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**CHARACTERISATION OF RYANODINE  
RECEPTOR EXPRESSION IN THE RAT COCHLEA**

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A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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## ABSTRACT

Increases in intracellular  $\text{Ca}^{2+}$  play a central role in cochlear function. The ryanodine receptor (RyR) intracellular  $\text{Ca}^{2+}$  release channel, a ubiquitous element of  $\text{Ca}^{2+}$  signalling, has been implicated in the regulation of sound transduction and auditory neurotransmission. Despite this, the molecular basis underlying RyR-mediated  $\text{Ca}^{2+}$  signalling in the cochlea has been limited.

This thesis investigates the molecular and functional characterisation of RyR expression in the cochlea. RT-PCR analysis showed expression of RyR1, RyR2 and RyR3 isoform mRNA transcripts in the rat cochlea and also in the spiral ganglion. Localisation of RyR protein revealed differential expression of these isoforms in the cochlea. Strong RyR immunolabelling for RyR1, RyR2 and RyR3 were detected in the spiral ganglion neuron (SGN) cell bodies. RyR3 labelling extended to the synaptic terminals innervating the inner and outer hair cells. RyR2 expression also occurred in the inner hair cells and supporting cells of the organ of Corti. Cells associated with ion homeostasis in the cochlea were also labelled, including RyR1 in spiral limbus interdental cells, and RyR2 and RyR3 in spiral prominence epithelial cells and stria vascularis basal cells. In the SGN cell bodies, confocal imaging of  $\text{Ca}^{2+}$  store release confirmed the presence of a functional RyR-gated  $\text{Ca}^{2+}$  store. Superfusion of glutamate and  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) evoked large  $\text{Ca}^{2+}$  responses in the SGN cell bodies that were dependent upon  $\text{Ca}^{2+}$  entry. However, subsequent depletion of SGN RyR-gated  $\text{Ca}^{2+}$  stores substantially reduced the glutamate- and AMPA-induced  $\text{Ca}^{2+}$  responses, demonstrating that the majority of the  $\text{Ca}^{2+}$  signal derived from RyR-gated  $\text{Ca}^{2+}$  stores via  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release. Involvement of the AMPA/Kainate-type glutamate receptor was confirmed by elimination of glutamate- and AMPA-induced  $\text{Ca}^{2+}$  responses with an AMPA/Kainate receptor antagonist.

These findings support a role for RyR in the regulation of auditory neurotransmission, sound transduction and cochlear electrochemical homeostasis. These data also demonstrate coupling between somatic AMPA-type glutamate receptors and RyR-gated  $\text{Ca}^{2+}$  stores, which is likely to influence auditory neurotransmission.

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## ABBREVIATIONS

|                                  |  |
|----------------------------------|--|
| [Ca <sup>2+</sup> ] <sub>i</sub> | intracellular Ca <sup>2+</sup> concentration                                 |
| ACh                              | acetylcholine  |
| AMPA                             | α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid                        |
| ATP                              | adenosine 5'-triphosphate  |
| BK                               | big conductance Ca <sup>2+</sup> -activated K <sup>+</sup> channel           |
| bs                               | basal cells  |
| bp                               | base pair  |
| Ca-F                             | Ca <sup>2+</sup> -free solution  |
| Ca <sup>2+</sup>                 | calcium ions   |
| CAP                              | compound action potential  |
| cc                               | Claudius' cells  |
| cDNA                             | complementary deoxyribonucleic acid  |
| CICR                             | calcium-induced calcium release  |
| CM                               | cochlear microphonic   |
| dc                               | Deiters' cells   |
| DHPR                             | dihydropyridine receptor   |
| DICR                             | depolarisation-induced Ca <sup>2+</sup> release                              |
| DMEM                             | Dulbecco's modified Eagle's medium   |
| DMSO                             | dimethyl sulfoxide   |
| DNA                              | deoxyribonucleic acid  |
| DNQX                             | 6,7-Dinitroquinoxaline-2,3-dione   |
| EC                               | excitation contraction   |
| EGTA                             | ethylene glycol-bis-(β-aminoethyl ether)- <i>N,N,N',N'</i> -tetraacetic acid |
| ER                               | endoplasmic reticulum  |

|                   |   |
|-------------------|---|
| GluR              | glutamate receptor  |
| HBSS              | hank's balance salt solution  |
| HRP               | horseradish peroxidase  |
| ICICR             | IP <sub>3</sub> -dependent Ca <sup>2+</sup> -induced Ca <sup>2+</sup> release |
| idc               | interdental cells   |
| iGluR             | ionotropic glutamate receptor   |
| IHC               | inner hair cell   |
| IP <sub>3</sub> R | inositol-1,4,5-trisphosphate receptor   |
| isc               | inner sulcus cells  |
| isp               | inner spiral plexus   |
| K <sup>+</sup>    | potassium ions  |
| Mg <sup>2+</sup>  | magnesium ions  |
| MET               | mechanoelectrical transduction  |
| mGluR             | metabotropic glutamate receptor   |
| mRNA              | messenger ribonucleic acid  |
| NGS               | normal goat serum   |
| NMDA              | <i>N</i> -methyl-D-aspartate  |
| NO                | nitric oxide  |
| o/C               | organ of Corti  |
| OCT               | optimal cutting tissue mount  |
| OHC               | outer hair cell   |
| OSF               | outer spiral fibre  |
| P                 | postnatal day   |
| PB                | phosphate buffer  |
| PBS               | phosphate buffered saline   |

|        |  |
|--------|--|
| pc     | pillar cell  |
| RT-PCR | Reverse Transcription-Polymerase Chain Reaction                    |
| RyR    | ryanodine receptor   |
| SGN    | spiral ganglion neuron   |
| SK     | small conductance $\text{Ca}^{2+}$ -activated $\text{K}^+$ channel |
| sl     | spiral limbus  |
| sp     | spiral prominence  |
| SR     | sarcoplasmic reticulum   |
| Std    | standard external solution   |
| sv     | stria vascularis   |
| tm     | tectorial membrane   |
| TMRD   | tetramethylrhodamine-conjugated dextran                            |
| UTP    | uridine 5'-triphosphate  |