STUDIES INTO THE RELEASE MECHANISMS

OF

HUMAN PLACENTAL LACTOGEN in vitro

by

VERNON JOHN CHOY

Postgraduate School of Obstetrics and Gynaecology

A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy from the University of Auckland

February 1977

ACKNOWLEDGMENTS

I express my thanks to the following:

Dr Wayne B. Watkins my thesis advisor, for his guidance and assistance;

the staff of the Delivery Suite at National Women's Hospital, for collecting placentas;

Messrs John Urry and Hugh Johansen for preparing the diagrams;

Mrs D. Choy and Miss Claudia Lindsay for braving my handwriting to prepare the draft copy of this thesis, and Miss Bonnie Ah Chee for typing the final manuscript;

my friends and colleagues in the Postgraduate School of Obstetrics and Gynaecology for moral support.

These studies were financed in part by a grant from the Medical Research Council of New Zealand to Dr W.B. Watkins, and by the University Grants Committee in the form of a Postgraduate Scholarship.

ABBREVIATIONS

Å	Angstrom
ACTH	adrenocorticotropic hormone
AD	assay diluent, BB containing 0.5 mg/ml human serum albumin
adenyl cyclase	adenylate cyclase or adenylyl cyclase
ADP	adenosine 5'-diphosphate
AMP	adenosine 5'-monophosphate
ATP	adenosine 5'-triphosphate
ATPase	adenosine 5'-triphosphatase
B	antibody-bound ^{125}I-labelled HPL
BB	0.05 M barbitone buffer, pH 8.6
c	centi, 10^{-2}
°C	degree Celsius
Ci	Curie
cpm	counts per minute
cyclic AMP	adenosine 3':5'-cyclic monophosphate
cyclic GMP	guanosine 3':5'-cyclic monophosphate
dbcAMP	N^6,$O^{2'}$-dibutyryl adenosine 3':5'-cyclic monophosphate
DMS	dimethylsulphoxide
D_2O	deuterium oxide
EDTA	ethylenediaminetetraacetate
EM	electron microscopic
F	free ^{125}I-labelled HPL
Fig.	figure
FSH	follicle stimulating hormone
g	gram
g	gravitational unit of acceleration
GH	growth hormone

h	hour
HCG	human chorionic gonadotropin
HCS	human chorionic somatomammotropin
HCT	human chorionic thyrotropin
HPL	human placental lactogen
^{125}I-HPL	^{125}I-labelled HPL
KRBG	Krebs-Ringer bicarbonate solution
KRBGA	KRBG containing 5.0 mM aminophylline
l	litre
LH	luteinising hormone
LMS	modified Locke's solution
m	metre
m	milli, 10^{-3}
M	molar (moles/litre)
min	minute
mRNA	messenger ribonucleic acid
n	nano, 10^{-9}
No.	number
n.s.	not significant
P	precipitated ^{125}I-labelled HPL
P	probability
PGA_1	prostaglandin A_1
PGE_1	prostaglandin E_1
pH	-log (hydrogen ion concentration)
PPI	rate expressed as a proportion of the rate during the previous incubation period
PrL	prolactin
RIA	radioimmunoassay
RR	rate ratio
RRF	rate ratio factor

s	second
SD	standard deviation
T	total ^{125}I-labelled HPL
Tris	tris(hydroxymethyl)aminomethane
TSH	thyroid stimulating hormone
U	international unit
v	volume
V	volt
w	weight

μ	micro, 10^{-6}
%	per cent
<	less than
>	greater than

Also used, the standard chemical symbols for elements, salts and ions

SUMMARY

1. A procedure to study the *in vitro* release of HPL was developed. Hormone release, partially uncoupled from hormone biosynthesis, was measured by incubating placental fragments in physiological salt solutions for short periods. The effect of test substances and different ionic environments on HPL release were tested to ascertain the roles of calcium ion and cyclic AMP in the release mechanism.

2. The HPL concentration of the incubation media was measured with a specific radioimmunoassay. Particular features of the assay were the use of high concentrations of reagents, and the separation of antibody-bound and free hormone by ethanol fractionation. The assay had a useful range of 75-800 ng HPL/ml with interassay and intra-assay variations of 14.7% and 3.2%, respectively.

3. Media with high Ca^{2+} (10 mM) caused an inhibition of HPL release, while Ca^{2+}-free media stimulated release. The presence of a depolarising concentration of K^{+} (54 mM), high and low concentrations of Na^{+} (261.9 mM and 24.9 mM, respectively), and substitution of Ca^{2+} by Ba^{2+} had no effect on hormone release. However, a ten-fold excess of Mg^{2+} (12 mM) inhibited release minimally in the presence of Ca^{2+}, and 5.0 mM La^{3+} markedly depressed the release rate. The calcium ionophore A-23187 had no effect on release.

4. The data from these experiments suggested that calcium was not required for HPL secretion *in vitro*. This leads one to conclude that HPL secretion is not a calcium related stimulus-secretion coupled process.

5. Perturbation of the placental adenyl cyclase/cyclic AMP system by dibutyryl cyclic AMP, aminophylline, fluoride ion, norepinephrine, ATP, prostaglandins, alcohol and transition metal ions yielded responses that suggested that HPL release in vitro was mediated by cyclic AMP.

6. The existence of an HPL release mechanism was discussed in the light of these findings.

TABLE OF CONTENTS

CHAPTER VI DISCUSSION AND CONCLUSIONS

LIST OF FIGURES

24. Effect of sodium ion concentration.

25. Effect of cation deficiency.

26. Effect of high sodium ion concentration.

27. Effect of high magnesium ion concentration.

28. Effect of barium ion.

29. Effect of transition metal ions.

30. Effect of aminophylline.

31. Effect of sodium fluoride.

32. Reversible stimulation by ethanol.

33. Hypothetical role of calcium and cyclic AMP in the release of HPL *in vitro*.

LIST OF TABLES