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The effects of serum, glucocorticoids, gender and gestational age on the inactivation, synthesis and secretion of surfactant phospholipids by cultured foetal rat type II pneumocytes.

by

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Declaration

I declare that this thesis is my own account of my own research and contains as its main content work that has not been previously submitted for the degree at any tertiary education institution.

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Abstract

Type II pneumocytes are responsible for the synthesis and secretion of pulmonary surfactant, which lines the alveoli of the lungs. Its presence as a monolayer at the air-liquid interface in the alveoli diminishes surface tension, thus reducing the tendency of alveoli to collapse during expiration. For this effect to be sustained, however, the integrity of the surfactant components needs to be maintained. In this study it has been demonstrated that when cultured type II pneumocytes are exposed to lipoprotein-free serum (LFS) the level of lyso-phosphatidylcholine (lyso-PC) in the secreted surfactant phospholipids is elevated. The lyso-PC content of secreted phospholipids in serum-supplemented cultures increased 5.4-fold with a concomitant decline in the level of phosphatidylcholine (PC) when compared to cells grown in absence of LFS. This effect is without doubt the result of hydrolysis of surfactant phosphatidylcholine by serum phospholipase A_2 (PLA_2)-like activity. Given that many cases of respiratory trauma are associated with leakage of serum into the alveoli, this enzyme may be a significant contributor to surfactant inactivation and the onset of adult respiratory distress syndrome (ARDS). Subjecting human LFS to DEAE-Sepharose chromatography demonstrated that the enzyme, which stimulates the production of lyso-PC, co-elutes with albumin and is biochemically distinct from the secretory form of PLA_2. Testing of commercially purified human serum albumin (HSA) also showed the presence of this enzymatic activity, indicating that the serum factor not only co-elutes with albumin but is possibly bound to it. This is supported by the fact that, when subjected to polyacrylamide gel electrophoresis, the activity associated with LFS and human serum

albumin (HSA) has an almost identical elution profile. Furthermore, when partially purified preparations of these were subjected to IEF electrophoresis, both yielded a similar double protein-banding pattern. Although the predominant band had similar migratory properties to that of albumin (pI of approximately 4.5) it was not possible, given that the IEF involves a denaturing gel, to assign the PLA_2-like activity to either of these two bands. The present study has also revealed that exposure of type II pneumocytes to recombinant human serum albumin (rHSA) stimulates the secretion of phospholipids and is almost as effective as LFS in enhancing the level of lyso-PC in the media. The latter observation implies that rHSA is able to directly generate lyso-PC and suggests that PLA_2 activity may be an innate activity of albumin.

Neonatal respiratory distress syndrome (NRDS) is a significant cause of mortality in preterm human infants. Because underdevelopment of the lungs is known to contribute to the pathogenesis of NRDS, corticosteroid treatment using synthetic glucocorticoids has been widely used to accelerate maturation of the type II alveolar cells and to prematurely initiate synthesis of surfactant. Dexamethasone has no effect upon the rate of synthesis of surfactant lipids when applied directly to cultured foetal type II cells. However, if type II cells are exposed to medium previously conditioned by cultured lung fibroblasts in the presence of dexamethasone, then there is a significant elevation (17.3% increase) in the rate of surfactant lipid synthesis. This indirect effect of dexamethasone, presumably acting via the production of fibroblast pneumocyte factor (FPF), is significantly greater if both the fibroblasts and type II cells are derived from female foetal rats (47.6% increase in PC synthesis) as compared to those derived from male foetuses (19.0% increase). The present study has confirmed that there are sex-specific and gestational age differences in the glucocorticoid receptor level of lung fibroblasts but not of type II cells, thus, regulation of the appearance of the

glucocorticoid receptor in foetal lungs in the later stages of gestation appears to be both sex- and cell-specific. The interaction of glucocorticoids with their specific receptor has also been shown to indirectly affect the rate of surfactant secretion. This study has demonstrated that β-adrenergic receptors are present and functional in cultures enriched with foetal type II pneumocytes. The β-adrenoreceptor activity is elevated if the type II cells are exposed to glucocorticoids but, as has been shown in this study, the elevation of receptor activity is significantly greater if the type II cells are exposed to media that had been previously conditioned by fibroblasts in the presence of glucocorticoids. Both of these effects are more pronounced if the type II cells were derived from female foetuses rather than from males, despite the cells being grown under similar conditions. The observed elevation in β-adrenergic receptor activity in type II cells exposed to either glucocorticoids or fibroblast-conditioned media has been shown to lead to a greater physiological response to the β-agonist, (—)-isoproterenol, as measured by surfactant lipid secretion. Indeed, there is a very strong correlation ($r = 0.962$) between the β-adrenergic receptor activity and the ability of agonists to stimulate phospholipid secretion from type II cells. Given that the enhanced β-adrenergic receptor activity of type II cells and the resultant cellular response to β-agonists is higher if the cells are treated with FCM rather than with dexamethasone, it is suggested that these glucocorticoid-induced responses may be indirect via an increased release of FPF from lung fibroblasts.

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Abbreviations

ADP	Adenosine 5'-diphosphate
AMP	Adenosine 5'-monophosphate
ATP	Adenosine 5'-triphosphate
ARDS	Adult Respiratory Distress Syndrome
BALF	Bronchioalveolar lavage fluid
BSS	Balanced Salt Solution
cAMP	Adenosine 3',5'-cyclic monophosphate
CoA	Coenzyme A
CDP	Cytidine 5'-diphosphate
CLSE	Calf lung surfactant extract
CRP	C-reactive protein
CTP	Cytidine 5'-triphosphate
DPPC	Dipalmitoyl phosphatidylcholine
DTNB	5,5'-Dithiobis-(2-nitrobenzoic acid)
DG	Diacylglycerol
EGF	Epidermal growth factor
EGTA	Ethylene glycol-bis (β-aminoethyl ether)
GRE	Glucocorticoid Response Element
FPF	Fibroblast pneumocyte factor
GR	Glucocorticoid receptor
GRP	Gastrin-releasing peptide
HDL	High density lipoproteins
HSA	Human serum albumin
HPLC	High performance liquid chromatography
IGF	Insulin-like growth factor
IgG	Immunoglobulin G
LCAT	Lecithin-cholesterol acyltransferase
LFS	Lipoprotein-free serum
MEM	Minimal essential medium

MG	Monoacylglycerol
MS	Mass spectrometry
MW	Molecular weight
NBCS	Newborn calf serum
NRDS	Neonatal Respiratory Distress Syndrome
NRG	Neuregulin
mRNA	Messenger ribonucleic acid
PAF-AH	Platelet-activating factor acetylhydrolase
PBS	Phosphate buffered saline
PC	Phosphatidylcholine
PI	Phosphatidylinositol
PLA_2	Phospholipase A_2
PKC	Protein kinase C
PMA	Phorbol 12-myristate 13-acetate
RDS	Respiratory Distress Syndrome
rHSA	Recombinant human serum albumin
SP	Surfactant-associated protein
VLDL	Very low-density protein

Units

°	degrees
°C	degrees Celsius
%	percentage
AU	absorbance unit
Ci	curie
cm	centimetre
g	centrifugal force
g	gram
kDa	kilodaltons
L	litre
M	moles litre^{-1} (molar)
m	metre
mCi	millicurie
mg	milligram
mL	millilitre
mM	millimoles litre $^{-1}$ (millimolar)
µM	micromoles litre $^{-1}$ (micromolar)
mm	millimetre
nm	nanometre
psi	pound per square mere
µCi	microcurie
µg	microgram
µL	microlitre
µm	micrometre
V	volt
v/v	volume/volume