Nitric Oxide Production in the Mammary Gland

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ABSTRACT

Although the effects of nitric oxide (NO) have been widely studied in many different cell and tissue types, very little is known of the role it plays in the mammary gland. Thus, the production of NO by mammary gland was investigated in a series of experiments. NO is a free radical gas which is produced by a wide variety of cells by the action of the enzyme nitric oxide synthase (NOS) on arginine. This results in the formation of citrulline and NO. The study first examined several methods for their suitability for the detection of NO or NOS. Evaluation of the methods revealed that the indirect measurement of NO production by the detection of nitrate and nitrite (NOx), the spontaneously produced metabolites of NO, was the most valid and reliable. The measurement of NOx was carried out in culture medium using a fluorescent-based assay, which was developed by the modification of published methods, during the course of this study. Comma-D cells (murine mammary epithelial cell line) were used to investigate the production of NOx by the inducible form of NOS (iNOS) following treatment with cytokines and cytotoxins. The cell's response was characterised and showed that mammary epithelial cells produce NOx in a dose dependent manner in response to interferon-γ (IFN-γ). Lipopolysaccharide (LPS), a component of bacterial cell walls, was employed to examine the response of the mammary epithelial cells to cytotoxins and it was found that the treated cells produced more NOx than the untreated ones, however, no dose response was apparent. The specific iNOS inhibitor, aminoguanidine (AG) and general NOS inhibitor N^{ω}-nitro-L-arginine (L-NNA) were both used to confirm that the NOx measured in the medium was produced by NOS. The production of NOx by the mammary gland was also examined in cultured explants of mammary tissue taken from pregnant (D 12-14 of pregnancy) and lactating (D 12-14 postpartum or D 17-18 postpartum) rats. A significant difference was found in the basal production of NOx between the different developmental stages. The method of euthanasia of the rats also affected the amount of NOx produced. The inclusion of prolactin (PRL) also increased the production of NOx from both Comma-D cells and explants of mammary tissue. Xanthine oxidase (XO), an enzyme responsible for the conversion of NOx to NO under anaerobic conditions, does not interfere with the determination

of NO production using the NOx assay. The measurement of NOx was carried out in the milk of cows following the intramammary infusion of *Streptococcus uberis* or interleukin-1β (IL-1β). By comparing the milk NOx concentration with the somatic cell count (SCC) and electrical conductivity (EC) of the milk, it was concluded that the source of the NOx in the milk could not be attributed entirely to the epithelium or the somatic cells. The experiments in this Thesis clearly show that the mammary gland is capable of the production of NO in response to a variety of situations and that the regulation of the production is very complex. The work also identifies some new areas of research, which if completed would further enhance the understanding of the role NO plays in the mammary gland.

For Grant,

My life, my love and my best friend

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ACh	acetylcholine
AG	aminoguanidine
cAMP	cyclic adenosine monophosphate
ANCOVA	analysis of covariance
ANOVA	analysis of variance
APS	ammonium persulphate
ATP	adenosine triphosphate
BAEC	bovine aortic endothelial cell
BCA	bicinchoninic acid
BMM	bone marrow derived monocytes
BSA	bovine serum albumin
CaM	calmodulin
CHAPS	3-[(3-cholamidopropyl)-dimethylammonio]-1-propane-sulfonate
CHX	cyclohexamide
cfu	colony forming units
D	day
DMEM	Dulbecco's modified Eagle's medium
DMEM:F12	Dulbecco's modified Eagle's medium:nutrient mixture F12
DMSO	dimethyl sulphoxide
DNA	deoxyribonucleic acid
cDNA	complementary deoxyribonucleic acid
EC	electrical conductivity
EDRF	endothelium derived relaxing factor
EDTA	ethylenediaminetetraacetic acid
EGTA	ethylene glycol-bis(β-amino-ethyl ether)
EGF	epidermal growth factor
FCS	foetal calf serum
bFGF	basic fibroblast growth factor
GLM	general linear model
HEPES	N-2-(hydroxyethyl) piperazine-N'-2-ethanessulfonic acid

HUVEC	human umbilical vein endothelial cells
IFN-γ	interferon-γ
hIFN-γ	human recombinant interferon-γ
mIFN-γ	mouse recombinant interferon-γ
rIFN-γ	rat recombinant interferon-γ
IL-1β	interleukin-1β
kb	kilobases
kDa	kilodaltons
LDL	lower detection limit
L-NAME	N^G-nitro-L-arginine methyl ester
L-NMMA	N^G-monomethyl-L-arginine
L-NNA	N^ω-nitro-L-arginine
LPS	lipopolysaccharide
MBF	mammary blood flow
MOPS	3-[N-morpholino]propane-sulphonic acid
n	number of samples
NADPH	β-Nicotinamide adenine dinucleotide phosphate, reduced form
NF-κB	nuclear factor-κB
NO	nitric oxide
NOS	nitric oxide synthase
cNOS	constitutive nitric oxide synthase
eNOS	endothelial nitric oxide synthase
iNOS	inducible nitric oxide synthase
nNOS	neuronal nitric oxide synthase
NOx	nitrite plus nitrate
OD	optical density
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
PBSE	phosphate buffered Saline & EDTA
$PGF_{2\alpha}$	prostaglandin-$F_{2\alpha}$
PMSF	phenylmethylsulfonyl fluoride
PRL	prolactin
PVP	polyvinylpyrrolidone

R^2	coefficient of determination
RNA	ribonucleic acid
mRNA	messenger ribonucleic acid
rRNA	ribosomal ribonucleic acid
SAS	Statistical Analysis System
SCC	somatic cell count
SDS	sodium dodecyl sulphate
SEM	standard error of the means
TBS	tris-buffered-saline
TEMED	N,N,N',N'-tetramethylethylenediamine
TPA	12-*O*-Tetradecanoylphorbol 13-acetate
Tris	tris(hydroxymethyl)aminomethane
TNF-α	tumor necrosis factor-α
VSMC	vascular smooth muscle cells
x *g*	times gravity
XD	xanthine dehydrogenase
XO	xanthine oxidase
XOR	xanthine oxidoreductase