Regulation of Glucose Transporters in Sheep Placenta

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ABSTRACT

Transplacental glucose transport is vital to fetal growth. Although the presence of glucose transporter-1 (GLUT1) and GLUT3 has been demonstrated in mammalian placenta, the factors regulating these genes remain unclear. Therefore, the overall aim of these studies was to clone ovine GLUT1 (oGLUT1) and oGLUT3 cDNAs, and to use these to investigate gene expression during ovine placental development and function.

Ovine GLUT1 (~2.2 kb) and oGLUT3 (483 bp) cDNAs were isolated and cloned. Sequence analysis demonstrated that oGLUT1 showed high homology (97 – 99%) with other mammalian species, whereas oGLUT3 did not (84 – 88%).

Northern analysis demonstrated that oGLUT1 mRNA abundance increased from d 45 to d 120 of gestation, then decreased towards term (d 145 ± 2), whereas oGLUT3 mRNA abundance increased throughout gestation. Western analysis showed oGLUT1 protein levels increased during late gestation, indicating post-transcriptional regulation of oGLUT1.

Localisation experiments revealed spatio-temporal differences in ovine placental GLUT expression. In early gestation (d 45), oGLUT1 protein was restricted to fetal trophoblast cells. By mid gestation oGLUT1 immuno-signal was predominantly localised to maternal villous and endometrial tissue. By late gestation oGLUT1 mRNA was most strongly localised to maternal syncytiotrophoblast and villous tissue, whereas oGLUT3 was predominantly localised to fetal trophoblast cells.

Placental oGLUT expression was regulated differently by acute (3 – 8 h) versus long-term (> 6 d) alterations in late gestation maternal glucose supply. No evidence was found for regulation of placental oGLUT gene expression by long-term maternal undernutrition, but oGLUT1 and oGLUT3 mRNA and oGLUT1 protein were elevated by short-term (24 – 48 h) maternal hypoglycemia. Acute maternal hyperglycemia transiently increased oGLUT1 and oGLUT3 mRNA abundance, whereas oGLUT1 protein (but not mRNA) levels increased after long-term maternal hyperglycemia.

Infusion studies provided no conclusive evidence for regulation of placental oGLUTs by long-term administration of growth hormone (GH) or insulin-like growth factor-1 (IGF-1) to the late gestation fetus. Following acute (4 h) fetal IGF-1 infusion, placental oGLUT3 mRNA

abundance was greater in growth restricted (placental embolisation) than in normal fetuses, although the reason for this difference remained equivocal.

This thesis describes isolation, cloning and sequence analysis of oGLUT1 and oGLUT3 cDNAs. These studies confirmed the presence of GLUT1 and GLUT3 mRNA in ovine placenta, and demonstrated ontogenetic and nutritional regulation of placental oGLUT1 and oGLUT3. In addition, these results indicated that regulation of placental oGLUTs may occur at both transcriptional and post-transcriptional levels.

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LIST OF ABBREVIATIONS

aa	amino acid
ACTH	adrenocorticotropic hormone
Ad Lib	ad libitum
ANOVA	analysis of variance
ARNT	aryl hydrocarbon nuclear translocator
ATP	adenosine-5'-triphosphate
BM	basal membrane
bp	base pair
BNC	binucleate cells
cAMP	cyclic adenosine-3',5'-monophosphate
CBS	carbonate buffered saline
cDNA	complementary DNA
cpm	counts per minute
CTP	cytidine-5'-triphosphate
d	day(s)
ddH_2O	double distilled water
Da	dalton
DAB	3'5' diaminobenzidine
DEPC	diethyl pyrocarbonate
DNA	deoxyribonucleic acid
ss DNA	salmon sperm DNA
dNTP	deoxyribonucleotide mix
DPX	distrene, dibutyl phthalate, xylene (mounting media)
ds	double stranded
DTT	dithiothreitol
ECL	enhanced chemiluminescence
E. coli	Escherichia coli
EDTA	ethylenediaminetetraacetic acid
FFA	free fatty acid
FRM	rich bacterial medium
g	gram (weight) or gravity (centrifugation)
GDM	gestational diabetes mellitus
GH	growth hormone
GLUT	glucose transporter
GTE	glucose, Tris, EDTA
GTP	guanidine-5'-triphosphate
h	hour(s)
HEPES	N-2-hydroxyethylpiperazine-N'-2-ethane sulphonic acid
IDDM	insulin dependent diabetes mellitus
IGF	insulin-like growth factor
IGFBP	insulin-like growth factor binding protein
IM	internal membrane protein fraction
ISH	in situ hybridisation
IPTG	isopropyl-β-D-thio-galactopyranoside
IUGR	intrauterine growth restriction
k	(prefix) kilo- (10^3)
kb	kilobase
LB	Luria Bertani media
μ	(prefix) micro- (10^{-6})
μCi	microCurie
m	(prefix) milli- (10^{-3})
min	minute(s)
M	mol/litre

MOPS	3-[N-Morpholino]propanesulfonic acid
mRNA	messenger ribonucleic acid
MVM	microvillous membrane
MW	molecular weight
n	(prefix) nano- (10^{-9}) or n = number of samples
NIDDM	non-insulin dependent diabetes mellitus
NRS	non-immune rabbit serum
p	(prefix) pico- (10^{-12})
p	p value (statistical)
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PEG	polyethylene glycol
pfu	plaque forming units
PM	plasma membrane protein fraction
PMSF	phenylmethylsulphonyl fluoride
Poly A+	polyadenylic acid
RCDMB	Research Centre for Developmental Medicine and Biology
RF	refeeding
RIA	radioimmunoassay
RNA	ribonucleic acid
RNase	ribonuclease
RPA	ribonuclease protection assay
rpm	revolutions per minute
rRNA	ribosomal ribonucleic acid
RT	room temperature
RT-PCR	reverse transcriptase polymerase chain reaction
SD	standard deviation
SDS	sodium dodecyl sulfate
sec	second(s)
SEM	standard error of the mean
SOB	bacterial medium
ss	single stranded
SSC	saline sodium citrate buffer
SSPE	saline sodium phosphate EDTA buffer
STZ	streptozotocin
TBE	tris, borate, EDTA
TBS	tris buffered saline
TE	tris EDTA buffer
TM	total post-nuclear protein fraction
Tris	tris[hydroxymethyl]aminomethane
tRNA	transfer ribonucleic acid
TTP	thymidine-5'-triphosphate
U	units
UN	undernutrition
UTP	uridine-5'-triphosphate
UTR	untranslated region
w/v	weight to volume ratio
w/w	weight to weight ratio