OVINE PLACENTAL LACTOGEN :

PURIFICATION AND PROPERTIES

by

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Thesis submitted to the University of Auckland for the degree of Doctor of Philosophy

October 1979

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ACKNOWLEDGEMENTS

I thank:

Dr W B Watkins, my supervisor, for his help, guidance and encouragement.

Dr Vernon Choy, Mr Lloyd Moore and Mr John Peek for their valuable assistance and moral support throughout the course of this investigation.

Mr Hugh Johanssen and Miss Virginia Bennett for preparing the diagrams with patience.

Mr John Urry and Mr Ian Foot for the photographic work.

Dr John France and Dr Steve Skinner for their constructive criticism of parts of the thesis.

Mr Tony Mekkelholt and Mr Vernon Jansen of the Animal Laboratory, Auckland School of Medicine, for their kind assistance with the experimental animals.

Miss Elizabeth West of the Greenlane Hospital Surgical Laboratory for care of the rabbits.

Professor G C Liggins and Dr J Kitterman for kindly providing sheep placental tissue and catheterized fetal and maternal sheep preparations for half-life studies.

Veterinary Staff, Ministry of Agriculture and Fisheries, Meat Division, Auckland Farmers' Freezing Co., Southdown, Penrose, Auckland for their co-operation in providing sheep, goat and cow placental tissue.

Dr Jim Peterson of the Ruakura Animal Research Centre, Hamilton for providing sheep placental tissue and pituitary glands from the goat and ox.

Dr Charles Small of the Greenlane Hospital Biochemistry Laboratory, Dr Ian Holdaway of the Endocrinology Department, Auckland Hospital and Associate Professor Denys Boshier of the Anatomy Department, Auckland School of Medicine for their helpful advice.

Staff of the Histology Department of Greenlane Hospital.

Mrs June Billings and Mrs Patricia James for their patient typing of the thesis.

Mr Warwick Henshall and Mr Glen Harvey for their help in the computer analysis of the half-life studies.

Members of the Postgraduate School of Obstetrics and Gynaecology for their friendship.

My wife for her patience and tolerance throughout the whole period of this study.

Financial support for these studies was provided by the Medical Research Council of New Zealand.

ABBREVIATIONS

ACTH	adrenocorticotrophic hormone
bGH	bovine growth hormone
bPL	bovine placental lactogen
bPRL	bovine prolactin
BSA	bovine serum albumin
c	centi, 10^{-2}
^{o}C	degree Celsius
Ci	Curie
CM	carboxymethyl
cPL	caprine placental lactogen
cpm	counts per minute
cyclic AMP	adenosine-3',5'-cyclic monophosphate
DEAE	diethylaminoethyl
EDTA	ethylenediaminetetraacetate
Fig., fig.	figure
FL	fetal lamb
FSH	follicle stimulating hormone
g	gram
g	gravitational unit of acceleration
GH	growth hormone
h	hour
H and E	haemotoxylin and eosin
hPL	human placental lactogen
hPRL	human prolactin
^{125}I-hGH	^{125}I-labelled human growth hormone
^{125}I-oPL	^{125}I-labelled ovine placental lactogen
^{125}I-oPRL	^{125}I-labelled ovine prolactin
I.U.	international unit

k	kilo, 10^3
K_{av}	partition coefficient
l	litre
LH	luteinizing hormone
m	milli, 10^{-3}
mA	milliamp
min	minute
M	molar (moles/litre)
n	nano, 10^{-9}
No	number
NPS	non-pregnant sheep
oPL	ovine placental lactogen
oPRL	ovine prolactin
PAS	periodic acid Schiff
PBS	phosphate-buffered saline
$PGF_{2\alpha}$	prostaglandin $F_{2\alpha}$
pH	- log (hydrogen ion concentration)
pI	isoelectric point
PMSG	pregnant mare serum gonadotrophin
PRL	prolactin
PS	pregnant sheep
QAE	quaternary aminoethyl
rPL	rat placental lactogen
s	second
SD	standard deviation
$t_1(\frac{1}{2})$	initial half-life
$t_2(\frac{1}{2})$	final half-life
TCA	trichloroacetic acid
Tris	tris(hydroxymethyl)aminomethane
U	unit
v	volume

V_e	elution volume
V_o	void volume of column
V_t	bed volume of column
w	weight
<	less than
>	greater than
%	percent
μ	micro, 10^{-6}

The standard chemical symbols for elements, salts and ions are also used.

SUMMARY

A procedure was developed to purify ovine placental lactogen (oPL) from fetal cotyledonary tissue of late gestation. A prolactin radioreceptor assay using mammary tissue membranes from the late pregnant rabbit was established and used to monitor lactogenic activity throughout the purification.

The cotyledonary tissue was extracted with 0.1 M NH_4HCO_3 at pH 8.5. The pH of the soluble fraction was reduced to 5.2 and the resulting precipitate discarded. The supernatant was adjusted to pH 7 and ammonium sulphate added to 60% saturation. The lactogenic activity in the ammonium sulphate precipitate was purified further by different column chromatographic procedures to determine the most convenient method for the purification of oPL. In the final method adopted, the ammonium sulphate precipitate was purified by successive chromatography on Sephadex G-100, carboxymethyl (CM) cellulose ion exchange and Sephadex G-100 columns. With this procedure 8 mg of oPL was obtained per kg wet weight of fetal placental tissue. The oPL possessed lactogenic activity equivalent to 1 mg ovine prolactin/mg protein and growth hormone-like activity equivalent to 0.8 mg human growth hormone/mg protein as determined by radioreceptor assays. The biological activity of oPL was confirmed using a rabbit intraductal mammary gland assay in vivo.

Polyacrylamide gel electrophoresis at pH 8.9 resolved oPL into one major and four minor components. All components appeared active in the prolactin radioreceptor assay. On analytical isoelectric focusing oPL had an isoelectric point of 8.2-8.4. Microimmunoelectrophoretic and immunodiffusion studies suggested that the oPL preparation was not contaminated by serum proteins.

Antibodies to oPL were raised in rabbits and immunocytochemical techniques (immunofluorescence and immunoperoxidase) were used to localize the hormone in sheep placental tissue at different stages of pregnancy. In both the cotyledonary and intercotyledonary tissue, the hormone was localized in the binucleate cells. These cells stained positively with the periodic acid Schiff (PAS) stain. In the placentome, the maternal syncytium also showed positive staining for oPL after day 80 of pregnancy. The proportion of binucleate cells showing positive staining for oPL and their distribution in the placentome were studied at different stages of gestation. Although the number of binucleate cells per cotyledon remained fairly constant from day 40 onwards, the number of hormone-containing cells increased between days 80 - 100. Before day 80 few binucleate cells showed positive staining for oPL. The distribution of binucleate cells within the cotyledon showed changes during pregnancy.

Ovine placental lactogen was labelled with radioactive iodine. The iodinated hormone bound to membranes prepared from mammary tissue and liver of the pregnant rabbit and could be displaced by oPL, ovine prolactin (oPRL) and human growth hormone (hGH). The plasma half-life of oPL was studied in pregnant, non-pregnant and fetal sheep after intravenous administration of ^{125}I-oPL. The disappearance of radioactivity in plasma and in trichloroacetic acid precipitates of plasma followed a biphasic exponential pattern with an initial half-life of 6 - 13 min and a second half-life of 44 - 196 min. When ^{125}I-oPL was administered into the fetal circulation, there was minimal transplacental passage of protein-associated radioactivity.

Ovine placental lactogen competed with ^{125}I-hGH for binding to membranes prepared from liver, mammary tissue, ovaries and uterus of the pregnant sheep.

The immunochemical relationship of oPL was investigated by reacting anti-oPL serum with tissue sections of the pituitary glands and placentae from a large number of phylogenetically diverse mammals by the immunofluorescence procedure. Placental extracts from various species were also reacted with anti-oPL serum by immunodiffusion. Positive reaction was obtained with placental sections from the sheep and cow. In addition some staining was observed in the placentae of the hanuman langur and white rhinoceros. There was no cross-reaction against sections from the pituitary glands. By immunodiffusion a strong precipitin line was only observed between anti-oPL serum and sheep placental extracts. These results suggested high specificity of the anti-oPL serum.

Radioreceptor assays for PRL and GH demonstrated the presence of lactogenic and growth hormone-like activities in placental extracts from several species.