillate
6.3 Modelling agonist-induced saliva secretion

but to travel in waves from one membrane to the other. These Ca$^{2+}$ waves have been seen in many cells types including cardiac myocytes, airway smooth muscle, pancreatic acinar cells, neurons [53] and parotid acinar cells [122]. Ca$^{2+}$ waves and oscillations are thought to be able to encode a larger amount of signalling information than a constant Ca$^{2+}$ concentration. Experimental and theoretical evidence suggests that frequency and amplitude are used to encode information in certain cell types [9, 25, 106]. It is not our focus here to investigate the genesis of Ca$^{2+}$ waves, for that see Sneyd et al. [98]. In this paper we seek to investigate how important the properties of Ca$^{2+}$ waves are for controlling the secretion of primary saliva.

Consideration of Ca$^{2+}$ waves, as opposed to homogeneous oscillations, requires consideration of amplitude, mean concentration, frequency and also the wave speed as mechanisms for signalling. Using a detailed spatial model of the Ca$^{2+}$ waves makes it difficult to change one property, say the wave speed, without affecting the others. A spatial modelling approach involves numerically solving partial differential equations throughout the cytosol. Yet as regards saliva secretion, Ca$^{2+}$ acts on ion channels which are located in the apical and basal membranes only. Hence using a spatial model generates far more information than is required and is not the approach taken here. Rather, we approximate Ca$^{2+}$ waves by using periodic functions for Ca$^{2+}$ at the basal and apical membrane. Using this approximation of Ca$^{2+}$ waves we are able to isolate Ca$^{2+}$ wave properties such as frequency, amplitude, wave speed and mean concentration to individually investigate their effect on saliva secretion.

6.3 Modelling agonist-induced saliva secretion

We use the mathematical model of the parotid acinar cell from Palk et al. [76]. Here saliva secretion is initiated by a raised Ca$^{2+}$ concentration which open K$^+$ and Cl$^-$ channels. This enables an ionic gradient to be maintained which allows water to flow by osmosis both transcellularly and paracellularly into the lumen. A schematic of the fluid secretion model can be seen in Figure 6.1.

Being un-buffered we assume that K$^+$, Cl$^-$ and Na$^+$ diffuse very quickly and therefore these ionic concentrations are homogeneous throughout the three sub-domains, the interstitium, the cytosol and the lumen.

Transmembrane ion fluxes are driven by Ca$^{2+}$ with the Ca$^{2+}$ concentration at the apical membrane affecting the open probability of the ion channels that reside in the apical membrane and similarly the Ca$^{2+}$ concentration at the basal membrane affecting the ion channels there.
Figure 6.1: A schematic of the movement of ions responsible for saliva secretion

Differential equations are written for the change in Cl\(^-\), K\(^+\), Na\(^+\), cell volume and the apical and basal membrane potentials. These are numerically solved using the Matlab routine \texttt{ode15s}. Foskett [38] show that volume changes are tightly correlated with changes in cytosolic Cl\(^-\). Our model assumes fluid flow to change instantaneously with ionic changes, and cell volume to follow directly. Details of the model equations are given in Section 6.9.1.

### 6.4 Simplified model of Ca\(^{2+}\) waves

We consider a periodic Ca\(^{2+}\) wave from the apical to basal membrane with a constant period such as seen experimentally by Zimmermann and Walz [127]. At any point throughout the cytosol the concentration of Ca\(^{2+}\) will be a periodic function with the same period and a possibly distinct mean and amplitude. We simulate a Ca\(^{2+}\) wave with the concentration being a periodic function at both the apical and basal membranes. We
can formally write this as follows,

\[ C_a = f(t), \]
\[ C_b = g(t + \delta), \]

where \( C_a \) is the \( \text{Ca}^{2+} \) concentration at the apical membrane and with \( C_b \) the basal \( \text{Ca}^{2+} \) concentration. Both \( f(t) \) and \( g(t) \) are assumed to be periodic with the same period \( T \) and both attain their minimum values at \( t = 0 \). The parameter \( \delta \) is a measure of synchronicity, when \( \delta = 0 \) \( \text{Ca}^{2+} \) oscillations are synchronous at the two membranes. When parameter \( \delta \) is non-zero there is a delay between \( \text{Ca}^{2+} \) peaking at the apical and basal membrane. This phase-shift can be used to simulate a \( \text{Ca}^{2+} \) wave with a given speed. Using this model we are free to change individual wave properties, for example the wave amplitude, without affecting the other wave properties.

![Graph](image-url)

**Figure 6.2:** \( \text{Ca}^{2+} \) concentrations at the apical and basal membrane numerically simulated with sine waves, period 7 s, with mean 200 nM and 50 nM amplitude. There is a 1 s time difference between the apical and basal \( \text{Ca}^{2+} \) peak which is equivalent to a 25 \( \mu \)m/s wave speed.

In Figure 6.2 we simulate an apical to basal \( \text{Ca}^{2+} \) wave, periodic with a period of 7 s, where the mean \( \text{Ca}^{2+} \) concentration and amplitude is the same at both membranes. Here a sine function was used to give the \( \text{Ca}^{2+} \) profile at both membranes. However, any periodic function with a similar profile to experimentally observed \( \text{Ca}^{2+} \) oscillations could have been used. For the remainder of the results presented a sine function is used to approximate the oscillations of \( \text{Ca}^{2+} \) at the apical and basal membranes. The \( \text{Ca}^{2+} \)
concentration $C$ is given by,

$$ C = C_{\text{norm}} + C_{\text{amp}} \sin \left( \frac{2\pi(t - \delta)}{\lambda} \right), $$

where $C_{\text{norm}}$ is the mean $Ca^{2+}$ concentration, $C_{\text{amp}}$ is the amplitude of the $Ca^{2+}$ oscillations, $\delta$ allows for the inclusion of a time delay and $\lambda$ is the period of oscillations.

### 6.5 Analysis of the effect of $Ca^{2+}$ wave speed on fluid flow

Previous work has investigated how the frequency of $Ca^{2+}$ oscillations may encode signalling information in the phosphorylation of a cellular substrate by protein kinase, Goldbeter et al. [42], and in hepatocytes, Larsen et al. [57]. We investigate the effect of wave speed on saliva secretion by varying the time difference between the peak in $Ca^{2+}$ at the apical and basal membranes. Experimentally, Won et al. [122] report a wave-speed of 27.81 $\mu$m/s with $Ca^{2+}$ peaking at the apical membrane approximately 1 second before the basal membrane. These measurements suggest the distance between the two membranes is 27.81 $\mu$m: for simplification this work uses a distance of 25 $\mu$m between membranes.

In Figure 6.2 we numerically simulate an apical to basal $Ca^{2+}$ wave having a 1 second time difference between the apical and basal $Ca^{2+}$ peaks. With our assumed cell size of 25 $\mu$m from the apical to basal membrane this is equivalent to a wave speed of 25 $\mu$m/s. If instead we ran a simulation with a 2 second time between the apical and basal membrane peak in $Ca^{2+}$ this would approximate a wave speed of 12.5 $\mu$m/s, assuming the same cell size. Using this idea of changing the time between apical and basal $Ca^{2+}$ peaks we can simulate a range of wave speeds and observe the effect on secretion.

In Figure 6.3 the effect of the time between apical and basal $Ca^{2+}$ peak can be seen on the average fluid flow rate. It is shown that maximum secretion occurs when the time difference is zero, implying synchronous $Ca^{2+}$ oscillations at the two-membranes, or equivalently a homogeneous rise and fall of $Ca^{2+}$ throughout the cytosol. A minimum secretion rate occurs at a time difference of 3.5 seconds. This is a time difference of exactly half the oscillation period and oscillations at the two membranes are out of phase.

For this result, and the remainder of the analysis, a sinusoidal function was used to approximate the $Ca^{2+}$ oscillations at each membrane. However the same result can be reproduced for several different periodic functions at both membranes. A mathematical
argument that a local maximum occurs when oscillations are synchronous for any periodic function is given in Section 6.9.2.

![Graph showing fluid flow rate against time difference between apical and basal Ca$^{2+}$ peaks.](image)

Figure 6.3: Fluid flow rate against time difference between apical and basal Ca$^{2+}$ peak. Maximum secretion occurs when the apical and basal oscillations are synchronous. Ca$^{2+}$ waves are approximated using a sine function with 150 nM mean, 100 nM amplitude and 7 s period.

The result that synchronous oscillations are most efficient appears to suggest that the experimentally observed Ca$^{2+}$ waves seen by Won et al. [122], with a 1 second time difference between apical and basal Ca$^{2+}$ peak, are less than efficient at signalling saliva secretion. There are, however, some assumptions made in the analysis above that we now explore. In particular the model currently has all ion channels operating at steady state. However, experimentally the ion channels have a time dependence. We will address this model shortfall in the following section.

### 6.6 Time-dependent Cl$^-$ channel gating

In Arreola et al. [2] the Cl$^-$ channels are found to react very quickly to changes in Ca$^{2+}$ at physiologically realistic membrane potentials and Ca$^{2+}$ concentrations. This quick opening and closing led us to initially use a steady-state model for the Cl$^-$ channel. We investigate whether adding the time dependence of the Cl$^-$ channel to the model affects the results relating to fluid secretion.
In Arreola et al. [2] a four-state model is given for the Cl\(^-\) channel with three closed and one open state as seen below,

\[
C_1 \xrightleftharpoons{\alpha_1}{\beta_1} C_2 \xrightleftharpoons{\alpha_1}{\beta_1} C_3 \xrightleftharpoons{\alpha_2}{\beta_2} O.
\]

Rates \(\alpha_1\) and \(\beta_1\) are faster than \(\alpha_2\) and \(\beta_2\) and their dependence on Ca\(^{2+}\) is not given explicitly in Arreola et al. [2]. Hence we simplify this model to a 2-state model using a rapid equilibrium approximation to group the three closed states, \(C_1, C_2\) and \(C_3\) into one new closed state \(C\).

\[
C \xrightleftharpoons{\alpha}{\beta_2} O.
\]

This two-state model simplification approximates the experimental data well (result not shown). Applying the two-state reduction we get a differential equation for the fraction of open Cl\(^-\) channels,

\[
\frac{dO}{dt} = \alpha C - \beta_2 O.
\]

Here \(\beta_2\) is the same reverse rate as seen in Arreola et al. [2]. The forward reaction rate, \(\alpha\), given in terms of the original rates \(K_1, K_2\) and \(\alpha_2\) in Arreola et al. [2], is shown below,

\[
\alpha = \frac{\alpha_2}{\left(1 + \frac{K_1}{C_a} + \frac{K_2}{C_a^2}\right)}.
\]

Here,

\[
K_1 = 214 \exp\left(-\frac{0.13FV_a}{RT}\right) \text{nM},
\]

\[
K_2 = 0.58 \exp\left(-\frac{0.24FV_a}{RT}\right),
\]

\[
\beta_2 = K_2\alpha_2 \text{ s}^{-1}, \quad \alpha_2 = 4.5 \text{ s}^{-1} \quad \text{and} \quad C_a \text{ is the Ca}\(^{2+}\) concentration at the apical membrane. \n\]

\(V_a\) is the membrane potential of the apical membrane. The total current through the Cl\(^-\) channels is then given by,

\[
I_{Cl} = g_{cl}O(V_a - V_{Cl}),
\]

where \(g_{Cl} = 31.4 \text{ nS}\) is the maximum whole cell conductance found by Arreola et al. [2]. \(V_{Cl}\) is the Nernst potential given by,

\[
V_{Cl} = \frac{RT}{z_{Cl}F} \log \left(\frac{[Cl]_l}{[Cl]_i}\right),
\]

where \([Cl]_l\) and \([Cl]_i\) are the Cl\(^-\) concentrations in the lumen and cytosol respectively.
and $z_{\text{Cl}} = -1$ is the valence of Cl$^-$, $R = 8.315$ J mol$^{-1}$ K$^{-1}$, $T = 310$ K and $F = 96490$ C mol$^{-1}$.

### 6.6.1 The effect of wave speed on fluid secretion rate in a model with time-dependent Cl$^-$ channels

The effect of wave speed on fluid flow is investigated using the same method of varying the time difference between the apical and basal Ca$^{2+}$ peaks described in Section 6.5. When time dependence of the Cl$^-$ channel is added to the model we find that a maximum secretion rate occurs with a small positive time difference between the Ca$^{2+}$ peaking at the apical and basal membranes (see Figure 6.4). Ca$^{2+}$ waves are simulated with a sinusoidal function with mean 150 nM, amplitude 100 nM and a period of 7 seconds.

![Figure 6.4: Fluid flow rate against time difference between apical and basal Ca$^{2+}$ peak in a model with time-dependent Cl$^-$ channels. Maximum fluid flow occurs when Ca$^{2+}$ peaks at the apical membrane 0.2 s before the basal membrane.](image)

In Figure 6.4 it can be seen that maximum secretion occurs when the Ca$^{2+}$ wave peaks at the apical membrane 0.2 s before the basal membrane. This roughly equates to an apical to basal wave with a speed of 125 $\mu$m/s, assuming a cell size of 25 $\mu$m from apical to basal membrane. This is much faster than the observed wave speed of 27.81 $\mu$m/s seen by Won et al. [122].
6.6.2 The effect of wave period on fluid secretion rate in a model with time-dependent Cl\(^-\) channels

In Figure 6.5 a sinusoidal function is used to simulate Ca\(^{2+}\) at the two membranes with a mean of 100 nM and an amplitude of 50 nM.

![Graph showing fluid flow rate against period of Ca\(^{2+}\) oscillations.](image)

Figure 6.5: Fluid flow rate against period of Ca\(^{2+}\) oscillations. The maximum fluid flow rate occurs with long period oscillations.

Figure 6.5 shows that as the period of the Ca\(^{2+}\) waves is increased the average saliva secretion rate increases. If Ca\(^{2+}\) oscillates quickly the time-dependent Cl\(^-\) channel will lag behind the current Ca\(^{2+}\) concentration. This results in less than maximum fluid flow. As the Ca\(^{2+}\) oscillation period is increased we find that fluid flow reaches a maximum. It should be noted that the rate of secretion changes very little despite large changes in oscillation period with the least efficient rate being only 96% of the most efficient period.

6.6.3 The effect of mean Ca\(^{2+}\) on fluid secretion rate in a model with time-dependent Cl\(^-\) channels

The effect of mean Ca\(^{2+}\) on fluid flow is seen in Figure 6.6. As the mean Ca\(^{2+}\) concentration increases the amount of secretion increases. The profile of fluid flow as Ca\(^{2+}\) increases takes a sigmoidal shape, initially increasing rapidly at low Ca\(^{2+}\) concentrations and levelling off as the ion channel open probability approaches one. A very large differ-
ence is observed in secretion rates with a high Ca\(^{2+}\) concentration secreting almost ten times the volume of low concentrations.

![Diagram showing fluid flow rate against mean Ca\(^{2+}\) concentration.](image)

Figure 6.6: Fluid flow rate against mean Ca\(^{2+}\) concentration. Larger mean Ca\(^{2+}\) concentrations increase secretion rate. Simulations are completed using a sine function with 20 nM amplitude and 7 s period.

### 6.6.4 The effect of oscillation amplitude on fluid secretion rate in a model with time-dependent Cl\(^{-}\) channels

The effect of oscillation amplitude on fluid flow is investigated. With a mean Ca\(^{2+}\) concentration of 100 nM, increasing the amplitude of oscillations increases the rate of secretion. This can be seen in Figure 6.7a. If instead the mean Ca\(^{2+}\) is increased to 300 nM the opposite is true, Figure 6.7b, with increasing oscillation amplitude reducing secretion. This result can be explained by the profile of fluid flow with mean Ca\(^{2+}\) seen in Figure 6.6. At low Ca\(^{2+}\) concentrations the function is convex. Jensen’s inequality states,

\[
E[f(x)] \geq f(E[x]),
\]

where \(E\) is the expectation and \(f\) is a convex function. If we consider an oscillating function of Ca\(^{2+}\), this inequality says that the average secretion rate for some oscillating function of Ca\(^{2+}\) is greater than the rate of secretion at the mean Ca\(^{2+}\) concentration.
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Figure 6.7: Fluid flow rate against amplitude of periodic Ca$^{2+}$ oscillations for two different mean Ca$^{2+}$ concentrations. The maximum fluid flow rate occurs with large amplitude oscillations for a 100 nM mean Ca$^{2+}$. With a larger 300 nM mean Ca$^{2+}$ the maximum secretion occurs with low amplitude oscillations. Both simulations are completed with a 7 s period sine function.

Or equivalently, we expect a larger amplitude to give us greater secretion than a constant Ca$^{2+}$ concentration.

With a larger mean Ca$^{2+}$ concentration the profile of secretion with Ca$^{2+}$ becomes concave and the converse is true with larger amplitude causing a reduction in secretion. At a mean Ca$^{2+}$ concentration of around 120 nM the secretion rate as a function of Ca$^{2+}$ is neither convex or concave. Here amplitude has no significant effect on secretion (result not shown).

6.7 Discussion

Ca$^{2+}$ signals involving oscillations are commonly found in biological systems, and are thought to enable a larger bandwidth of signalling. We have found that each of the investigated properties of Ca$^{2+}$ waves are capable of altering the rate of saliva secretion to differing degrees.

We find Ca$^{2+}$ oscillation frequency to be inefficient at regulating secretion rate, with only a 4% change in secretion over a large range of frequencies. Any difference in secretion is due to the time-dependence of the Cl$^{-}$ channel as no change is observed when this is absent from the model. Gray [44] find that given a large range of applied agonist concentrations the parotid acinar cells oscillated with a reasonably constant frequency, potentially supporting the idea that frequency encoding is unimportant in the parotid
acinar cell. It is worth noting the shape of Figure 6.5. As the period is increased a plateau is reached. Bruce et al. [14] report 7-11 Ca$^{2+}$ oscillations per minute in parotid acinar cells giving a period of 5.5-8.5 seconds. This physiologically realistic range for oscillations lies just at the start of the plateau maximising efficiency of secretion.

As the Ca$^{2+}$ wave speed is changed a noticeable change in secretion rate occurs with the least efficient wave speed secreting 83% of the maximum secretion rate that is obtained with the most efficient wave speed. Our model with time-dependent Cl$^{-}$ channels predicts apical to basal Ca$^{2+}$ waves to be the most efficient, however the most efficient secretion is predicted for a wave speed much faster than observed experimentally by Won et al. [122]. The model used for this analysis has Cl$^{-}$ channels in the apical membrane and K$^{+}$ in the basal membrane. There is evidence, both experimental and theoretical [1, 76] that apical K$^{+}$ channels are found in parotid acinar cells. These K$^{+}$ channels are thought to be of the maxi-K type. It is possible that the addition of these K$^{+}$ channels to the model with their time dependence could make slower wave speeds more efficient to parotid acinar cell function. Future work would require a detailed study of the activation of maxi-K channels by Ca$^{2+}$ in order for this to be properly resolved.

The experimentally observed wave speed of 27.81 $\mu$m/s seen by Won et al. [122] is found to remain roughly constant in parotid acinar cells for varying amounts of stimulation, and therefore it seems unlikely that wave speed is used as a signalling mechanism. This wave speed is very similar to Ca$^{2+}$ waves observed in other mammalian cell types by Jaffe [53], with only cardiac myocytes displaying much greater wave speeds. It seems possible that a similar wave generation mechanism in different cell types might limit wave speeds to this narrow range. One might conjecture that Ca$^{2+}$ waves travel at a speed which maximises fluid secretion or, alternatively, that the ion channels responsible for fluid regulation have adapted to maximise secretion for this constrained wave speed.

The effect of Ca$^{2+}$ oscillation amplitude on secretion is dependent on the mean Ca$^{2+}$ concentration, with increasing amplitude increasing secretion at low Ca$^{2+}$ concentrations and decreasing secretion as the mean Ca$^{2+}$ increases. Gray [44] reports a large range of oscillation amplitudes seen experimentally and thus it is unclear what role amplitude might have in signalling.

By far the most significant mechanism for signalling is the mean Ca$^{2+}$ concentration. Here the flow rate for low Ca$^{2+}$ concentration is less than 10% what is seen for the highest concentration. Foskett and Melvin [36] find a resting level of Ca$^{2+}$ as 59 nM, increasing to 474 nM when stimulated with carbachol. According to our model this would result in a 10-fold increase in secretion. Experimentally an increase of between 6 and 13 fold is seen between resting and stimulated salivary glands [7, 49].
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By avoiding a detailed spatial model, wave properties are easily isolated and investigated for their effect on secretion. Several assumptions are made in using this simplified approach. Changing a global variable, such as the wave speed, is assumed to affect the apical and basal regions equally and not to affect other variables. If we were to alter the wave speed experimentally, perhaps by inhibiting the \( \text{Ca}^{2+} \) release channels, we might expect the profile of the oscillations at the two membranes to change. It is also likely that the frequency, amplitude and mean \( \text{Ca}^{2+} \) concentration would also be changed.

In Section 6.6 we consider the time-dependence of the \( \text{Cl}^- \) channel gating using the experimental data and model of Arreola et al. [2]. There are other time-dependent processes that have not been included in this analysis. As previously mentioned, further data is needed for the inclusion of time-dependent maxi-K channels. Membrane mechanics and fluid dynamics are also likely to add time-dependent effects to the model, but are not considered due to their complexity.

The overall aim of our research is to understand the regulation of saliva secretion across temporal and spatial scales from individual ion channels to whole gland secretion rates. To create a multiscale model of saliva secretion we must decide what detail to include and what to simplify. Given that \( \text{Ca}^{2+} \) waves are found experimentally, a spatial modelling approach might be taken using partial differential equations to solve for \( \text{Ca}^{2+} \). However, unless we are particularly interested in how \( \text{Ca}^{2+} \) waves arise then this detailed spatial model will be numerically costly and produce large amounts of data which are not required. A conclusion from this analysis is that a detailed model of \( \text{Ca}^{2+} \) waves is unlikely to result in improved results relating to the rate of fluid secretion. By far the most important signalling mechanism is found to be the mean \( \text{Ca}^{2+} \) concentration. Therefore it is our opinion than a compartment model using ordinary differential equations with homogeneous \( \text{Ca}^{2+} \) oscillations is sufficient when considering secretion rate as the most important model variable. Going further, if mean secretion rate is the only model concern and extreme computational constraints existed, perhaps considering a whole-organ model, it would even be possible to ignore all oscillations completely and just consider \( \text{Ca}^{2+} \) as a constant function of agonist stimulation.

On the topic of signal transduction we might hypothesise that the process of salivation does not require the complex signal encoding that is seen in some other cell types. It seems unlikely that we must signal for an exact saliva secretion rate. If accuracy in the flow rate is not required then an increase in mean \( \text{Ca}^{2+} \) might be all that is required as a signalling mechanism. We might further hypothesise that the other experimentally observed wave properties, such as oscillation frequency and wave speed, might be tuned to values which offer the maximum efficiency in secretion for a given mean \( \text{Ca}^{2+} \).
6.8 Acknowledgements

This work was supported by National Institutes of Health Grant R01-DE19245.

6.9 Appendix

6.9.1 Model equations

Fluid flow model

A summary of the main differential equations in the fluid flow model is included below. We use the following subscript notation with $[\text{Cl}]_i$, $[\text{Cl}]_l$ and $[\text{Cl}]_e$ denoting the Cl$^-$ concentration in the cytosol, lumen and interstitium respectively. For full details and parameter values see Palk et al. [76], we choose to not include apical K$^+$ channels in this analysis and therefore set $\alpha_K = 1$. Differential equations for the cytosolic concentrations are as follows,

\begin{align*}
\frac{d([\text{Cl}]_i w)}{dt} &= -\frac{I_{\text{Cl}}}{z_{\text{Cl}} F} + 2J_{\text{NKCC}}, \quad (6.1) \\
\frac{d([\text{Na}]_i w)}{dt} &= -3J_{\text{NaK}} + J_{\text{NKCC}}, \quad (6.2) \\
\frac{d([\text{K}]_i w)}{dt} &= 2J_{\text{NaK}} + J_{\text{NKCC}} - \frac{I_K}{z_K F}, \quad (6.3)
\end{align*}

where

$$I_{\text{tight}} = \frac{V_a - V_b}{R_{\text{tight}}}$$

is the current through the tight junction.

Luminal ionic concentrations are given by the following differential equations,

\begin{align*}
w_L \frac{d([\text{Na}]_l)}{dt} &= \frac{g_{\text{Na,Na}} I_{\text{tight}}}{z_{\text{Na}} F} - q_{\text{tot}} [\text{Na}]_l, \quad (6.4) \\
w_L \frac{d([\text{K}]_l)}{dt} &= \left(1 - \frac{g_{\text{Na,Na}}}{z_{\text{K}}} \right) \frac{I_{\text{tight}}}{F} - q_{\text{tot}} [\text{K}]_l, \quad (6.5) \\
w_L \frac{d([\text{Cl}]_l)}{dt} &= \frac{I_{\text{Cl}}}{z_{\text{Cl}} F} - q_{\text{tot}} [\text{Cl}]_l. \quad (6.6)
\end{align*}
Equations for the basal and apical membrane potentials are,

\[ C_m \frac{dV_b}{dt} = -I_K - FJ_{NaK} + I_{\text{tight}}, \]  
\[ (6.7) \]

\[ C_m \frac{dV_a}{dt} = -I_{Cl} - I_{\text{tight}}. \]  
\[ (6.8) \]

The fluid flow across the apical membrane is,

\[ q_a = RTL_{Pa} \left( [\text{Cl}]_l + [\text{Na}]_l + [\text{K}]_l - \left( [\text{Cl}]_i + [\text{Na}]_i + [\text{K}]_i + [\text{Ca}]_i + \frac{x}{w} \right) \right), \]

where \([\text{Ca}]_i\) is the mean \(\text{Ca}^{2+}\) concentration throughout the cytosol given by,

\[ [\text{Ca}]_i = \frac{C_a + C_b}{2}, \]

where \(C_a\) and \(C_b\) are the apical and basal \(\text{Ca}^{2+}\) concentrations respectively.

The basal fluid flow \(q_b\) and paracellular fluid flow \(q_{\text{tight}}\) are given respectively,

\[ q_b = RTL_{Pb} \left( [\text{Cl}]_i + [\text{Na}]_i + [\text{K}]_i + [\text{Ca}]_i + \frac{x}{w} - ([\text{Cl}]_e + [\text{Na}]_e + [\text{K}]_e) \right), \]

\[ q_{\text{tight}} = RTL_{Pt} \left( [\text{Cl}]_l + [\text{Na}]_l + [\text{K}]_l - ([\text{Cl}]_e + [\text{Na}]_e + [\text{K}]_e) \right). \]

The total secretion is then given by the sum of the paracellular and transcellular components,

\[ q_{\text{tot}} = q_a + q_{\text{tight}}. \]

The cell volume is governed by the balance of incoming and outgoing fluid flow,

\[ \frac{dw}{dt} = q_b - q_a. \]  
\[ (6.9) \]

Full details of the fluxes and parameters used can be seen in Palk et al. [76].

\textbf{Cl}^{−} \text{ channels}

For the analysis in Section 6.5 a steady-state model of Cl\textsuperscript− channel gating from Arreola et al. [2] is used (for details of the non steady-state model see Section 6.6). Here the Cl\textsuperscript− channel steady-state open probability is given as,

\[ P_{\text{Cl}} = \frac{1}{1 + K_2(K_1^2/C_a^2 + K_1/C_a + 1)}, \]
Model parameter values

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<td>( 6.8 \times 10^8 ) ohms</td>
<td>( g_{\text{t,Na}} )</td>
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</tbody>
</table>

<table>
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<th>Ionic valence</th>
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<tr>
<td>( z_{\text{Cl}} )</td>
<td>-1</td>
<td>( z_{\text{K}} )</td>
</tr>
<tr>
<td>( z_{\text{Na}} )</td>
<td>+1</td>
<td></td>
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<table>
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<th>Interstitial concentrations</th>
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<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>([\text{Cl}]_e)</td>
<td>102.6 mM</td>
<td>([\text{Na}]_e)</td>
<td>140.2 mM</td>
</tr>
</tbody>
</table>

Table 6.1: * from Arreola et al. [2], ** from Thompson and Begenisich [109], other parameters are physical constants or model fits chosen to give the correct steady state concentrations and membrane potentials.

Where \( C_a \) is the Ca\(^{2+}\) concentration at the apical membrane and

\[
K_1 = 214 \exp \left( \frac{-0.13 F V_a}{RT} \right) \text{nM},
\]

\[
K_2 = 0.58 \exp \left( \frac{-0.24 F V_a}{RT} \right).
\]

Here \( V_a \) is the membrane potential of the apical membrane. Total current through the Cl\(^-\) channels is then given by,

\[
I_{\text{Cl}} = g_{\text{Cl}} P_{\text{Cl}} (V_a - V_{\text{Cl}}),
\]

\( g_{\text{Cl}} \) is the maximum whole cell conductance, 31.4 nS found by Arreola et al. [2]. \( V_{\text{Cl}} \) is the Nernst potential given by,
\[
V_{\text{Cl}} = \frac{RT}{z_{\text{Cl}}F} \log \left( \frac{[\text{Cl}]_i}{[\text{Cl}]_e} \right),
\]

\(z_{\text{Cl}} = -1\) is the valence of \(\text{Cl}^-\), \(R = 8.315 \text{ J mol}^{-1} \text{ K}^{-1}\), \(T = 310 \text{ K}\) and \(F = 96490 \text{ C mol}^{-1}\).

**K\(^+\) channels**

We use the model of Takahata et al. [105]. The steady-state open probability of the K\(^+\) channel at the basal membrane is given as,

\[
P_K = \frac{1}{1 + (K_d/C_b)^nH},
\]

where \(C_b\) is the Ca\(^{2+}\) concentration at the basal membrane and \(nH = 2.54\) and \(K_d = 0.182 \mu\text{M}\). \(K_d\) is modified from the value found by Takahata et al. [105] of \(K_d = 0.43 \mu\text{M}\) to give a small open probability at steady state Ca\(^{2+}\) concentrations.

The current through the K\(^+\) channel at the basolateral membrane, \(I_K\), is given by,

\[
I_K = g_K P_K (V_b - V_K),
\]

where \(g_K\) is the maximum whole cell conductance of 14 nS, the value found by Thompson and Begenisich [109]. \(V_K\) is the Nernst potentials of the basolateral membrane given by,

\[
V_K = \frac{RT}{z_K F} \log \left( \frac{[\text{K}]_e}{[\text{K}]_i} \right),
\]

here \(z_K = +1\) is the valence of K\(^+\).

**Na\(^+\) - K\(^+\) - ATPase simplification**

As in Palk et al. [76] a simplified model of the Na\(^+\) - K\(^+\) - ATPase is used with the steady-state flux as follows,

\[
u_{\text{NaK}} = r \frac{[\text{K}]_e^2 [\text{Na}]_i^3}{[\text{K}]_e^2 + \alpha [\text{Na}]_i^3}.
\]  \hspace{1cm} (6.10)

With \(r = 1.305 \times 10^6 \text{ s}^{-1} \text{ mM}^{-3}\) and \(\alpha = 0.647 \text{ mM}^{-1}\). \(J_{\text{NaK}} = \alpha_{\text{NaK}} \nu_{\text{NaK}}\), where \(\alpha_{\text{NaK}} = 2.236 \times 10^{-17} \text{ mol}\) is the density of the Na\(^+\) - K\(^+\) - ATPase exchanger.
Na\textsuperscript{+} - K\textsuperscript{+} - 2Cl\textsuperscript{−} cotransporter simplification

As in Palk et al. [76] a simplified model of the Na\textsuperscript{+} - K\textsuperscript{+} - 2Cl\textsuperscript{−} cotransporter is used with the steady-state flux as follows,

\[ \nu_{\text{NKCC}} = r_{\text{NKCC}} \left( 1 - \alpha_1 [\text{Na}\textsubscript{i}] [\text{K}\textsubscript{i}]^2 [\text{Cl}\textsubscript{i}]^2 \right) / \left( K_{\text{NKCC}} + \alpha_2 [\text{Na}\textsubscript{i}] [\text{K}\textsubscript{i}] [\text{Cl}\textsubscript{i}]^2 \right). \]  

(6.11)

Where \( r_{\text{NKCC}} = 4.31 \text{ s}^{-1} \), \( \alpha_1 = 1.2755 \times 10^5 \), \( \alpha_2 = 3.7894 \times 10^4 \) and \( K_{\text{NKCC}} = 0.0282 \text{ mM} \). \( J_{\text{NKCC}} = \nu_{\text{NKCC}} \alpha_{\text{NKCC}} \) where \( \alpha_{\text{NKCC}} = 3.2 \times 10^{-17} \text{ mol} \) is the membrane density of the cotransporter.

6.9.2 Approximate analysis of model equations: synchronous Ca\textsuperscript{2+} waves produce a local maximum for fluid secretion

Here we seek to show that the fluid secretion model with steady-state ion channels seen in Section 6.3 has a maximum secretion rate when Ca\textsuperscript{2+} oscillations are synchronous at apical and basal membranes. In order to do this we must make some assumptions. First we make the assumption that the membrane potentials are at quasi-steady-state, which we can justify given the very small membrane capacitance \( C_m \). This gives,

\[ C_m \frac{dV_a}{dt} = -I_{\text{Cl}} - I_{\text{tight}} = 0 \]  

(6.12)

and

\[ C_m \frac{dV_b}{dt} = -I_K - FJ_{\text{Nak}} + I_{\text{tight}} = 0. \]  

(6.13)

Now we substitute the definitions for the currents, \( I_K \), \( I_{\text{Cl}} \) and \( I_{\text{tight}} \) into equations 6.12 and 6.13, giving,

\[ -g_{\text{cl}} P_{\text{cl}} (V_a - V_{\text{Cl}}) = (V_a - V_b) / R_{\text{tight}} \]

and

\[ g_k P_k (V_b - V_k) + FJ_{\text{Nak}} = (V_a - V_b) / R_{\text{tight}}. \]

If we solve both these equations simultaneously we can get expressions for the membrane potentials \( V_a \) and \( V_b \). During simulations it is found that, for near isosmotic fluid secretion, fluid flow is proportional to the current through the tight junction, see Maclaren et al. [63]. The tight junctional current is given by \( (V_a - V_b) / R_{\text{tight}} \), we use this
as follows,

$$\text{flow} \propto \frac{V_a - V_b}{R_{\text{tight}}} = \frac{P_{CL}P_K(V_{Cl} - V_k) + P_{CL}FJ_{Nak}}{P_K + P_KR_{\text{tight}}P_{CL} + P_{CL}}.$$ 

Here we have used the notations $P_{CL} = P_{cl}g_{Cl}$ and $P_K = P_kg_k$.

**Periodic functions**

We will make the assumption that during the course of one Ca$^{2+}$ wave both $P_{CL}$ and $P_K$ are periodic functions. We take this assumption further to make these both the same periodic function with a phase difference $\delta$. We also assume $V_{Cl}$ and $V_k$ stay approximately constant, a valid assumption if changes in ionic concentrations are small.

We then denote, $f(t) = \frac{1}{P_K(V_{Cl} - V_k)}$, $f(t + \delta) = \frac{1}{P_{CL}(V_{Cl} - V_k)}$, $\gamma = FJ_{Nak}$ and $A = \frac{R_{\text{tight}}}{(V_{Cl} - V_k)}$.

The expression for fluid flow then becomes,

$$\text{flow} \propto \frac{1 + \gamma f(t)}{f(t) + f(t + \delta) + A},$$

where $A$ and $\gamma$ are positive constants and $f(t)$ is any periodic function, period $T$. Now we would like to see how the average flow over the course of one period, $T$, depends on the phase difference $\delta$.

We define $I$ to be the total flow over a period, $T$,

$$I = \int_0^T \frac{1 + \gamma f(t)}{A + f(t) + f(t + \delta)} dt.$$

We would like to find when this expression has a maximum and minimum. Taking the derivative with respect to $\delta$,

$$\frac{\partial I}{\partial \delta} = -\int_0^T \frac{(1 + \gamma f(t))f'(t + \delta)}{(A + f(t) + f(t + \delta))^2} dt.$$ 

We predict a maximum at $\delta = 0$, so looking at the partial derivative here,

$$\frac{\partial I}{\partial \delta} \bigg|_{\delta=0} = -\int_0^T \frac{(1 + \gamma f(t))f'(t)}{(A + 2f(t))^2} dt = \frac{1}{2(A + 2f(t))} - \frac{\gamma}{4} \ln(2f(t) + A) - \frac{\gamma A}{4(2f(t) + A)} \bigg|_0^T.$$ 

As, $2f(0) = 2f(T)$, being a periodic function, this gives,

$$\frac{\partial I}{\partial \delta} \bigg|_{\delta=0} = 0,$$

and therefore a maximum or minimum occurs when there is no phase difference. To determine whether this is a maximum or minimum we look at the second derivative.
\[
\frac{\partial^2 I}{\partial \delta^2} \bigg|_{\delta=0} = - \int_0^T \frac{(1 + \gamma f(t)) f''(t)}{(A + 2f(t))^2} dt + 2 \int_0^T \frac{(1 + \gamma f(t))(f'(t))^2}{(A + f(t))^3} dt \equiv I_1 + I_2 + I_3 + I_4,
\]

where,

\[I_1 = \int_0^T \frac{-f''(t)}{(A + 2f(t))^2} dt,\]

\[I_2 = 2 \int_0^T \frac{(f'(t))^2}{(A + f(t))^3} dt,\]

\[I_3 = \int_0^T \frac{\gamma f(t)f''(t)}{(A + 2f(t))^2},\]

and

\[I_4 = \int_0^T \frac{2\gamma f(t)(f'(t))^2}{(A + 2f(t))^3}.\]

Evaluating the first integral, \(I_1\) using integration by parts,

\[I_1 = - \frac{f'(t)}{(A + 2f(t))^2} \bigg|_0^T - \int_0^T \frac{4(f'(t))^2}{(A + 2f(t))^3} dt = 0 - 4 \int_0^T \frac{(f'(t))^2}{(A + 2f(t))^3} dt.\]

Evaluating the integral \(I_3\) using integration by parts,

\[I_3 = \int_0^T \frac{-\gamma f(t)f''(t)}{(A + 2f(t))^2} = - \frac{\gamma f(t)f'(t)}{(A + 2f(t))^2} \bigg|_0^T + \int_0^T \frac{\gamma (f'(t))^2}{(A + 2f(t))^3} dt - \int_0^T \frac{4\gamma f(t)(f'(t))^2}{(A + 2f(t))^3} dt = 0 + \int_0^T \frac{\gamma (f'(t))^2}{(A + 2f(t))^2} dt - \int_0^T \frac{4\gamma f(t)(f'(t))^2}{(A + 2f(t))^3} dt.\]

Now,

\[\frac{\partial^2 I}{\partial \delta^2} \bigg|_{\delta=0} = - \int_0^T \frac{2(f'(t))^2}{(A + 2f(t))^2} dt - \int_0^T \frac{2\gamma f(t)(f'(t))^2}{(A + 2f(t))^2} dt + \int_0^T \frac{\gamma (f'(t))^2}{(A + 2f(t))^2} dt = -(2 - \gamma A) \int_0^T \frac{(f'(t))^2}{(A + 2f(t))^2} dt.\]

If we assume \(A\) is a positive constant and that \(f(t)\) is a positive function, both valid assumptions, then here we are taking the integral of an expression that is strictly positive. The sign of the second derivative is therefore determined by the expression \((2 - \gamma A)\). Looking back to the original notation, \(\gamma A = F J_{Nak} \frac{R_{\text{right}}}{(V_{\text{crit}} - V_0)}.\) Under physiological
condition the various terms are of the following magnitude,

\[(V_{Cl} - V_K) \sim O(10^{-2}), \]
\[R_{tight} \sim O(10^8), \]
\[FJ_{Nak} \sim O(10^{-12}). \]

Therefore \(\gamma A \sim O(10^{-2})\) and,

\[\frac{\partial^2 I}{\partial \delta^2} \bigg|_{\delta=0} = -(2 - \gamma A) \int_0^T \frac{(f'(t))^2}{(A + 2f(t))^3} dt < 0.\]

By proving the second derivative is negative we have shown that \(\delta = 0\) is a local maximum and thus there is a local maximum in secretion when \(Ca^{2+}\) waves are synchronous at the apical and basal membranes.
Chapter 7

A mathematical study to determine the role of IP$_3$ in HSY cell calcium oscillations

IP$_3$ and the IP$_3$R play a vital role in Ca$^{2+}$ signalling, providing the link between neuronal stimulation and the release of Ca$^{2+}$ from internal stores. As a consequence, the IP$_3$R is perhaps the most important element of a Ca$^{2+}$ model. When we constructed the Ca$^{2+}$ model of parotid acini seen in Chapter 4 we used the latest model of the IP$_3$R from Gin et al. [41]. Transitions between IP$_3$R states were rapid, with the receptor spending at most a few milliseconds in the open state. Oscillation of Ca$^{2+}$ in parotid acini are found to have a period of between 5-9 seconds [14]. With the large difference in timescales, the IP$_3$R kinetics were discounted from generating Ca$^{2+}$ oscillations. Instead, oscillations were assumed to be the result of Ca$^{2+}$ feedback on the IP$_3$ dynamics, where IP$_3$ oscillation were required for generating Ca$^{2+}$ oscillations.

A more recent study of the IP$_3$R by Siekmann et al. [90] resulted in a different receptor model. A six-state model is divided into two modes. Transitions between states of a given mode are rapid. The transitions between modes are slower, with the receptor spending up to 50 seconds in a given mode for certain Ca$^{2+}$ concentrations. These slow transition rates allow for the possibility of longer period oscillations being generated by IP$_3$R kinetics. In their experimental work on HSY cells, Tanimura et al. [107] argue that oscillations of IP$_3$ passively followed the Ca$^{2+}$ oscillations, and were not required for the generation of Ca$^{2+}$ oscillations. In the following chapter we will look at the role of IP$_3$ in HSY cell Ca$^{2+}$ oscillations. Our work will incorporate the latest Siekmann IP$_3$R model. What follows is a manuscript draft awaiting experimental validation from Akihiko Tanimura in the Department of Pharmacology, School of Dentistry at the University of
Chapter 7. A mathematical study to determine the role of IP$_3$ in HSY cell calcium oscillations

Hokkaido.
7.1 Abstract

Recent experimental results show coupled oscillations of Ca^{2+} and IP_3 in HSY cells, a salivary duct cell line. We present a mathematical model in which IP_3 oscillations are not required for the generation of Ca^{2+} oscillations. The model uses the latest single channel IP_3R data, and incorporates Ca^{2+} feedback on the production of IP_3. The inclusion of passive IP_3 oscillations is shown to increase the Ca^{2+} oscillation frequency range and be consistent with the experimental data. Long period oscillations, with a plateau between Ca^{2+} spikes, are hypothesised to be the result of rapid Ca^{2+} re-uptake and IP_3 degradation following a Ca^{2+} spike, followed by a period of slow Ca^{2+} release and IP_3 production. A number of experimental tests are proposed which could confirm this hypothesis. Numerical simulations predict an absence of long period oscillations in cells where an IP_3 buffer is introduced. Ca^{2+} and IP_3 photo-release is predicted to bring about a quick Ca^{2+} spike often followed by a delay before the return to regular oscillations. The period and amplitude of oscillations following a photo-release is unaffected.

7.2 Introduction

Oscillations in the concentration of free cytoplasmic Ca^{2+} have been found to exist in many cell types. These Ca^{2+} oscillations are important for a range of cellular functions such as muscle contraction, neuronal signalling, cell growth and saliva secretion. Several mechanisms have been proposed for the genesis of Ca^{2+} oscillations with detailed mathematical models used to explain the experimental data, see Dupont et al. [30] for a recent review. In many cell types the concentration of IP_3 is also found to oscillate. While the use of fluorescent Ca^{2+} indicators has allowed time-course measurements of Ca^{2+} to be taken since the 1990s, until recently it was not possible to measure inositol trisphosphate (IP_3) in the same manner and there has been debate around the function of IP_3 in Ca^{2+} oscillations. Recent experimental data from HSY cells produces time-course data for both Ca^{2+} and IP_3 [107].

HSY cells are a salivary duct cell line from parotid glands. The parotid gland is the largest of the major salivary glands, with duct cells responsible for modifying the ionic concentration of primary saliva after it has been secreted from the parotid acinar cells. Inadequate saliva secretion is a problem faced by many people with sufferers having difficulty speaking and eating and problems with dental cavities. A gene therapy targeting salivary duct cells has recently been shown to be an effective therapy for hyposalivation adding new importance to the understanding of duct cells [6].

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Models of Ca\(^{2+}\) dynamics are often divided into two classes \([47]\). In Class I models oscillations arise from Ca\(^{2+}\) feedback on the inositol trisphosphate receptor (IP\(_3\)R) dynamics. These Class I models should be capable of exhibiting oscillations in Ca\(^{2+}\) with constant IP\(_3\) concentration. In Class II models it is the feedback of Ca\(^{2+}\) on the IP\(_3\) production and/or degradation that is the cause of the oscillations. In a Class II model oscillations in IP\(_3\) are required to produce oscillations in Ca\(^{2+}\). The presence of both IP\(_3\) and Ca\(^{2+}\) oscillations in experimental data does not necessarily imply that a Class II mechanism is responsible for the oscillations. It is equally possible that a Class I mechanism is the cause of the Ca\(^{2+}\) oscillations, where IP\(_3\) oscillates passively due to Ca\(^{2+}\) feedback on IP\(_3\) production or degradation.

Tanimura et al. \([107]\) hypothesise that IP\(_3\) oscillations in HSY cells are passive reflections of the Ca\(^{2+}\) oscillations, predicting a Class I mechanism is responsible for Ca\(^{2+}\) oscillations. Using the latest data on the dynamics of the IP\(_3\)R, we present a quantitative realisation which is consistent with this hypothesis. Oscillation in IP\(_3\) are considered to be passive reflections of the Ca\(^{2+}\) concentration that enable a larger frequency range of Ca\(^{2+}\) oscillations to be observed. Sneyd et al. \([99]\) and Politi et al. \([82]\) propose experimental protocols using IP\(_3\) photo-release and IP\(_3\) buffering to gain an understanding of the Ca\(^{2+}\) oscillation mechanisms. We numerically predict the outcome of these experimental tests in HSY cells. We hope the confirmation of these predictions in physical experiments will later help to confirm the role of IP\(_3\) in HSY cell Ca\(^{2+}\) oscillations.

### 7.3 A mathematical model of HSY cell Ca\(^{2+}\) oscillations

#### 7.3.1 Modelling aims and considerations

Figure 7.1 shows the feedback mechanisms proposed in the generation of Ca\(^{2+}\) oscillations. Red arrows show forward feedback, where an increase in one variable positively affects the rate of change of the other. Blue arrows show negative feedback. In a Class I model Ca\(^{2+}\) opens the IP\(_3\)R at low concentrations but shuts the IP\(_3\)R at higher concentrations. A negative feedback loop between the IP\(_3\)R and cytosolic Ca\(^{2+}\) is able to produce oscillations at constant IP\(_3\) concentration. The bold arrows in Figure 7.1a show the necessary feedback mechanisms for Class I Ca\(^{2+}\) oscillations. In Class II models IP\(_3\) oscillations are required for Ca\(^{2+}\) oscillations. Here IP\(_3\) opens the IP\(_3\)R leading to a release of Ca\(^{2+}\). Ca\(^{2+}\) is known to influence both the production and degradation of IP\(_3\) \([19, 72]\). Gouzé \([43]\) show that monotone networks require a negative feedback loop
7.3. A mathematical model of HSY cell Ca\(^{2+}\) oscillations

for the existence of stable periodic orbits. Ca\(^{2+}\) models cannot be considered monotone networks due to the existence of positive and negative feedback between variables. Nevertheless, in Section 7.7.2 we present an argument that negative feedback, with Ca\(^{2+}\) causing IP\(_3\) degradation, is required for oscillations in a Class II model.

Where IP\(_3\) oscillations are observed experimentally we have three candidates to model the phenomena. The first candidate is a model where Class I mechanisms generate Ca\(^{2+}\) oscillations and IP\(_3\) oscillates passively due to Ca\(^{2+}\) feedback on the IP\(_3\) production rate. We shall call this Class I Positive. The second candidate, which we call Class I Negative, is where a Class I mechanism is responsible for Ca\(^{2+}\) oscillations and where IP\(_3\) oscillates passively due to Ca\(^{2+}\) feedback on the IP\(_3\) degradation rate. Finally, a Class II model could generate Ca\(^{2+}\) oscillations. Here oscillations in IP\(_3\) are required for Ca\(^{2+}\) oscillations, with Ca\(^{2+}\) feedback on the degradation of IP\(_3\). Where we use the term Class II model in the remainder of article we necessarily imply a Class II Negative model according to the argument in Section 7.7.2.

Experimental data from HSY cells show IP\(_3\) oscillations have the same period as the Ca\(^{2+}\) oscillations. Upon stimulation with agonist, the concentration of Ca\(^{2+}\) increases first with IP\(_3\) following. During oscillations the peak in the Ca\(^{2+}\) concentration always precedes the peak of IP\(_3\). The rise of Ca\(^{2+}\) before IP\(_3\) suggests that Ca\(^{2+}\) feedback on IP\(_3\) production is the dominant feedback mechanism. Were a negative feedback to be dominant, with Ca\(^{2+}\) primarily affecting IP\(_3\) degradation, we would expect a rise in Ca\(^{2+}\) concentration to cause a fall in the IP\(_3\) concentration. We present a Class I Positive model of Ca\(^{2+}\) dynamics, including the latest model of the IP\(_3\)R from single data [90], to quantitatively test the predictions of Tanimura et al. [107]. Later in Section 7.5 we compare results with a Class II model and show a Class II model is inconsistent with experimental data from HSY cells.

In constructing our model there are several experimental phenomena that we must consider. Firstly, Ca\(^{2+}\) peaks are seen to precede IP\(_3\) peaks, with both concentrations oscillating with the same period. Next, a large range of oscillations periods, from 25 to 120 seconds, are seen in HSY cells. We look for the model to reproduce and explain this. Finally, in contrast to HSG cells (a submandibular cell line), HSY cells show sustained oscillations of Ca\(^{2+}\) in the absence of external Ca\(^{2+}\) [59]. This constrains the model such that oscillations must not require the influx of external Ca\(^{2+}\). In Section 7.4.5 we discuss the effect of plasma membrane Ca\(^{2+}\) fluxes.

An increasingly studied branch of Ca\(^{2+}\) signalling is that of Ca\(^{2+}\) sparks, quarks and puffs [18]. Ca\(^{2+}\) sparks are releases of Ca\(^{2+}\) that are highly localised in both space and time. The modelling of Ca\(^{2+}\) sparks involves stochastically simulating of small numbers
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Figure 7.1: Class I and Class II feedback mechanisms. Red arrows show positive feedback and blue, thin, arrows show negative feedback. Ca$^{2+}$ opens the IP$_3$R at low Ca$^{2+}$ concentration (positive feedback) and closes the receptor at high Ca$^{2+}$ concentration (negative feedback). With the IP$_3$R open the concentration of cytosolic Ca$^{2+}$ increases, a positive feedback. Experimentally, both positive and negative feedback of Ca$^{2+}$ on the IP$_3$ dynamics are found to exist by affecting either the IP$_3$ production or degradation. IP$_3$ has a positive affect on the IP$_3$R, increasing the receptors open-probability. Bold arrows show the feedback mechanisms that are strictly necessary for Ca$^{2+}$ oscillations in a given model, with faded arrows showing feedback mechanisms that may exist, but are not responsible for the Ca$^{2+}$ oscillations.

of Ca$^{2+}$ release channels. Data from HSY cells shows regular global Ca$^{2+}$ oscillations and this stochastic approach will not be taken here. Instead, we assume a large density of Ca$^{2+}$ release channels and a deterministic model is used. Our model is compartmental, with Ca$^{2+}$ considered either stored in the endoplasmic reticulum (ER), in the cytosol or external to the cell. As previously mentioned, experimental data shows that Ca$^{2+}$ oscillations in HSY cells do not require external Ca$^{2+}$ and the oscillations must therefore be caused by the periodic release and re-uptake of Ca$^{2+}$ from the ER.

Class I model of Ca$^{2+}$ dynamics

The Class I model presented here is an adaptation of compartmental Ca$^{2+}$ models seen previously in Gin et al. [40] and Palk et al. [76] with variables, $C$, the Ca$^{2+}$ concentration in the cytosol and $C_{ER}$, the Ca$^{2+}$ concentration in the ER. Ca$^{2+}$ is released from the ER into the cytosol via the IP$_3$R, the ryanodine receptor (RyR) and a leak term. Re-uptake of Ca$^{2+}$ into the ER is via the sarco/endoplasmic reticulum Ca$^{2+}$-ATPase (SERCA). A schematic of the Ca$^{2+}$ model is shown in Figure 7.2.

When stimulated with an agonist cells generate phospholipase C (PLC) which increases the production of IP$_3$. The increased IP$_3$ concentration opens the IP$_3$Rs releasing Ca$^{2+}$ from the ER into the cytosol. High cytosolic Ca$^{2+}$ closes the IP$_3$Rs, re-uptake of Ca$^{2+}$ occurs and cytosolic Ca$^{2+}$ is reduced. When the Ca$^{2+}$ concentration is reduced sufficiently the IP$_3$Rs re-open and more Ca$^{2+}$ is released. Through this feedback mechanism
7.3. A mathematical model of HSY cell Ca\(^{2+}\) oscillations

periodic oscillations in cytosolic Ca\(^{2+}\) can occur.

Figure 7.2: A schematic of the Class I HSY cell model. IP\(_3\) production is agonist induced and Ca\(^{2+}\) dependent. Release of Ca\(^{2+}\) from the ER is via the IP\(_3\)R, a RyR and a leak term. Re-uptake is via a SERCA. An agonist dependent influx of Ca\(^{2+}\), J\(_{in}\), and Ca\(^{2+}\) dependent pump, J\(_{pm}\), are present on the plasma membrane. Solid black arrows denote Ca\(^{2+}\) fluxes, dotted arrows show feedback mechanisms with grey arrows showing the IP\(_3\) production and degradation.

We model the Ca\(^{2+}\) fluxes as follows, with the IP\(_3\)R function given in the later Section 7.3.3. The re-uptake of Ca\(^{2+}\) by the SERCA is modelled by a Hill-function,

\[
J_{SERCA} = \frac{V_{SERCA} C^{3}}{K_{SERCA}^{3} + C^{3}},
\]

where \(V_{SERCA}\) is the SERCA density and \(K_{SERCA}\) is the half-maximal activation. Similarly we use a Hill-function to model the ryanodine receptor with the RyR flux given by,

\[
J_{RyR} = V_{RyR} \frac{C^{3}}{K_{RyR}^{3} + C^{3}}(C_{er} - C),
\]

where \(V_{RyR}\) is the density of the RyRs and \(K_{RyR}\) is the half-maximal activation. Finally we allow for a leak of Ca\(^{2+}\) from the ER given by,

\[
J_{leak} = k_{er}(C_{er} - C).
\]

The inward Ca\(^{2+}\) flux across the plasma membrane is given as a leak term plus an
 agonist dependent influx,

\[ J_{\text{in}} = \alpha_1 + \alpha_2 \mu, \]

where \( \mu \) is the concentration of applied agonist. The outward flux is modelled with a Hill-function,

\[ J_{\text{pm}} = V_{\text{pm}} \frac{C^2}{C^2 + K_{\text{pm}}^2}. \]

The size of the plasma membrane fluxes, in proportion to the fluxes from the ER, is given by the small parameter, \( \epsilon \). The concentration of Ca\(^{2+} \) in the cytosol is then given by,

\[ \frac{dC}{dt} = J_{\text{IPR}} + J_{\text{RyR}} + J_{\text{leak}} - J_{\text{SERCA}} + \epsilon (J_{\text{in}} - J_{\text{pm}}). \]

The total Ca\(^{2+} \) in the cell changes as a result of fluxes across the plasma membrane,

\[ \frac{dC_t}{dt} = \epsilon (J_{\text{in}} - J_{\text{pm}}). \]

The ER is estimated to have a volume around five times smaller than that of the cytosol. Any Ca\(^{2+} \) ions that are contained in the cell, but not contained in the cytosol must be in the ER. The Ca\(^{2+} \) concentration in the ER is thus given by,

\[ C_{\text{er}} = \gamma (C_t - C), \]

where \( \gamma \) is the ratio of cytosolic to ER volume. In some cases we shall assume the plasma membrane fluxes are negligible. Here we take the limit \( \epsilon \rightarrow 0 \) and the total concentration in the cell, \( C_t \), is constant. All parameters for the Class I model are given in the Table 7.1.

<table>
<thead>
<tr>
<th>Parameter values of the Class I model</th>
</tr>
</thead>
<tbody>
<tr>
<td>( V_{\text{SERCA}} ) 3800 nM/s</td>
</tr>
<tr>
<td>( V_{\text{RyR}} ) 0.033 s(^{-1} )</td>
</tr>
<tr>
<td>( k_{\text{ER}} ) 1.9673 ( \times 10^{-5} ) s(^{-1} )</td>
</tr>
<tr>
<td>( \alpha_1 ) 0.04988 nM/s</td>
</tr>
<tr>
<td>( V_{\text{pm}} ) 20 nM/s</td>
</tr>
<tr>
<td>( \gamma ) 5.405</td>
</tr>
<tr>
<td>( k_{\text{plc}} ) 1500 nM</td>
</tr>
<tr>
<td>( \tau_p ) 1</td>
</tr>
<tr>
<td>( \psi_1 ) 287 nM s(^{-1} )</td>
</tr>
<tr>
<td>( \psi_2 ) 400 nM s(^{-1} )</td>
</tr>
</tbody>
</table>

Table 7.1: All parameters are model fits
7.3. A mathematical model of HSY cell Ca\(^{2+}\) oscillations

### 7.3.2 IP\(_3\) equations

We present a Class I Positive model which assumes IP\(_3\) production is an increasing function of the Ca\(^{2+}\) and agonist concentrations. The IP\(_3\) production rate is given by,

\[
V_{\text{plc}} = v_{\text{plc}}(\mu) \left( \frac{C^2}{C^2 + K_{\text{plc}}^2} \right),
\]

where \(v_{\text{plc}}(\mu)\) is the agonist dependent production of IP\(_3\). May et al. [67] find the production of IP\(_3\) increases almost exponentially at low levels before levelling off at higher applied agonist concentrations. We model this as follows,

\[
v_{\text{plc}}(\mu) = \psi_1 \frac{\mu^5}{\kappa_1^5 + \mu^5} + \psi_2 \frac{\mu^4}{\mu^4 + \kappa_2^4},
\]

where \(\mu\) is the applied agonist concentration. The degradation of IP\(_3\) is assumed to be constant,

\[
V_{\text{deg}} = r_{\text{deg}} P.
\]

The IP\(_3\) concentration changes as a result of the production and degradation of IP\(_3\),

\[
\frac{dP}{dt} = \tau_p (V_{\text{plc}} - V_{\text{deg}}).
\]

The speed of the IP\(_3\) dynamics is given by the parameter \(\tau_p\). When \(\tau_p\) is large the IP\(_3\) dynamics are fast. If we take the limit \(\tau_p \to 0\) then the IP\(_3\) concentration stays constant.

### 7.3.3 A model of the IP\(_3\)R

In constructing our mathematical model of Ca\(^{2+}\) oscillations we use the latest single-channel model of the IP\(_3\)R from Siekmann et al. [90]. Here the IP\(_3\)R is divided into two modes, park and drive, Figure 7.3. In the park mode the receptor opens rarely and the Ca\(^{2+}\) release from the ER is small. In the drive mode the receptor is predominantly open and large Ca\(^{2+}\) release occurs. In each mode transitions between states are fast and independent of the Ca\(^{2+}\) concentration, the rates constants are shown in Table 7.2.

The transition rates between park and drive are Ca\(^{2+}\) dependent and fitted as follows,

\[
q_{24} = a_{24} - \left( \frac{V_{24} C_m^{m_1}}{k_{24}^{m_1} + C_m} \right) + \left( \frac{V_{n24} C_m^{m_2}}{k_{n24}^{m_2} + C_m} \right),
\]

\[
q_{42} = a_{42} + \left( \frac{V_{42} C_m^{m_1}}{k_{42}^{m_1} + C_m} \right) - \left( \frac{V_{n42} C_m^{m_2}}{k_{n42}^{m_2} + C_m} \right).
\]
Chapter 7. A mathematical study to determine the role of IP$_3$ in HSY cell calcium oscillations

Figure 7.3: The six-state Siekmann IP$_3$R model divided into two ‘modes’, park and drive. Figure reproduced from Siekmann et al. [90].

<table>
<thead>
<tr>
<th>Siekmann IP$_3$R rate constants</th>
</tr>
</thead>
<tbody>
<tr>
<td>$q_{12}$ 1.24 ms$^{-1}$</td>
</tr>
<tr>
<td>$q_{23}$ 0.003 ms$^{-1}$</td>
</tr>
<tr>
<td>$q_{26}$ 10.5 ms$^{-1}$</td>
</tr>
<tr>
<td>$q_{45}$ 0.011 ms$^{-1}$</td>
</tr>
</tbody>
</table>

Table 7.2: Rate constants of the Siekmann IP$_3$R model

<table>
<thead>
<tr>
<th>Park/drive transition parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{24}$ 300 s$^{-1}$</td>
</tr>
<tr>
<td>$V_{n_{24}}$ 300 s$^{-1}$</td>
</tr>
<tr>
<td>$m_1$ 6</td>
</tr>
<tr>
<td>$a_{24}$ 300 s$^{-1}$</td>
</tr>
<tr>
<td>$V_{42}$ 30 s$^{-1}$</td>
</tr>
<tr>
<td>$V_{n_{42}}$ 30 s$^{-1}$</td>
</tr>
<tr>
<td>$n_1$ 3</td>
</tr>
<tr>
<td>$a_{42}$ 0.05 s$^{-1}$</td>
</tr>
</tbody>
</table>

Table 7.3: Fitted parameters of the mode transitions rates $q_{24}$ and $q_{42}$.

We have altered the mode transition rates from those in Siekmann et al. [90] in order to better fit the HSY cell experimental data. Parameters for the mode transitions rates are shown in Table 7.3. The experimental data shows that transitions within a park or
drive mode are rapid. In comparison the rate transitions between park and drive are slow. We make the modelling assumption that the transitions in either park or drive happen instantaneously and the park and drive modes are at steady-state. We write a differential equation for the fraction of receptors in the drive mode, $D$.

$$\frac{dD}{dt} = q_{42}(1 - D) - q_{24}D.$$  

A given IP$_3$R must either be in park or drive, therefore the fraction of receptors in park is given by $1 - D$. The steady-state open probability of the drive mode is given by,

$$O_D = \frac{q_{12}q_{32}q_{26}}{q_{12}q_{32}q_{26} + q_{62}q_{23}q_{12} + q_{62}q_{32}q_{12} + q_{62}q_{32}q_{21}},$$  

with the steady-state open probability of the park mode given by,

$$O_P = \frac{q_{45}}{q_{45} + q_{54}}.$$  

We assume the flux through the IP$_3$Rs is given by the fraction of IP$_3$Rs in the drive mode multiplied by the open probability of this mode, plus the remaining fraction of receptors in the park mode multiplied by the park mode open probability. The IP$_3$ dependence of the IP$_3$R is incorporated using a Hill function. The receptor flux is also proportional to the difference in between the ER and cytosolic Ca$^{2+}$ concentration. The whole cell flux through the IP$_3$Rs is given by,

$$J_{IPR} = k_{IPR}(O_D D + O_P (1 - D))(C_{er} - C)\left(\frac{P}{K_P + P}\right),$$

where $k_{IPR}$ is the density of the IP$_3$Rs.

7.4 Model results

In Figure 7.4a we simulate the application of three different concentrations of agonist, looking to reproduce the experimental results of Tanimura et al. [107]. At low agonist concentrations long period oscillations are seen, with the period reducing as the agonist concentration is increased. These results can be compared to the experimental results shown in Figure 7.4b. The model behaviour agrees well with the experimental data. Ca$^{2+}$ and IP$_3$ increases slowly before spiking, with the peak of the Ca$^{2+}$ spikes occurring just before the peak in IP$_3$. 

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Figure 7.4: The Class I model (a) is compared with experimental data (b). In (a) Ca\(^{2+}\) oscillations are followed with passive IP\(_3\) oscillations. The application of agonist is simulated with \(\mu = 4\) mM for \(0 < t < 800\) s, \(\mu = 7\) mM for \(800 < t < 1100\) s and \(\mu = 30\) mM for \(1100 < t < 1200\) s. IP\(_3\) oscillations are shown with black (dash-dot) lines with Ca\(^{2+}\) oscillations in red (solid) lines. (b) Experimental Ca\(^{2+}\) and IP\(_3\) oscillations in HSY cells stimulated with ATP. Ca\(^{2+}\) oscillations (red) can be seen to peak just before IP\(_3\) oscillations (black). Experimental trace reproduced from Tanimura et al. [107].

7.4.1 Ca\(^{2+}\) feedback on IP\(_3\) production increases the period range of Ca\(^{2+}\) oscillations

In Figure 7.5 the period of the Ca\(^{2+}\) oscillations is plotted versus the agonist induced IP\(_3\) production rate \(v_{\text{plc}}\). We investigate how the strength of Ca\(^{2+}\) feedback on the IP\(_3\) production rate affects the range of oscillation periods. We assume plasma membrane
Figure 7.5: Ca\(^{2+}\) oscillation period plotted against the agonist induced IP\(_3\) production \(v_{plc}\). Ca\(^{2+}\) feedback on IP\(_3\) production increases the frequency range. When there is no Ca\(^{2+}\) feedback on IP\(_3\) production, \(k_{plc} = 0\) nM the period ranges between 17 and 35 seconds. As \(k_{plc}\) increases a larger range of oscillation period is observed with \(k_{plc} = 700\) nM giving a maximum oscillation period of around 67 seconds. \(k_{plc} = 1500\) nM gives a very large range of oscillation periods.

Ca\(^{2+}\) fluxes are negligible taking the limit \(\epsilon \to 0\) with \(C_t = 1.855 \times 10^4\) nM. When \(k_{plc} = 0\) the production of IP\(_3\) is not dependent on Ca\(^{2+}\) and the IP\(_3\) concentration is a constant function of the applied agonist. Here only a small range of high frequency oscillations are seen. When the Ca\(^{2+}\) feedback is increased, by increasing \(k_{plc}\), we see a much larger range of oscillation frequencies.

If we consider a Ca\(^{2+}\) oscillation in three phases we can explain how Ca\(^{2+}\) feedback on the production of IP\(_3\) enables long period Ca\(^{2+}\) oscillations to be generated. We shall call phase one, where the Ca\(^{2+}\) spike is just commencing, the upstroke. Here the IP\(_3\)Rs are open, the Ca\(^{2+}\) concentration increases quickly with IP\(_3\) following passively. In phase two, the downstroke, Ca\(^{2+}\) has reached its peak. With the very high concentration of Ca\(^{2+}\) the IP\(_3\)Rs are predominantly in the park mode, Ca\(^{2+}\) release is slow and Ca\(^{2+}\) re-uptake into the ER occurs through the SERCA. The rate of SERCA pumping is dependent on the cytosolic Ca\(^{2+}\) concentration. When Ca\(^{2+}\) levels are high the re-uptake is fast. In this second phase the Ca\(^{2+}\) drops rapidly with the IP\(_3\) concentration following. In phase three a low plateau occurs whilst Ca\(^{2+}\) and IP\(_3\) concentrations are both small. During this phase the IP\(_3\)Rs stay shut due to the low IP\(_3\) concentration. The cytosolic Ca\(^{2+}\)
increases slowly with little Ca\(^{2+}\) re-uptake but also very limited Ca\(^{2+}\) release. As the Ca\(^{2+}\) concentration slowly increases so does the IP\(_3\), before reaching a threshold value and another spike occurs.

During an upstroke, Ca\(^{2+}\) and IP\(_3\) positively affect each other. As we increase the Ca\(^{2+}\) feedback on IP\(_3\) production we increase this positive feedback, increasing the amplitude of the Ca\(^{2+}\) oscillations. A large peak in Ca\(^{2+}\) will cause a very fast re-uptake of Ca\(^{2+}\) into the ER. Therefore a large spike is followed by a descent to a low trough. The lower the Ca\(^{2+}\) and IP\(_3\) concentrations fall after the spike the longer an oscillation spends in the plateau and the longer the period of the oscillation.

### 7.4.2 Modelling the effect of IP\(_3\) buffer on Ca\(^{2+}\) oscillations

Experimental evidence from the HSY cells shows IP\(_3\) peaks occur very quickly after Ca\(^{2+}\) peaks, as shown in Figure 7.4b. A change in Ca\(^{2+}\) is followed rapidly by a change in IP\(_3\) suggesting the IP\(_3\) dynamics are fast. Class I models are capable of exhibiting Ca\(^{2+}\) oscillations with constant IP\(_3\) concentration. However, as we have just seen in Section 7.4.1, oscillating IP\(_3\) with Ca\(^{2+}\) feedback on IP\(_3\) production facilitates a large range of oscillation periods. We investigate how the speed of the IP\(_3\) dynamics affects the Ca\(^{2+}\) oscillation period.

In Figure 7.6 we plot the oscillation period against the agonist induced IP\(_3\) production \(v_{\text{plc}}\). When the IP\(_3\) dynamics are fast, such as with \(\tau_p = 1\), the Ca\(^{2+}\) feedback on the IP\(_3\) production is able to produce a large range of frequencies. When the IP\(_3\) dynamics are slower a smaller range of frequencies is predicted.

When the IP\(_3\) dynamics are fast, the fast reduction of Ca\(^{2+}\) during a downstroke is matched passively by IP\(_3\). This causes both concentrations to drop to low levels and a large time to occur in the plateau phase before the following spike. If the IP\(_3\) dynamics are slower there will be a lag between a change in Ca\(^{2+}\) and a change in IP\(_3\). Following a spike, Ca\(^{2+}\) will fall rapidly whilst the IP\(_3\) concentration stays high. This higher IP\(_3\) concentration allows the release of Ca\(^{2+}\) through the IP\(_3\)R and the cytosolic concentration of both Ca\(^{2+}\) and IP\(_3\) do not fall to such low levels. When the IP\(_3\) dynamics are slower, the oscillation minimum is increased and, subsequently, longer period oscillation are not observed.

The addition of an IP\(_3\) buffer will slow the IP\(_3\) dynamics of the cell. Politi et al. [82] show that the experimental addition of an IP\(_3\) buffer can be represented by reducing the IP\(_3\) time-constant, \(\tau_p\), in the model. We simulate Ca\(^{2+}\) oscillations in an IP\(_3\) buffered cell to establish whether it is indeed the fast IP\(_3\) dynamics, with Ca\(^{2+}\) feedback on IP\(_3\) production, that are responsible for generating the long period oscillations of Ca\(^{2+}\) in
7.4. Model results

Figure 7.6: IP₃ dynamics must be fast to passively increase the frequency range of Ca²⁺ oscillations. We see that when the IP₃ dynamics are slow $\tau_p = 0.1$ and $\tau_p = 0.5$ only a small range of frequencies is observed. As $\tau_p$ is increased to 1 we see a large range of oscillation frequencies. All results are simulated with $k_{plc} = 1500$ nM.

When we simulate the application of three different agonist concentrations in an IP₃ buffered cell (with $\tau_p = 0.1$) we see a large delay before the onset of oscillations at low agonist concentrations (Figure 7.7). The slower IP₃ dynamics in an IP₃ buffered cell reduce the agonist dependent production of IP₃. This slow IP₃ production rate causes a delay before the onset of oscillations. Once Ca²⁺ oscillations occur, the period is seen to be shorter that in the non-buffered simulations shown in Figure 7.4a. The oscillation period for both low and medium agonist levels ($\mu = 4$ mM and $\mu = 7$ mM) are similar. Oscillations do not persist for the larger applied agonist of $\mu = 30$ mM.

We make the prediction that the introduction of an IP₃ buffer to the HSY cells will both show an absence of very long period oscillations and a delay before the onset of the first periodic solution.

7.4.3 Modelling Ca²⁺ photo-release during sustained Ca²⁺ oscillations

Sneyd et al. [99] present an experimental test to distinguish Class I and Class II models
Chapter 7. A mathematical study to determine the role of IP$_3$ in HSY cell calcium oscillations

Figure 7.7: Ca$^{2+}$ and IP$_3$ oscillations simulated in an IP$_3$ buffered cell with $\tau_p = 0.1$. Applied agonist $\mu = 4$ mM for $0 < t < 800$ s, $\mu = 7$ mM for $800 < t < 1100$ s and $\mu = 30$ mM for $1100 < t < 1200$. IP$_3$ oscillations shown with black (dash-dot) lines and Ca$^{2+}$ oscillations with red (solid) lines.
7.4. Model results

Based on the photo-release of IP$_3$ and Ca$^{2+}$ during sustained Ca$^{2+}$ oscillations. Here a delay or change in frequency of Ca$^{2+}$ oscillations following photo-release is used to determine the active oscillation mechanism. Further work by Harvey et al. [47] demonstrated that the test is not as easy to interpret as the authors claim. We aim to use photo-release, not to distinguish between Class I and Class II, but to understand how IP$_3$ oscillations facilitate long period Ca$^{2+}$ oscillations.

In Section 7.4.1 we hypothesise that the long plateau phase between Ca$^{2+}$ spikes is due to the limited Ca$^{2+}$ release from the ER thanks to low IP$_3$ concentration, and a low rate of IP$_3$ production as a consequence of the low cytosolic Ca$^{2+}$ concentration. Here we make predictions regarding the photo-release of Ca$^{2+}$ in HSY cells during this plateau phase. In the model we define the photo-release, $s(t)$ as,

$$s(t) = MH(t - t_{\text{pulse}})H(t_{\text{pulse}} + t_{\text{width}} - t),$$

where $H$ is a heaviside function and M is the photo-release (or pulse) strength. For all of the following we set $t_{\text{pulse}} = 300$ s and $t_{\text{width}} = 3$ s. The photo-release term is then added to the differential equation for Ca$^{2+}$ such that,

$$\frac{dC}{dt} = J_{\text{IPR}} + J_{\text{RyR}} + J_{\text{leak}} - J_{\text{SERCA}} + \epsilon (J_{\text{m}} - J_{\text{pm}}) + s(t).$$

Figure 7.8: The Class I Positive model is pulsed with a 3 s small (a) and medium (b) Ca$^{2+}$ pulse at $t = 300$ s. The photo-release of Ca$^{2+}$ directly causes a Ca$^{2+}$ spike. After this a latency is observed before the next spike, this is particularly noticeable for $M = 1200$. Following this the oscillations continue as before the Ca$^{2+}$ pulse. The period and amplitude of subsequent Ca$^{2+}$ spikes is not affected.

When Ca$^{2+}$ is pulsed between Ca$^{2+}$ spikes, the increased Ca$^{2+}$ causes an increase in
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The Ca$^{2+}$ spike induced by the photo-release will often have a larger amplitude than the regular Ca$^{2+}$ peaks. The increased peak of Ca$^{2+}$ is followed by a large re-uptake of Ca$^{2+}$ through the SERCA and a fast degradation of IP$_3$. Therefore the large peak is followed by a trough which is lower than during the regular oscillations. As both the Ca$^{2+}$ and IP$_3$ concentrations are low there is limited release of Ca$^{2+}$ and a delay occurs before the next spike. In mathematical terms, the Ca$^{2+}$ photo-release causes the following orbit to pass very close to a saddle-node causing a latency. We define the latency of the system to be the time between the spike of Ca$^{2+}$ following the photo-release and the resumption of regular Ca$^{2+}$ oscillations.

This latency can be easily observed in Figure 7.8b where a pulse strength of $M = 1200$...
is used. In contrast, a pulse strength of $M = 400$ shows regular period oscillations return with a negligible latency (Figure 7.8a). The size of the latency depends on the size of the Ca$^{2+}$ pulse. In Figure 7.9 the latency is plotted against the size of the Ca$^{2+}$ pulse. A maximum latency occurs when $M = 1150$. Here the Ca$^{2+}$ photo-release causes the resulting orbit to pass very close to a saddle-node where the Ca$^{2+}$ and IP$_3$ dynamics are very slow. The orbit spends a long time in the plateau phase before the Ca$^{2+}$ concentration reaches its critical value and initiates another spike.

Beyond $M = 1150$ the latency is reduced as the pulse-strength increases. If the Ca$^{2+}$ photo-release is very large the very large Ca$^{2+}$ release that follows causes a large Ca$^{2+}$ re-uptake. This happens very quickly and the Ca$^{2+}$ returns to low levels before the IP$_3$ has time to fully degrade. Whilst the degradation continues the IP$_3$ concentration is able to open some IP$_3$Rs and the trough that occurs is not as low as found with a medium sized pulse.

We predict that a Ca$^{2+}$ photo-release in HSY cells will always show an immediate spike in Ca$^{2+}$ and IP$_3$. A latency before the return of oscillations is expected for some photo-release strengths. Unlike results seen in Sneyd et al. [99], no long term effect was seen on the oscillation period or amplitude following a photo-release.

### 7.4.4 Modelling IP$_3$ photo-release during sustained Ca$^{2+}$ oscillations

Similar effects can be seen with the photo-release of IP$_3$ as were seen with the photo-release of Ca$^{2+}$. This time the differential equation for IP$_3$ is modified,

$$\frac{dP}{dt} = \tau_p (V_{plc} - V_{deg}) + s(t),$$

where $s(t)$ is the photo-release as previously defined.

In Figure 7.10 a photo-release of IP$_3$ is simulated at 300 s shortly after a Ca$^{2+}$ spike. Two different strength pulses are both seen to initiate an immediate Ca$^{2+}$ spike. For a pulse strength of $M = 400$ a latency is observed before the return to regular oscillations (Figure 7.10a). This is in contrast to Figure 7.10b where $M = 1200$ and the return to regular oscillations occurs very quickly. Here the latency is shorter than the period of the regular Ca$^{2+}$ oscillations. For $M = 1200$ a large IP$_3$ photo-release causes a large spike of both Ca$^{2+}$ and IP$_3$, the increased Ca$^{2+}$ concentration quickly shutting the IP$_3$Rs. The subsequent fast re-uptake of Ca$^{2+}$ occurs leaving some IP$_3$ yet to degrade. The non-degraded IP$_3$ is able to initialise another Ca$^{2+}$ spike and advance the return of regular oscillations.
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Figure 7.10: The Class I model is pulsed with a 3 s small (a) and medium (b) IP$_3$ pulse at $t = 300$ s. The pulse of IP$_3$ is seen to bring about the next Ca$^{2+}$ spike very quickly. Following this spike the oscillations are seen to return. In (a) with $M = 400$ a latency occurs before regular oscillations resume. In contrast for $M = 1200$ (b), the following spike occurs after a time much shorter than the oscillation period. Regular oscillations in both cases continue as before the photo-release with the period and amplitude unaffected.

Figure 7.11: The latency following a photo-release is plotted agonist the strength of the IP$_3$ photo-release. The photo-release is simulated with a 3 s pulse at $t = 300$ s, shortly after a preceding peak. At an optimal pulse of $M = 400$ the maximum delay is observed before the return to regular oscillations. At this value the IP$_3$ photo-release causes the following trajectory to pass very close to a saddle-node and as such travel very slowly before restoring oscillation to their normal. For $M > 800$ regular oscillations are advanced with the latency shorter than the oscillation period.
7.5. A comparison to active IP$_3$ oscillations in a Class II model

In Figure 7.11 the latency is plotted against the size of the IP$_3$ photo-release. For a critical range of IP$_3$ photo-release strength a large latency can be observed, largest for $M = 400$. For $M > 800$ the return to regular oscillations is advanced and occurs faster than the regular period of oscillations.

As with the Ca$^{2+}$ photo-release we predict a photo-release of IP$_3$ between spikes will bring about a new spike almost immediately. Small IP$_3$ photo-release shows a latency before regular oscillations resume. For large IP$_3$ photo-release regular oscillations are predicted to resume quickly. No long term effects are expected on the oscillation frequency or amplitude.

7.4.5 The importance of plasma membrane Ca$^{2+}$ fluxes

The Class I model we have presented generates cytosolic Ca$^{2+}$ oscillations with the periodic release and re-uptake of Ca$^{2+}$ across the ER membrane. It is theoretically possible for periodic influx and efflux of Ca$^{2+}$ across the plasma membrane to generate Ca$^{2+}$ oscillations in a similar manner. Several mathematical models of Ca$^{2+}$ oscillations are reliant on this periodic influx and efflux [27, 29, 40, 42, 99]. The Ca$^{2+}$-free test, where Ca$^{2+}$ is removed from the external solution surrounding the cells is useful to determine the importance of external Ca$^{2+}$ fluxes. With a Ca$^{2+}$-free solution we expect no Ca$^{2+}$ influx into the cell. This can be represented in the model by setting $J_{\text{in}} = 0$. If Ca$^{2+}$ oscillations continue under Ca$^{2+}$-free conditions we can discount plasma membrane fluxes as the generator of the Ca$^{2+}$ oscillations.

In Figure 7.12a we can see that Ca$^{2+}$ oscillations in HSY cells persist under Ca$^{2+}$-free conditions. We can therefore assume external Ca$^{2+}$ fluxes have a negligible effect on the Ca$^{2+}$ oscillations. We fit our model to this data setting the external Ca$^{2+}$ fluxes to be small with $\epsilon = 0.001$. Our model is able to reproduce these results with a Ca$^{2+}$-free solution simulated by setting the Ca$^{2+}$ influx $J_{\text{in}} = 0$.

7.5 A comparison to active IP$_3$ oscillations in a Class II model

In Section 7.4 we presented a model of HSY cell Ca$^{2+}$ oscillations. This was a Class I model with IP$_3$R kinetics responsible for the oscillations of cytosolic Ca$^{2+}$. Passive oscillations of IP$_3$, caused by the feedback of Ca$^{2+}$ on the production of IP$_3$, were responsible for increasing the frequency range of oscillations. Using the Class II model from Palk et al. [76] we present the differences between a Class I and Class II model and show
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Figure 7.12: Removing external Ca$^{2+}$ is shown to have negligible effect on the oscillations of cytosolic Ca$^{2+}$ in both the model and experimental data. Upper trace (a) shows experimental results. Figure reproduced from Tojo et al. [111]. The lower trace (b) shows the model result. Oscillations are induced with applied agonist $\mu = 10$ mM. Ca$^{2+}$-free conditions are reproduced by setting $J_{in} = 0$ where shown with the dashed line.

that a Class II model is inconsistent with experimental data from HSY cells. The model equations can be found in Section 7.7.1.

In Figure 7.13 we show oscillations in Ca$^{2+}$ for the Class II model when IP$_3$ is both unbuffered ($\tau_p = 1$) and buffered ($\tau_p = 0.1$). The first difference to note between these
results and the Class I model results presented in Section 7.4 is the timing of the Ca$^{2+}$ to IP$_3$ peak. In the HSY cells it is seen that the Ca$^{2+}$ peak precedes the IP$_3$ peak (Figure 7.4b). This agrees well with the Class I model results (Figure 7.4a). In the Class II model the IP$_3$ peak is seen to precede the Ca$^{2+}$ peak. We argue in Section 7.7.2 that Class II models can only be of the negative feedback type, where Ca$^{2+}$ feeds back on the IP$_3$ degradation. Therefore Class II models will always have IP$_3$ peaks preceding those of Ca$^{2+}$.

Figure 7.13: IP$_3$ buffering increases the period of Ca$^{2+}$ oscillations in the Class II model. Oscillations are stimulated with the IP$_3$ production rate $\mu = 5100$ nM/s. (a) Oscillations in a simulation without buffer, $\tau_p = 1$, are short period. (b) When an IP$_3$ buffer is simulated with $\tau_p = 0.1$ longer period oscillations are observed.

The second difference to notice between the Class I and Class II models can be observed when looking at the IP$_3$ buffering predictions. Earlier in Section 7.4 we saw that, when buffered, the Class I Positive model showed a reduction in the range of oscillation period with only short period oscillations occurring. In Figure 7.13 it can be seen that the Class II model shows longer period oscillations under IP$_3$ buffering. This is more evident in the bifurcation diagram seen in Figure 7.14 where it is easy to see the range of oscillation periods is increased in the Class II model with IP$_3$ buffering.

A Class II model assumes the majority of the Ca$^{2+}$ feedback is on IP$_3$ degradation. Here Ca$^{2+}$ and IP$_3$ are connected in a negative feedback loop. Whilst once variable increases the other variable falls. Unlike the Class I Positive, the phase-space does not include a saddle-node for solutions to pass close to. Ca$^{2+}$ and IP$_3$ photo-release in a Class II model, therefore, does not show any latency. Result not shown here.
Chapter 7. A mathematical study to determine the role of IP$_3$ in HSY cell calcium oscillations

Figure 7.14: A bifurcation diagram showing the period of stable period orbits of the Class II model plotted against the IP$_3$ production rate $\nu$. As the IP$_3$ dynamics are slowed ($\tau_p$ is reduced) the range of oscillation frequencies is increased with $\tau_p = 0.1$ showing long period oscillations.

7.6 Discussion

We have presented a model of Ca$^{2+}$ oscillations in HSY cells. Oscillations are caused by the feedback of Ca$^{2+}$ on the IP$_3$R kinetics and are not dependent on oscillations of IP$_3$. Passive oscillations in IP$_3$, caused by the feedback of Ca$^{2+}$ on the production of IP$_3$, allow for longer period Ca$^{2+}$ oscillations to be found. The model agrees well with the experimental data from the HSY cells showing the Ca$^{2+}$ peaks preceding those of IP$_3$.

Long period oscillations are reliant on fast IP$_3$ dynamics. In the presence of an IP$_3$ buffer cells are predicted to lack long period oscillations and only show the faster oscillations. Politi et al. [82] show that passive oscillations in IP$_3$ can also show long period oscillations when Ca$^{2+}$ feedback is on IP$_3$ degradation. In this case the IP$_3$ dynamics must be slow. We discount this mechanism in the HSY cells. When Ca$^{2+}$ feedback is on the IP$_3$ degradation, the IP$_3$ peak must precede the Ca$^{2+}$ peak. This is not seen in experimental data from HSY cells.

Long period oscillations in Ca$^{2+}$ show a delay between spikes where both Ca$^{2+}$ and IP$_3$ concentrations are low. Here the production of IP$_3$ is limited by the low Ca$^{2+}$ concentration, and the release of Ca$^{2+}$ is small due to the low IP$_3$ concentration. IP$_3$ and Ca$^{2+}$ photo-release between peaks is predicted to bring about a Ca$^{2+}$ spike very quickly, even for a small photo-release. For a range of Ca$^{2+}$ and IP$_3$ photo-release
strengths a latency is predicted before normal oscillations resume. With very large IP$_3$ photo-release, Ca$^{2+}$ oscillations were advanced, returning quickly without a latency. Our model results show no evidence of a frequency or amplitude change following Ca$^{2+}$ or IP$_3$ photo-release.

In some cell types it is possible that external Ca$^{2+}$ fluxes are responsible for modulating the Ca$^{2+}$ oscillation frequency. The significance of the external Ca$^{2+}$ fluxes on oscillations is easy to observe with the removal of external Ca$^{2+}$. In systems where the external Ca$^{2+}$ fluxes are of importance we would expect a noticeable change in Ca$^{2+}$ oscillations when the external Ca$^{2+}$ is removed. In HSY cells oscillations in cytosolic Ca$^{2+}$ continue unaltered following the removal of external Ca$^{2+}$, suggesting plasma membrane Ca$^{2+}$ fluxes have a negligible affect on the system. In contrast Liu et al. [59] show oscillations of cytosolic Ca$^{2+}$ in HSG cells cease when external Ca$^{2+}$ is removed.

We use the latest single channel model of the IP$_3$R. The transition rates between park and drive are modified slightly from how they appear in Siekmann et al. [90]. This slows the receptor dynamics and allows a better fit to the HSY cell data. There is some uncertainty in the fit of the rate constants at Ca$^{2+}$ concentrations where the receptor is either always in park or always in drive. Rate constant are also constrained by a lower bound related to the length of the experimental trace. In future work we will present evidence that it is not the opening of the IP$_3$R at low Ca$^{2+}$ concentrations that is of maximum importance. Instead it is the closing of the receptor at high Ca$^{2+}$ concentrations that has the greatest affect on the dynamics of the Ca$^{2+}$ oscillations.

In our model the IP$_3$ dependence of the IP$_3$R is incorporated using a Hill function, an increase in the IP$_3$ concentration increasing the flux through the IP$_3$R. In reality a change in the IP$_3$ concentration will change the transition rates, subsequently affecting both the IP$_3$R flux and the IP$_3$R dynamics. Siekmann et al. [90] fit the transition rates at two IP$_3$ concentrations. Given more experimental data, interpolation could be used to produce an expression for the rate constants at any given Ca$^{2+}$ and IP$_3$ concentration, as was seen in Palk et al. [76]. It would be interesting to see if this had any effect on the results presented here.

We make the distinction between Class I and Class II models and go further to distinguish positive and negative feedback of Ca$^{2+}$ on the IP$_3$ dynamics. Domijan et al. [27] show that where Class I and Class II mechanisms jointly exist the behaviour can be approximated by either a Class I or Class II model, depending on which is the more dominant. All feedback mechanisms presented here are likely to exist in all cells. As such this work should be taken as a study to find the most important mechanisms regarding the genesis of Ca$^{2+}$ oscillations and the modulation of their frequency.
We present an argument that Class II models where Ca$^{2+}$ feedback is exclusively on IP$_3$ production are unable to generate stable periodic oscillations. Examples in the literature [27, 40, 99] where oscillations in Ca$^{2+}$ are erroneously classified as Class II Positive models are due to oscillations not in IP$_3$, but in the total cellular Ca$^{2+}$. Setting external Ca$^{2+}$ fluxes to zero in these models results in the immediate halt of Ca$^{2+}$ oscillations.

We show a Class II model is inconsistent with Ca$^{2+}$ oscillations in HSY cells. The time between IP$_3$ and Ca$^{2+}$ peaks gives us useful information into the feedback mechanisms that exist. Ca$^{2+}$ spikes preceding IP$_3$ spikes suggest a positive feedback mechanism exists, with Ca$^{2+}$ affecting the IP$_3$ production rate. As we show in Section 7.7.2, Class II oscillations require Ca$^{2+}$ feedback on IP$_3$ degradation. When experimental data shows Ca$^{2+}$ peaks preceding IP$_3$ peaks we can assume oscillations are Class I Positive. Where IP$_3$ measurements aren’t possible the determination of positive or negative feedback mechanisms should be possible using IP$_3$ buffer [82]. Models with positive feedback of Ca$^{2+}$ on the IP$_3$ dynamics show a lack of long period oscillations when IP$_3$ is buffered. The reverse is true for negative feedback where an IP$_3$ buffer will increase the period of oscillations.

IP$_3$ spikes preceding Ca$^{2+}$ spikes would suggest a negative feedback exists, with Ca$^{2+}$ feedback more dominant on the rate of IP$_3$ degradation. Here a Class I Negative model and a Class II model could both explain the data. The use of either IP$_3$ buffer or photo-release is unable to distinguish between Class I and Class II systems where both have strong negative feedback. If the IP$_3$ dynamics could be made sufficiently slow we might expect a Class I mechanism to continue showing oscillations in Ca$^{2+}$ following the removal of agonist. This is theoretically impossible in a Class II mechanism. It is unclear whether IP$_3$ buffer can sufficiently slow the IP$_3$ dynamics to make this test possible.

Acknowledgments

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7.7 Appendix

7.7.1 Class II Negative model

Here we present a summary of the Class II Ca$^{2+}$ model from Palk et al. [76]. The rate of change of IP$_3$ can be expressed in the following differential equation,
\[
\frac{dP}{dt} = (\mu - J_{\text{IP3deg}}) \tau_p,
\]

where \( \mu \) is the agonist dependent production rate and IP\(_3\) degradation is Ca\(^{2+} \) dependent and given as follows,

\[
J_{\text{IP3deg}} = \left( k_{5p} + k_{3K} \frac{C^2}{C^2 + k_{\text{deg}}^2} \right) P.
\]

### IP\(_3\) parameter values

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( k_{3K} )</td>
<td>40 s(^{-1} )</td>
</tr>
<tr>
<td>( k_{5p} )</td>
<td>0.005 s(^{-1} )</td>
</tr>
<tr>
<td>( k_{\text{deg}} )</td>
<td>400 nM</td>
</tr>
</tbody>
</table>

Table 7.4: IP\(_3\) degradation rate constants

The IP\(_3\)R is considered at steady-state to rule out Class I mechanisms. Here the steady-state open probability is given by,

\[
P_{\text{IPR}} = \frac{q_{12}q_{32}q_{24}}{q_{12}q_{32}q_{24} + q_{42}q_{23}q_{12} + q_{42}q_{32}q_{12} + q_{42}q_{32}q_{21}}.
\]

The rate constants between the states were investigated for their dependence on both the concentration of Ca\(^{2+} \) and IP\(_3\) and are given by,

\[
q_{12} = 0.74 \text{ ms}^{-1}, \quad q_{21} = \Phi_{21}(P) \text{ ms}^{-1}, \quad q_{23} = \alpha_{23} \psi_{23}(C) \Phi_{23}(P) \text{ ms}^{-1}, \quad q_{32} = \alpha_{32} \psi_{32}(C) \Phi_{32}(P) \text{ ms}^{-1}, \quad q_{42} = 3.6 \text{ ms}^{-1},
\]

where

\[
\Phi_{21}(P) = \frac{VP_{21}}{1 + kp_{21}P^3} + bp_{21},
\]

\[
\psi_{23}(C) = a_{23} - \left( \frac{V_{23}}{k_{23}^3 + C^2} + b_{23} \right) \left( \frac{Vm_{23}C^5}{km_{23}C^7 + bm_{23}} \right),
\]

\[
\Phi_{23}(P) = \frac{VP_{23}}{1 + kp_{23}P^3} + bp_{23},
\]

\[
\psi_{32}(C) = \left( \frac{V_{32}}{k_{32}^3 + C^3} + b_{32} \right) \left( \frac{Vm_{32}C^7}{km_{32}C^7 + bm_{32}} \right),
\]

\[
\Phi_{32}(P) = \frac{VP_{32}P^3}{1 + kp_{32}P^3} + bp_{32}.
\]
IP<sub>3</sub> receptor parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$b_{p21}$</td>
<td>0.11 ms&lt;sup&gt;-1&lt;/sup&gt;</td>
</tr>
<tr>
<td>$b_{p23}$</td>
<td>0.001 ms&lt;sup&gt;-1&lt;/sup&gt;</td>
</tr>
<tr>
<td>$b_{p32}$</td>
<td>0</td>
</tr>
<tr>
<td>$k_{p21}$</td>
<td>5 × 10&lt;sup&gt;-10&lt;/sup&gt; nM&lt;sup&gt;-3&lt;/sup&gt;</td>
</tr>
<tr>
<td>$k_{p23}$</td>
<td>5 × 10&lt;sup&gt;-9&lt;/sup&gt; nM&lt;sup&gt;-3&lt;/sup&gt;</td>
</tr>
<tr>
<td>$k_{p32}$</td>
<td>1.5 × 10&lt;sup&gt;-10&lt;/sup&gt; nM&lt;sup&gt;-3&lt;/sup&gt;</td>
</tr>
<tr>
<td>$V_{P21}$</td>
<td>0.0949 nM&lt;sup&gt;3&lt;/sup&gt; ms&lt;sup&gt;-1&lt;/sup&gt;</td>
</tr>
<tr>
<td>$V_{P23}$</td>
<td>0.162 nM&lt;sup&gt;3&lt;/sup&gt; ms&lt;sup&gt;-1&lt;/sup&gt;</td>
</tr>
<tr>
<td>$V_{P32}$</td>
<td>3 × 10&lt;sup&gt;-12&lt;/sup&gt; nM&lt;sup&gt;3&lt;/sup&gt; ms&lt;sup&gt;-1&lt;/sup&gt;</td>
</tr>
<tr>
<td>$a_{23}$</td>
<td>1/1.023 ms&lt;sup&gt;-1&lt;/sup&gt;</td>
</tr>
<tr>
<td>$a_{23}$</td>
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</tr>
<tr>
<td>$a_{32}$</td>
<td>50</td>
</tr>
<tr>
<td>$V_{m23}$</td>
<td>0.0949 nM&lt;sup&gt;3&lt;/sup&gt; ms&lt;sup&gt;-1&lt;/sup&gt;</td>
</tr>
<tr>
<td>$k_{m23}$</td>
<td>72 nM</td>
</tr>
<tr>
<td>$b_{m23}$</td>
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</tr>
<tr>
<td>$V_{m32}$</td>
<td>7 × 10&lt;sup&gt;6&lt;/sup&gt; nM&lt;sup&gt;3&lt;/sup&gt; ms&lt;sup&gt;-1&lt;/sup&gt;</td>
</tr>
<tr>
<td>$k_{m32}$</td>
<td>520 nM</td>
</tr>
<tr>
<td>$b_{m32}$</td>
<td>0.005 ms&lt;sup&gt;-1&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Table 7.5: All parameters taken from Gin et al. [41] with the following exceptions, * are scaling parameters, # are scaling parameters, * parameters have been modified from original values in Gin et al. [41] to ensure correct scaled rates and a closed IPR in the absence of IP<sub>3</sub>.

The flux through the IP<sub>3</sub>R is given by,

$$J_{IPR} = k_{IPR}P_{IPR}(C_{er} - C).$$

where $k_{IPR}$ is the density of the IP<sub>3</sub>R. The flux through the RyR is,

$$J_{RyR} = k_{RyR}P_{RyR}(C_{er} - C),$$

where $k_{RyR} = 0.01$ s<sup>-1</sup> is the receptor density and $P_{RyR}$ is the steady state open probability given by

$$P_{RyR} = \frac{w^\infty (1 + (C/K_b)^3)}{1 + (K_a/C)^4 + (C/K_b)^3},$$

and

$$w^\infty = \frac{1 + (K_a/C)^4 + (C/K_b)^3}{1 + 1/K_c + (K_a/C)^4 + (C/K_b)^3}.$$

The leak from the ER is given by,

$$J_{er} = k_{er}(C_{er} - C),$$

with the SERCA re-uptake,

$$J_{SERCA} = \frac{V_{SERCA}C^2}{K_{SERCA}^2 + C^2}.$$

The plasma membrane fluxes are given by,

$$J_{pm} = \frac{V_{pm}C^3}{K_{pm}^3 + C^3}.$$
and

\[ J_{\text{in}} = \alpha_1 + \alpha_2 \mu. \]

The rate of change of Ca\(^2+\) is,

\[ \frac{dC}{dt} = J_{\text{IPR}} + J_{\text{RyR}} + J_{\text{er}} - J_{\text{SERCA}} + J_{\text{in}} - J_{\text{pm}} \]

with the concentration of Ca\(^2+\) in the ER,

\[ \frac{dC_{\text{er}}}{dt} = -\gamma(J_{\text{IPR}} + J_{\text{RyR}} + J_{\text{er}} - J_{\text{SERCA}}). \]

All model parameters are given in Table 7.6.

### Ca\(^{2+}\) parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(k_{\text{IPR}})</td>
<td>0.04 s(^{-1})</td>
</tr>
<tr>
<td>(V_{\text{SERCA}})</td>
<td>(1 \times 10^{-9}) nmol s(^{-1})</td>
</tr>
<tr>
<td>(V_{\text{pm}})</td>
<td>15 nmol s(^{-1})</td>
</tr>
<tr>
<td>(\alpha_1)</td>
<td>0.231 nmol s(^{-1})</td>
</tr>
<tr>
<td>(\gamma)</td>
<td>5.405</td>
</tr>
<tr>
<td>(k_{\text{RyR}})</td>
<td>0.01 s(^{-1})</td>
</tr>
<tr>
<td>(k_{\text{SERCA}})</td>
<td>1.554 \times 10^{-4} s(^{-1})</td>
</tr>
<tr>
<td>(K_{\text{pm}})</td>
<td>200 nM (^{*})</td>
</tr>
<tr>
<td>(K_{\text{SERCA}})</td>
<td>400 nM (^{*})</td>
</tr>
</tbody>
</table>

### RyR parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(k^+_a)</td>
<td>1500 ((\mu M))(^{-4}) s(^{-1})</td>
</tr>
<tr>
<td>(k^-_a)</td>
<td>28.8 s(^{-1})</td>
</tr>
<tr>
<td>(K_a)</td>
<td>372 nM</td>
</tr>
<tr>
<td>(k^+_b)</td>
<td>1500 ((\mu M))(^{-3}) s(^{-1})</td>
</tr>
<tr>
<td>(k^-_b)</td>
<td>385.9 s(^{-1})</td>
</tr>
<tr>
<td>(K_b)</td>
<td>636 nM</td>
</tr>
<tr>
<td>(k^+_c)</td>
<td>1.75 s(^{-1})</td>
</tr>
<tr>
<td>(k^-_c)</td>
<td>0.1 s(^{-1})</td>
</tr>
<tr>
<td>(K_c)</td>
<td>0.0057</td>
</tr>
</tbody>
</table>

Table 7.6: \(^{*}\) parameters from Keizer and Levine [54], \(^{*}\) from Lytton et al. [61], \(^{**}\) taken from Camello et al. [16], all remaining receptor densities and parameters are fitted to recreate experimental Ca\(^{2+}\) oscillations.

#### 7.7.2 Stable periodic solutions are not possible in a 2D Class II Positive model but are possible in a Class II Negative model

Here we present an argument that a Class II model, where IP\(_3\) oscillations are required for Ca\(^{2+}\) oscillation cannot exhibit stable oscillations if the feedback of Ca\(^{2+}\) is only on the IP\(_3\) production. We then show Class II Negative models can demonstrate stable periodic orbits.

We make the following assumptions which are the basis for most existing calcium models and are justified by experimental evidence.
Chapter 7. A mathematical study to determine the role of IP$_3$ in HSY cell calcium oscillations

![Graphs of calcium and IP$_3$ functions](image)

Figure 7.15: ER Ca$^{2+}$ functions.

- The IP$_3$R is considered to be fast acting and so at quasi-steady state. This rules out a Class I mechanism as the cause of Ca$^{2+}$ oscillations.

- The cell is closed and therefore the only variable of the system are Ca$^{2+}$ ($C$) and IP$_3$ ($P$).

- Leak from the ER is assumed to be a positive, monotonically decreasing function of cytosolic Ca$^{2+}$. The leak function is positive at zero cytosolic Ca$^{2+}$.

- The SERCA function is assumed to be zero when cytosolic Ca$^{2+}$ is zero, increasing monotonically with Ca$^{2+}$. This is in agreement with Lytton et al. [61].

- IP$_3$R release is zero with zero cytosolic Ca$^{2+}$, then increases to a peak before gradually decreasing as cytosolic Ca$^{2+}$ increases. This is in experimental agreement with Gin et al. [41]. This is assumed to occur at all concentrations of IP$_3$ making the Ca$^{2+}$ dependency separable.

- IP$_3$R release increases monotonically with IP$_3$, which is shown by Gin et al. [41].

- For simplicity the flux through the RyR is considered negligible.

We plot some representative Ca$^{2+}$ fluxes in Figure 7.15.

We look to determine the shape of the Ca$^{2+}$ and IP$_3$ nullclines. When the rate of change of Ca$^{2+}$ is zero, $\frac{dC}{dt} = J_{IPR}(C, P) + J_{serca}(C) - J_{leak}(C) = 0$ we have,

$$J_{IPR}(C, P) = J_{serca}(C) - J_{leak}(C).$$

We can break the IP$_3$R function into a Ca$^{2+}$ and IP$_3$ dependent function given by $RC$ and $RP$ respectively,

$$RP(P) \cdot RC(C) = J_{serca}(C) - J_{leak}(C).$$
Given that the Ca\(^{2+}\) leak is a small monotonically decreasing function and the SERCA is a monotonically increasing function we can determine the shape of the \(J_{\text{serca}}(C) - J_{\text{leak}}(C)\), see Figure 7.16.

![Figure 7.16: Difference between SERCA uptake and Ca\(^{2+}\) leak.](image)

We can then use,

\[
RP(P) = \frac{J_{\text{serca}}(C) - J_{\text{leak}}(C)}{RC(C)}.
\]

Again we can predict the shape of the function on the right hand side of this expression and we know the flux through the IP\(_3\)R increases monotonically with IP\(_3\), we can now predict the shape of the Ca\(^{2+}\) nullcline. In Figure 7.17 the Ca\(^{2+}\) nullcline is shown in orange.

In a Class II forward feedback model the rate of IP\(_3\) production is an increasing function of the Ca\(^{2+}\) concentration. The rate of degradation is proportional to the concentration of IP\(_3\).

\[
\frac{dP}{dt} = \text{prod}(C) - \text{deg} \cdot P.
\]

Solving to find the nullcline we have,

\[
P = \frac{\text{prod}(C)}{\text{deg}}.
\]

Therefore the IP\(_3\) nullcline will be an increasing function of Ca\(^{2+}\) crossing the origin. Assuming the production of IP\(_3\) is monotonic, the Ca\(^{2+}\) and IP\(_3\) nullclines will either
have 1 or 3 intersections. The green curve in 7.17 shows the IP₃ nullcline.

Figure 7.17: Ca²⁺ (orange) and IP₃ (green) nullclines of a Class II Positive model. Arrows show the direction of solutions. Four regions are labelled A, B, C and D. It can easily be seen that region B is a trapping region, all solutions that enter the region are contained there. This prevents periodic solutions orbiting all three equilibrium points. Region D and the two unlabelled region are also trapping regions.

The nullclines shown in Figure 7.17 are calculated using a modified model from Atri et al. [3]. Given the model assumptions on the Ca²⁺ fluxes, the nullclines for Ca²⁺ and IP₃ will always take a form similar to that shown. Thus Figure 7.17 can be considered representative of all Class II Positive models.

**Trapping regions and the non-existence of oscillations in Class II forward feedback models**

We divide the quarter plane into regions divided by the nullclines. Figure 7.17 shows the nullclines in the case when we have three equilibria. Two sinks exist at low and high
Ca\(^{2+}\) concentration and a saddle point exists in between. Looking at region B, Due to the nature of the nullclines it can easily be seen that all solutions that enter region B are contained in this region and therefore head to the the equilibrium point at \(C = 0.45\). The same is true of region D, here again all solution head to the same equilibrium at \(C = 0.45\).

In index theorem a stationary point is given an index based on its type. Sinks or sources are assigned an index +1, saddle points are given an index −1. The region enclosed by a periodic orbit must contain at least one stationary point with the sum of the indices equal to +1 [45]. In the situation shown in Figure 7.17 index theorem tells us that periodic orbits may be possible around either of the source terms, or around all three equilibria. Due to the trapping regions it is easy to see that none of these periodic orbit will exist in this Class II Positive phase plane. In Figure 7.17 we show the nullclines when three equilibria exist. When the nullclines intersect once the plane will again be divided into distinct trapping regions preventing the existence of stable periodic orbits.

There are example of Class II Positive models in the literature which claim to exhibit stable periodic solutions [27, 40, 99]. We have just demonstrated that Ca\(^{2+}\) feedback on IP\(_3\) production cannot generate stable oscillations in Ca\(^{2+}\). The models presented in Domijan et al. [27], Gin et al. [40], Sneyd et al. [99] are erroneously classified as Class II Positive models. Oscillations of Ca\(^{2+}\) are actually the result of periodic oscillations in the total cellular Ca\(^{2+}\) concentration. Setting the external membrane Ca\(^{2+}\) fluxes to zero in these models results in the immediate halt of Ca\(^{2+}\) oscillations (result not shown here).

**A Class II Negative model can generate stable periodic oscillations**

In a Class II Negative model the IP\(_3\) degradation an increasing function of Ca\(^{2+}\), for simplicity the IP\(_3\) production is assumed constant.

\[
\frac{dP}{dt} = \text{prod} - \text{deg}(C) \cdot P.
\]

Solving to find the nullcline we have,

\[
P = \frac{\text{prod}}{\text{deg}(C)}.
\]

The IP\(_3\) degradation is assumed to increase linearly with Ca\(^{2+}\), with zero degradation when the Ca\(^{2+}\) concentration is zero. The IP\(_3\) nullcline will start at infinity when Ca\(^{2+}\) is zero, decreasing monotonically with Ca\(^{2+}\).
Figure 7.18: Ca\(^{2+}\) (orange) and IP\(_3\) (green) nullclines of the Class II Negative model. Arrows show the direction of solutions. Periodic solutions are possible around the intersection of the nullclines, following the solution arrows.

In Figure 7.18 the nullclines of the Class I Negative feedback model are plotted. In comparison to the forward feedback model, no trapping regions are seen. Stable periodic orbits around the intersection of the nullclines are possible. For model equations of a Class II Negative model see Section 7.7.1 or Palk et al. [76].
Chapter 8

Conclusions and future work

In this thesis we used a range of mathematical modelling techniques and analysis to help improve our understanding of the regulation of saliva secretion. Here we will present a summary of the work, focussing on how the results tie together and suggest directions for future work. For a more detailed discussion of the results of individual chapters see Sections 4.5, 5.6, 6.7 and 7.6.

8.1 Fluid secretion and ion transport

We developed a dynamic model of saliva secretion in parotid acini. The distribution of K$^+$ channels was investigated for its effect on the efficiency of saliva secretion. Where localisation studies and most current models placed K$^+$ channels exclusively in the basolateral membrane, our model predicted the presence of apical K$^+$ channels could increase saliva secretion. Following the publication of this work, Almassy et al. [1] found experiment evidence of apical K$^+$ channels using localised Ca$^{2+}$ release. The combined theoretical and experimental results provide compelling evidence that apical K$^+$ channels not only exist, but also help aid the efficiency of saliva secretion.

Saliva secretion in our parotid acinar model is driven by the transepithelial movement of Cl$^-$ ions into the lumen. Secretion in Na$^+$- K$^+$- 2Cl$^-$ cotransporter knockout mice is reduced by up to 85%, providing evidence that this Cl$^-$-driven secretion is dominant. The fact that some saliva secretion continues suggests an alternative mechanism exists which Melvin et al. [70] propose is HCO$_3^-$-driven. In Future work we would like to develop a model of HCO$_3^-$-driven secretion. This would require modelling the pH in the cell in a manner similar to that completed in cardiac myocytes [22].

Radiation therapy, used for the treatment of throat and neck cancer, results in the damage of acinar cells. Baum et al. [6] show gene transfer of aquaporins to salivary duct
Chapter 8. Conclusions and future work

cells as an effective treatment for hyposalivation that commonly occurs. The authors hypothesise that a KHCO$_3$ gradient from the interstitium, through the duct cells, to the lumen drives water transport. A mathematical model of duct cell electrolyte exchange has been developed by Patterson et al. [80]. A combined duct and acinar model, incorporating water transport through aquaporins, could provide real insight into how this gene therapy enables increased saliva secretion.

8.2 Ca$^{2+}$ dynamics

Much of the work in this thesis has been focussed on the role of Ca$^{2+}$. A Class II Ca$^{2+}$ model is used to explain Ca$^{2+}$ oscillations in parotid acinar cells, where oscillations in IP$_3$ are required for oscillations in Ca$^{2+}$. In Chapter 5 the Class II model of Ca$^{2+}$ oscillations in parotid acini is spatially extended. We show an inhomogeneous distribution of Ca$^{2+}$ channels is capable of producing periodic apical to basal Ca$^{2+}$ waves, in agreement with experimental data. Later, we show duct cell Ca$^{2+}$ oscillations are consistent with a Class I model, where IP$_3$ oscillations passively follow Ca$^{2+}$ oscillations and increase the frequency range. The original Class II model used the IP$_3$R model of Gin et al. [41]. Here the receptor moved between states rapidly, spending at most a few milliseconds in the open state. A Class I model with these rapid IP$_3$R kinetics was incapable of producing the 5-9 second Ca$^{2+}$ oscillation period seen in parotid acinar cells [14], hence a Class II model was used. A new study into the IP$_3$R dynamics by Siekmann et al. [90] showed slower kinetics, with transitions between modes occurring as little as once every 50 seconds. We show a Class I model with the Siekmann IP$_3$R model is capable of producing long period oscillations at constant IP$_3$ concentration. Time-course measurements of IP$_3$ in HSY cells provided evidence that a Class I mechanism was consistent with the Ca$^{2+}$ oscillations in this cell type. No such measurements have been made in parotid acinar cells. It is, therefore, unclear whether oscillation in IP$_3$ are required for Ca$^{2+}$ oscillation in salivary acinar cells.

We argue that positive feedback, with Ca$^{2+}$ affecting IP$_3$ production, cannot alone generate oscillations in a Class II model. Where IP$_3$ spikes are seen shortly after Ca$^{2+}$ spikes we assume a positive feedback is present, and therefore a Class I mechanism must be the driver of the Ca$^{2+}$ oscillations. If IP$_3$ measurements are not possible, we are able to distinguish positive and negative feedback using IP$_3$ buffering. If the presence of IP$_3$ buffer reduces the frequency range, leaving only short period oscillations, a positive feedback mechanisms is hypothesised to exist. If a negative feedback mechanism was predicted, either by measuring IP$_3$ or by observations with IP$_3$ buffer, we do not find an
infallible test to distinguish between Class I and Class II mechanisms. With very slow IP₃ dynamics, a Class I model is capable of generating Ca²⁺ oscillations following the removal of agonist. Here IP₃ would degrade slowly, following the removal of agonist, but could not oscillate, ruling out Class II mechanisms. If IP₃ buffer was able to sufficiently slow the IP₃ dynamics then perhaps this might serve as a useful test.

The saliva secretion process, as with most physiological processes, is inherently multiscale. Individual Ca²⁺ release receptors, whose size is a few nanometres, release Ca²⁺ from internal stores. Our modelling has focussed on the individual cells at the micron scale. These individual cells are clustered into acini and make up a whole gland, whose diameter is several centimetres. A proper understanding of the regulation of saliva secretion requires understanding all of these scales. A future goal is to build a multiscale model of an entire secretory gland.

In Chapter 4 we estimate over 20 million acinar cells are needed to produce the secretion that results from a 100 g parotid gland. Cells are coupled with their neighbours through IP₃ diffusion and by secreting into a shared lumen [63]. Simulating 20 million interacting cells would present extreme numerical difficulties. If we then add dynamics within each cell, such as ionic concentrations that diffuse and interact with each other, and Ca²⁺ receptors that rapidly transition between multiple states, we can easily see that model simplifications, where possible, are necessary for generating a whole organ model.

In Chapter 6 we analysed how Ca²⁺ wave and oscillation properties might be used for signal encoding. We found that oscillation amplitude and frequency had little effect on the saliva secretion rate, with the Ca²⁺ wave speed having a slightly greater effect. By far the most important property was the mean cytosolic Ca²⁺ concentration. With respect to a whole organ model, we suggest Ca²⁺ waves are of limited importance and need not be included. Instead, homogeneous oscillations throughout the cytosol could be used without a great loss in the accuracy of solutions. If heavily constrained by numerical cost it would also be possible to assume the cytosolic Ca²⁺ concentration was a constant function of the applied agonist. This would greatly reduce the computational cost.

The work presented in this thesis has been entirely deterministic. As experimental measurements become more precise, extremely localised releases of Ca²⁺ are being observed. Thurley et al. [110] demonstrate that Ca²⁺ spikes are a Poisson process. They show the standard deviation of the inter-spike interval is proportional to the mean, with a gradient close to one. Stochastic simulation requires modelling Ca²⁺ release from individual Ca²⁺ release channels. These releases are called blips. A blip from one release
channel can instigate the release from its neighbours, causing a combined release from a cluster of receptors, called a puff. The orchestration of puffs from several clusters can then lead to a global Ca$^{2+}$ release. The time between puffs is shown to be an order of magnitude less than the time between global Ca$^{2+}$ spikes [110]. This allows for the possibility that long period global Ca$^{2+}$ oscillations can result from fast local IP$_3$R kinetics. As a result of the coupling between receptors in a cluster, and then clusters in the cytosol, stochastic Ca$^{2+}$ models are numerically intensive. It is not clear what behaviours can be found in these stochastic simulations that cannot be found in deterministic models. Using the Siekmann IP$_3$R model, it would be interesting to compare the two modelling approaches to see if any qualitative change in solutions is found.

8.3 Summary

As experimental procedure and equipment continues to improve, the importance of mathematical modelling, as a tool to both explain and predict phenomena, will grow. Increases in imaging resolution and sampling rate, combined with the ability to make time-course measurements of IP$_3$, provides new Ca$^{2+}$ signalling insight. Where mathematical Ca$^{2+}$ models originated to explain global cytosolic Ca$^{2+}$ oscillations. New stochastic and spatial modelling now looks to provide a link between the localised Ca$^{2+}$ release from individual channels and global Ca$^{2+}$ waves. The mystery surrounding the role of IP$_3$ in Ca$^{2+}$ signalling will continue to be revealed and new questions will no doubt be asked, which mathematical models can look to answer.

The commencement of clinal studies involving the gene transfer of aquaporins to patients following radiation therapy provides exciting new directions for mathematical modelling of saliva secretion. A whole organ model, investigating secretion before irradiation, after radiation therapy, and following the transfer of aquaporins could provide invaluable information. Not only answering how this treatment works, but also if it comes at any unknown cost.
Appendices
Appendix A

Finite difference scheme to solve the spatial Ca$^{2+}$ model

Here we present details of the finite difference method used to solve the Ca$^{2+}$ wave model seen in Chapter 5.

A.1 Model discretisation

We use a semi-implicit finite difference method. Space and time derivatives are approximated by a centered difference and backwards Euler’s method respectively. We discretise Equations (5.1) from Section 5.3 as follows,

$$
\frac{C_j^{(m)} - C_j^{(m-1)}}{\Delta t} = D_c \frac{C_{j+1}^{(m)} - 2C_j^{(m)} + C_{j-1}^{(m)}}{(\Delta x)^2} + \left( J_{IPR} + J_{ER} + J_{RyR} - J_{SERCA} + J_m - J_{pm} \right) \bigg|_{C=C_j^{(m)}, P=P_j^{(m)}, S=S_j^{(m)}} ,
$$

$$
\frac{S_j^{(m)} - S_j^{(m-1)}}{\Delta t} = D_s \frac{S_{j+1}^{(m)} - 2S_j^{(m)} + S_{j-1}^{(m)}}{(\Delta x)^2} - \frac{1}{\gamma} \left( J_{IPR} + J_{ER} + J_{RyR} - J_{SERCA} \right) \bigg|_{C=C_j^{(m)}, P=P_j^{(m)}, S=S_j^{(m)}} ,
$$

$$
\frac{P_j^{(m)} - P_j^{(m-1)}}{\Delta t} = D_p \frac{P_{j+1}^{(m)} - 2P_j^{(m)} + P_{j-1}^{(m)}}{(\Delta x)^2} + \left( J_{IP3prod} - J_{IP3deg} \right) \bigg|_{C=C_j^{(m)}, P=P_j^{(m)}} .
$$

(A.1)

Superscript, $m$, denotes the discretised time step with subscript, $j$, denoting the spatial grid point. Note all fluxes in the above are evaluated at the current time and space.
point, e.g. $C_j^{(m)}$, $S_j^{(m)}$ and $P_j^{(m)}$. We introduce the following notation for the Ca$^{2+}$ fluxes and IP$_3$ dynamics evaluated at a given spatial grid point and time step.

\[
(J_{IPR} + J_{ER} + J_{RyR} - J_{SERCA} + J_{in} - J_{pm}) \bigg|_{C=C_j^{(m)}, P=P_j^{(m)}, S=S_j^{(m)}} = \Phi(C_j^{(m)}, S_j^{(m)}, P_j^{(m)})
\]

\[
= \Phi_j^{(m)}.
\]

\[
\frac{1}{\gamma} (J_{IPR} + J_{ER} + J_{RyR} - J_{SERCA}) \bigg|_{C=C_j^{(m)}, P=P_j^{(m)}, S=S_j^{(m)}} = \rho(C_j^{(m)}, P_j^{(m)}, S_j^{(m)})
\]

\[
= \rho_j^{(m)}.
\]

\[
(J_{IP3prod} - J_{IP3deg}) \bigg|_{C=C_j^{(m)}, P=P_j^{(m)}} = \Psi(C_j^{(m)}, P_j^{(m)}) = \Psi_j^{(m)}.
\]

We can rewrite (A.1) using the above notation and the following, $k_C = D_c \frac{\Delta t}{(\Delta x)^2}$, $k_S = D_s \frac{\Delta t}{(\Delta x)^2}$ and $k_P = D_p \frac{\Delta t}{(\Delta x)^2}$, giving,

\[
C_j^{(m)} - C_j^{(m-1)} = k_C \left(C_{j+1}^{(m)} - 2C_j^{(m)} + C_{j-1}^{(m)}\right) + \Delta t \cdot \Phi_j^{(m)},
\]

\[
S_j^{(m)} - S_j^{(m-1)} = k_S \left(S_{j+1}^{(m)} - 2S_j^{(m)} + S_{j-1}^{(m)}\right) - \Delta t \cdot \rho_j^{(m)},
\]

\[
P_j^{(m)} - P_j^{(m-1)} = k_P \left(P_{j+1}^{(m)} - 2P_j^{(m)} + P_{j-1}^{(m)}\right) + \Delta t \cdot \Psi_j^{(m)}.
\]

**A.1.1 Boundary conditions**

We assume zero flux on the apical and basal membrane, assuming any plasma membrane fluxes occur perpendicular to the spatial coordinate, $x$. We discretise Equations (5.2) from Section 5.3 as follows,

\[
\frac{\partial C}{\partial x} \bigg|_{x=0, L} \approx \frac{C_{j+1} - C_{j-1}}{2\Delta x} = 0,
\]

\[
\frac{\partial S}{\partial x} \bigg|_{x=0, L} \approx \frac{S_{j+1} - S_{j-1}}{2\Delta x} = 0,
\]

\[
\frac{\partial P}{\partial x} \bigg|_{x=0, L} \approx \frac{P_{j+1} - P_{j-1}}{2\Delta x} = 0.
\]

At the left hand boundary, $j = 1$ and $C_0 = C_2$, $S_0 = S_2$, $P_0 = P_2$, where $C_0$, $S_0$ and $P_0$ are imaginary points one grid point outside of the boundary which ensure the
A.1. Model discretisation

The no-flux condition on the boundary can be met. The same is completed at the right-hand boundary at \( j = N \), where \( N \) is the number of grid points, \( C_{N+1} = C_{N-1}, S_{N+1} = S_{N-1}, P_{N+1} = P_{N-1} \).

A.1.2 Matrix form

Using (A.5 and A.6) we can write the numerical scheme in a matrix form. Below is an example for the cytosolic Ca\(^{2+}\) equations in a system with \( N = 4 \) grid points.

\[
\begin{pmatrix}
1 + 2k_C & -2k_C & 0 & 0 \\
-k_C & 1 + 2k_C & -k_C & 0 \\
0 & -k_C & 1 + 2k_C & -k_C \\
0 & 0 & -2k_C & 1 + 2k_C
\end{pmatrix}
\begin{pmatrix}
C_1^{(m)} \\
C_2^{(m)} \\
C_3^{(m)} \\
C_4^{(m)}
\end{pmatrix}
= 
\begin{pmatrix}
(C_1^{(m-1)} + \Delta t \cdot \Phi_1^{(m)}) \\
(C_2^{(m-1)} + \Delta t \cdot \Phi_2^{(m)}) \\
(C_3^{(m-1)} + \Delta t \cdot \Phi_3^{(m)}) \\
(C_4^{(m-1)} + \Delta t \cdot \Phi_4^{(m)})
\end{pmatrix}
\] (A.7)

With a small time step we can assume the reaction term, \( \Phi_j^{(m)} \approx \Phi_j^{(m-1)} \). Use of this approximation changes the scheme from being fully-implicit to semi-implicit. After this approximation the only unknown in (A.7) is the Ca\(^{2+}\) concentration in the LHS vector. To find the unknown Ca\(^{2+}\) concentration vector we solve the linear system with a tridiagonal solver, the Thomas Algorithm, at each time step to find the new concentration values. The same approach can be taken to solve for \( S \) and \( P \). It is easy to extend the scheme for \( N > 4 \) grid points.

In order to solve the 1D spatial Ca\(^{2+}\) model seen in Chapter 5, we use \( N = 126, \Delta x = 0.2 \mu m \) and \( \Delta t = 0.01 \) s. Diffusion coefficients are estimated as \( D_c = 15 \mu m^2/s \), \( D_s = 7.5 \mu m^2/s \) and \( D_p = 300 \mu m^2/s \). We initialise the model with homogeneous initial conditions,

\[
C_{j=1..N}^{(0)} = 50 \text{ nM}, \\
S_{j=1..N}^{(0)} = 9.7 \times 10^4 \text{ nM}, \\
P_{j=1..N}^{(0)} = 0 \text{ nM},
\]

We implement the finite difference scheme using a Fortran code. Higher order methods, such as the Crank-Nicolson method, could also be used.
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