DNA synthesis in mammary epithelial cells of Swiss mice during lactation

A thesis presented in partial fulfilment of
the requirements for the degree of
Doctor of Philosophy in Animal Science

at
Massey University
Palmerston North
New Zealand

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2003

Abstract

Proliferation of extra secretory epithelial cells in mammary glands during lactation could potentially increase milk production, with flow-on benefits such as improved weaning weights of young or increased exports from the dairy cow industry. The primary objective of the research reported in this thesis was to increase proliferation of mammary epithelial cells during lactation so that the mechanisms associated with this phenomenon could be studied. Induction of increased proliferation in mammary glands was attempted by applying challenges to mice which were used as a laboratory model for agriculturally important species such as cows and pigs.

The experiments reported in this thesis also included refinement of methodologies developed to study proliferation of secretory cells in mammary glands during lactation. The first was to improve techniques for describing the chemical composition of mammary glands collected during lactation. This was achieved by collecting and analysing the composition of mouse milk at 3 stages of lactation (Chapter 3). While milk protein and milk fat remained constant throughout, the concentration of lactose increased with time. These data were critically important for correcting the weights of mammary glands for milk content. A second investigation was carried out to compare different methods of calculating the milk production of mice (Chapter 3). Three methods were evaluated with the best based on calculating the maintenance energy requirements of the metabolic weight of the litter which was added to the energy required for measured litter growth. The total energy required was then converted to a quantity of milk. The third methodology developed during the course of the work in this thesis was sample preparation for analysis of lactating mammary cells using flow cytometry.

One approach to increasing proliferation of mammary epithelial cells during lactation was to increase the suckling intensity of the mice. This challenge was accomplished by either increasing litter size (Chapter 6) or by increasing the ratio of pups per gland by taping over 5 of the 10 glands (Chapter 5). Suckling intensity was increased to 2 pups per gland but the effect was to accelerate mammary gland development in terms of cell number and milk synthesis status. Once a suckling intensity of >1 pup per gland was reached, there was no additive effect on the size of mammary glands or

milk production at mid lactation. Mammary glands appeared to have a limit on their size and output which is reached at a suckling intensity of 1 pup per gland. Manipulation of suckling intensity did not produce a suitable model of elevated proliferation of mammary epithelial cells during lactation.

Another approach tested was to use exogenous steroids as these had previously caused increased proliferation in mammary glands (Nagasawa and Yanai, 1978; Knight and Peaker, 1982d). The work reported herein showed that the response of mammary glands of mice to administration of steroids was dependent on stage of lactation and the dose (Chapter 4). In mid lactation, mammary glands were unresponsive for the parameters measured but in late lactation, incorporation of [^{3}H] thymidine into DNA increased and milk production decreased in response to higher doses of estrogen. The high estrogen dose did not however yield a suitable model for the study because the elevated incorporation of [^{3}H] thymidine was associated with early involution of mammary glands rather than proliferation leading to a net increase of epithelial cells.

The most promising method of analysis came from histological studies of lactating glands of mice labelled for DNA synthesis. Labelling indices of epithelial cells were >1.5 times greater on the edges of glands on D1 of lactation compared to the inner zones of glands. This within mouse variation was much greater than any between mouse variation arising from the suckling intensity and steroid experiments. An attractive feature is that tissues are derived from the same gland and have therefore been exposed to the same factors such as systemic mitogens and nutrition. In addition, the differences in labelling indices were measured in glands of mice suckling litters of 10 pups which is an easily repeatable treatment compared to some of the more complicated treatments tested during the course of this thesis. Dissection of mammary glands into outer and inner zones could provide useful tissue for the study of local factors involved with increased DNA synthesis of epithelial cells during lactation. Histological studies also revealed that following labelling of mammary epithelial cells for DNA synthesis on the day after parturition, the proportion of cells labelled decrease at a constant rate over the next 23 days (Chapter 8).

This project has increased the knowledge of manipulations of mouse mammary glands during lactation. It was found that growth of mouse mammary glands during lactation is difficult to increase experimentally and may have limited application as a model system to study regulation of growth of mammary glands during lactation. However, the work completed in this thesis will allow similar work to continue, with a high chance of success of investigating factors involved in mitosis of epithelial cells in lactating mammary glands.

Acknowledgements

Thank you for input at various stages from supervisors Associate Professor Dr Duncan Mackenzie (Institute of Food, Human Health and Nutrition, Massey University, New Zealand), Dr Kevin Nicholas (Department of Natural Resources and Environment, Victoria, Australia then University of Melbourne, Australia), Dr Jerome Demmer (AgResearch, Hamilton, New Zealand then Genesis, Auckland, New Zealand) and Dr Tom McFadden (AgResearch, Hamilton, New Zealand then Vermont University, Vermont, United States of America).

Thank you very much to the Dairy Research and Development Corporation of Australia for kindly awarding me the scholarship and for its encouragement. Thank you to AgResearch, Ruakura, New Zealand for providing research facilities and technical support.

A big thank you to the people who taught me new skills and assisted during the write up period. Thank you to Megan Callaghan for teaching me the ropes of the protein chemistry laboratory and the cell culture laboratory. Thank you to Ric Broadhurst, Glenda Smith and Bobby Smith at the Small Animal Colony for teaching me animal handling skills required to conduct my mouse experiments. Thank you to the statisticians and to Dr Neil Cox in particular, for his endless patience. Thank you to the librarians, especially Jean Brown, for her special detective work in sourcing many odd articles. A very appreciative thank you to Dr Chris Morris and the Genetics group, who kindly lent me an office for most of the writing up period.

Thank you to many friends at work who understood the ups and downs experienced. Thank you to lunch time diners Megan, Gina and Angela for their support and friendship. Thank you for the welcomed distractions from the tennis players, Vicki, Kuljeet, Tom, Judy and Megan, the ex-office mates, Megan, Anita and Velletta, and the fellow thesis writers Brett and Sally-Anne. Thank you to my friends and family overseas for their regular contacts by email and telephone.

My biggest thank you is to my three boys. To Martin, my husband, for his continual encouragement and support, and to our two wonderful little boys, Lachlan and Callum, who kept everything in perspective for me. My family is my biggest achievement and I look forward to spending more time with all my boys.

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UNITS

S.I. (Syteme International d'Unites) abbreviations for units are listed below.

cpm	counts per minute
g	rcf = gravitational force
J, kJ, MJ	joule, kilo joule, mega joule
kDa	kilo Dalton
n	number of observations
nm	nano metres
°C	degrees Celcius
rpm	revolutions per minute
sec, min, h, D	second, minute, hour, day
v/v	volume per volume
w/v	weight per volume
µCi	micro Currie
µg, mg, g, kg	micro gram, milli gram, gram, kilo gram
µl, ml, l	micro litre, milli litre, litre
µM, mM, M	micro molar, milli molar, molar

ABBREVIATIONS

[^{3}H] thymidine	[methyl-^{3}H] thymidine
BCA	bicinchoninic acid
BrdU	5-bromo-2'-deoxyuridine
BSA	bovine serum albumin
CDK	cyclin dependent kinase
CMF	calcium magnesium free media
CO_2	carbon dioxide
DAB	3,3'-diaminobenzidine tetrahydrochloride
DNA	deoxyribonucleic acid
DPX	DPX mountant
E	estrogen
EDTA	ethylenediaminetetra-acetic acid
EGF	epidermal growth factor
FCS	fetal calf serum
FdU	5-fluoro-2'-deoxyuridine
FITC	fluorescein isothiocynate
GH	growth hormone
IGF-1	insulin like growth factor, type 1
JAK	Janus kinase
Milli Q	Milli Q water
MOPS	3-N-morpholino propanesulfonic acid
mRNA	messenger ribonucleic acid
MW	molecular weight
NAD	nicotinamide adenine dinucleotide
NADH	nicotinamide adenine dinucleotide, reduced form
P	progesterone

ABBREVIATIONS continued

PBS	phosphate buffered saline
PBSE	phosphate buffered containing EDTA
PBSF	phosphate buffered saline containing formalin
PCNA	proliferating cell nuclear antigen
PE	phyco erythrin
PI	propidium iodide
PL	placental lactogen
PNA	peanut agglutinin
Prl	prolactin
RNA	ribonucleic acid
RSD	residual standard deviation
RT	room temperature
SDS	sodium dodecyl sulphate
SED	standard error of difference
SEM	standard error of the mean
STAT	signal transducer and activator of transcription
TUNEL	terminal deoxynucleotide end labelling
TCA	trichloroacetic acid
TEB	terminal end bud
tris	tris(hydroxymethyl) aminomethane
UV	ultra violet
wt	weight