OXYGEN SENSING IN THE SHEEP ADRENAL MEDULLA

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December 2002.

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THESIS SUMMARY

In the fetus, prior to the development of adrenal innervation, hypoxia acts directly on the adrenal medulla to stimulate catecholamine secretion which triggers a set of physiological responses that are imperative for intrauterine survival. This direct response to hypoxia is suppressed upon development of the splanchnic innervation, but reappears if the gland is denervated. In the glomus cells of the carotid body, hypoxia evokes the release of dopamine by closing K_{O_2} channels, leading to membrane depolarisation, Ca^{2+} entry through voltage-gated Ca^{2+} channels and subsequent catecholamine secretion. K_{O_2} channels also exist in the adrenal medulla, but it is unknown whether these channels play a role in initiating the response by depolarising the cell or what intracellular mechanisms enable O_2 levels to control K_{O_2} channel function. The means by which adrenal gland innervation is able to suppress the direct hypoxic response also remains unclear, though it is likely due to the actions of a substance released from the nerve terminals, as the direct hypoxic response returns rapidly upon denervation of the gland.

The aims of this study therefore, were: 1) to identify the channel(s) which cause the responses to hypoxia observed in the adrenal medulla. 2) investigate the different types of Ca^{2+} channels which are present, their contribution to Ca^{2+} entry and whether any particular Ca^{2+} channels modulate K^+ channels in these cells. 3) find the intracellular pathways which transmit the decrease in extracellular O_2 levels to the membrane bound ion channels. 4) find whether the actions of opioid peptides, which are released from nerve terminals innervating the adrenal medulla, account for the suppression of the direct response upon innervation by altering either K^+ or Ca^{2+} channel function.

The whole-cell patch clamp method was utilised to measure Ca^{2+} currents, characterise the K_{O_2} channel(s) and identify their contribution in initiating membrane

depolarisation, to study the modulation of K^+ and Ca^{2+} currents by opioid agonists, and look at the effect of reactive oxygen species (ROS) levels on K^+ current. Fluorescence of Fluo-3 AM loaded cells was used to measure changes in intracellular Ca^{2+} levels caused by K^+ channel blockers, solutions containing high external $[K^+]$, or during hypoxia in the presence or absence of opioid receptor agonists.

In fetal adrenal chromaffin cells, SK and BK channels were both identified as O_2-sensitive. During episodes of hypoxia, closure of SK channels lead to depolarisation of the cell while closure of BK channels potentiated the entry of Ca^{2+} initiated by SK channel closure. While these cells were found to contain L-, N-, P/Q- and T-type Ca^{2+} currents, there was not a specific association of Ca^{2+} influx through any one of these channels and activation of $K_{(Ca)}$ current. Disruption of mitochondrial function reduced the response of chromaffin cells to hypoxia, most likely because of a reduction in ROS production, indicating that the mitochondria act as an O_2-sensor in these cells The stimulation of μ- and κ-type opioid receptors decreased the hypoxia-evoked $[Ca^{2+}]_i$ increase in single cells and abolished hypoxia-induced catecholamine secretion from the whole perfused adrenal gland. It appears that alterations in the resting membrane potential and reduced activation of voltage-dependent Ca^{2+} channels accounted for the actions of these opioid agonists as both μ- and κ-type receptor activation similarly decreased Ca^{2+} current and μ-type activation increased K^+ conductance to such a degree as to offset the decrease in K^+ conductance during hypoxia. The application of apamin blocked this effect, revealing that μ-type opioid receptor activation increases SK channel conductance. As SK channels have been found to close during hypoxia and initiate membrane depolarisation, the increased opening of these channels by μ-type opioid receptor stimulation provides a logical explanation for the suppression of the direct hypoxic response upon innervation of the adrenal medulla.

COMMONLY USED ABBREVIATIONS

A B

4-AP	4-aminopyridine
A	Adrenergic
ACh	Acetylcholine
ACTH	Aderenocorticotrophic hormone
AMCC	Adrenal medullary chromaffin cells
Ang II	Angiotensin II
ANOVA	Analysis of variance
AP	Action potential
ATP	Adenosine triphosphate
BK	Large conductance calcium dependent potassium channel
BK_i	Non-inactivating BK channel
BK_s	Fast-inactivating BK channel

C D

$[Ca^{2+}]_i$	Intracellular calcium concentration
cAMP	Cyclic adenosine 3', 5'-monophosphate
CO	Carbon monoxide
DALDA	[D-Arg^2, Lys^4]-dermorphin-(1-4)-amide H-Tyr-DArg-Phe-Lys-NH_2
DHEA	Dehydroepiandrosterone

DHP	Dihydropyridine
DMEM	Dulbecco's modified Eagles medium
DNA	Deoxyribose nucleic acid
DPDPE	[D-Pen^{2,5}]-enkephalin
DPI	Diphenylene iodonium
DTT	Dithiotreitol

E F G

EGTA	Ethylene glycol-bis(beta-aminoethyl ether)-N,N,N',N'-tetraacetic acid
Enk	Enkephalin
E_{rev}	Reversal potential
ETC	Electron transport chain
FITC	Fluorescein isothiocyanate
GSH	Reduced glutathione
GSSH	Oxidised glutathione

H

HEPES	N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid)
HPV	Hypoxic pulmonary vasoconstriction
HO	Heme oxidase
HO-1	Heme oxidase-1
HO-2	Heme oxidase-2
HVA	High voltage activating

Hz	Hertz

I K L

IC_{50}	Half the maximum inhibiting concentration
IP_3	Inositol trisphosphate
IK	Intermediate conductance potassium channel
I_{KN}	Non-inactivating potassium current
I-V	Current-voltage
$[K^+]$	Potassium concentration
K_{ATP}	ATP sensitive potassium channel
$K_{(Ca)}$	calcium-dependent potassium channel
KO_2	Oxygen-sensitive potassium channel
K_m	Substrate concentration for ½ the maximum rate of the reaction
Kv	Voltage-gated potassium channel
LVA	Low voltage activating

M N

MΩ	Megaohm
μM	Micromolar
mAChR	Muscarinic acetylcholine receptor
mM	Millimolar
mmHg	Millimeters of mercury
mRNA	Messenger ribonucleic acid

ms	Millisecond
mV	Millivolt
NA	Noradrenergic
nAChR	Nicotinic acetylcholine receptor
NADH	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
NDS	Normal donkey serum
NEB	Neuroepithelial body
nm	Nanometer
NPPB	5-Nitro-2-(3-phenylpropylamino) benzoic acid
NO	Nitric oxide
NOS	Nitric oxide synthase
nS	Nanosiemens

O P O R

OCT	Optimal cutting temperature compound
pA	Picoamperes
PACAP	Pituitary adenylate cyclase activating peptide
PASMC	Pulmonary artery smooth muscle cell
PBS	Phosphate buffered saline
PCMBS	P-chloromercuribenzene sulphonic acid
PKA	Protein kinase A
PKC	Protein kinase C
PLC	Phospholipase C

PNMT	Phenylethanolamine N-methyl transferase
Po_2	Partial pressure of oxygen
pS	Picosiemens
PVR	PACAP/VIP receptors
ROS	Reactive oxygen species

S T U V

SEM	Standard error of mean
SGC	Small granule-containing cells
SK	Small conductance calcium-dependent potassium channel
SOD	Superoxide dismutase
SP	Substance P
STREX	Stress axis-regulated exon
TEA	Tetraethylammonium
TH	Tyrosine hydroxylase
TRITC	Tetramethylrhodamine isothiocyanate
TTX	Tetrodotoxin
V_{max}	Maximum velocity of an enzyme catalysed reaction
VGCC	Voltage-gated calcium channel
VIP	Vasoactive intestinal polypeptide