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GENE EXPRESSION IN PRESYMPATHETIC NEURONS OF THE ROSTRAL VENTROLATERAL MEDULLA OF THE RAT

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ABSTRACT

The rostral ventrolateral medulla (RVL) contains a population of bulbospinal neurons which are critically involved in the tonic and reflex control of blood pressure. They project to the intermediolateral cell column of the thoracolumbar spinal cord and excite sympathetic preganglionic neurons. The RVL region also contains the C1 adrenergic group of neurons, some of which are also bulbospinal RVL vasomotor neurons. The objective of this study was to investigate RVL neurons at the cellular and molecular level, to gain a better understanding of the properties of this heterogeneous population of neurons. Two main approaches were used to achieve this. The first studied the expression of several genes in RVL micropunches and single acutely dissociated RVL neurons using the reverse transcription-polymerase chain reaction (RT-PCR). The second utilised antisense oligodeoxyribonucleotides (aODNs) to reduce expression of two adrenaline-synthesising genes, tyrosine hydroxylase (TH) or phenylethanolamine N-methyltransferase (PNMT), in C1 adrenergic neurons in the RVL.

Semi-nested RT-PCR analysis of tissue micropunched from the RVL region of adult rats established the expression of mRNA transcripts for glyceraldehyde 3-phosphate dehydrogenase (GAPDH), neuron-specific enolase (NSE), TH, PNMT, the glucocorticoid receptor (GR), mineralocorticoid receptor (MR), noradrenaline transporter (NET), glycine transporter 2 (GLYT2), neuronal glutamate transporter (EAAC1) and glial glutamate transporter (GLT). The expression of mRNA for glutamic acid decarboxylase (GAD67) and the gamma-amino butyric acid (GABA) transporter (GAT-1) was also detected in rat brainstem. The micropunch technique combined with RT-PCR offers a simple non-radioactive way to identify genes being expressed in the RVL and other medullary regions.

Single neurons were acutely dissociated from the RVL of 13 - 21 day old rats, and classified as spinally projecting or non-spinal by the presence or absence of retrogradely-transported fluorescent beads injected into the upper thoracic segments. Dissociated bulbospinal RVL neurons did not exhibit any spontaneous firing, and there was no evidence of any pacemaker-like properties. A fast-activating (spike-like) inward current could be elicited by depolarising voltage steps, and could be abolished by application of tetrodotoxin. Extracellular application of either kainic acid or L-glutamic acid onto RVL cells under voltage-clamp conditions elicited dose-dependent inward currents. Individual neurons were collected by aspiration into a glass

micropipette and the cell contents analysed by RT-PCR. Detection of either GAPDH or NSE mRNA was used as the criterion for selecting cells for further analysis, and 80% of the neuron samples tested expressed one of these genes. A subpopulation of spinally-projecting neurons expressed PNMT or TH (50%), indicating that they were catecholaminergic. Bulbospinal RVL neurons were also found to express mRNA for the MR (45% of those tested), the GR (5%), NET (10%), and EAAC1 (58%). GLT, GAD67 and GAT-1 were not expressed in any bulbospinal neurons, but they were detected in 5 - 10% of non-spinal neurons tested. Expression of mRNA for MR, EAAC1 and NET was also observed in subpopulations of PNMT-positive and -negative bulbospinal neurons. Expression of NET protein, assessed by immunohistochemistry, was found to be similar to NET mRNA expression in C1 adrenergic neurons in the RVL.

These results indicate that single-cell RT-PCR is a powerful method for elucidating the functional characteristics of a defined neuronal population, and that it is possible to perform whole-cell patch-clamp recording prior to RT-PCR analysis, allowing linkage of the molecular analysis of mRNA expression to the electrophysiological properties of these neurons. The method is very sensitive, enabling low copy-number mRNA transcripts to be detected, and can be used to provide key information about blood-pressure regulation at the molecular and cellular level.

The final part of this study used aODNs to attempt to knockdown expression of TH or PNMT in C1 neurons in the RVL. The aODNs were either injected or infused unilaterally into the RVL of adult rats *in vivo*, and 4 - 14 days later the animals were sacrificed and the expression of TH or PNMT protein assessed using immunohistochemistry. Immunoreactivity for TH was unchanged by injection or infusion of an aODN targeting TH mRNA. Immunoreactivity for PNMT was decreased in 2 out of 10 infusion experiments, but this was achieved using an aODN with one base pair mismatch to the PNMT mRNA sequence, and could not be repeated with aODNs of the correct sequence. This study was the first attempt to target the PNMT gene with aODNs, and although preliminary in nature, it provided some useful information for future studies of this system. Antisense ODN suppression of genes in the RVL may prove to be an interesting future avenue for investigating the functional significance of specific genes being expressed in the RVL region.

PREFACE

Some of the contents of this thesis have been prepared for publication elsewhere as articles or abstracts:

ARTICLES

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ABBREVIATIONS

11β-HSD	11β-hydroxysteroid dehydrogenase
ACSF	artificial cerebrospinal fluid
ALDO	aldosterone
aODN	antisense oligodeoxyribonucleotide
AP	area postrema
bNET	bovine noradrenaline transporter
bp	base pair(s)
cDNA	copy deoxyribonucleic acid
C_m	membrane capacitance
CNS	central nervous system
CO_2	carbon dioxide
CORT	corticosterone
CSF	cerebro-spinal fluid
CVL	caudal ventrolateral medulla
DAB	diaminobenzidine
DEX	dexamethasone
DNA	deoxyribonucleic acid
dNTPs	deoxyribonucleotide tri-phosphates
DTT	dithiothreitol
EAAC1	neuronal glutamate transporter
EDTA	ethylenediamine tetra-acetic acid
EGTA	ethyleneglycol-bis-(β-aminoethyl ether) N, N, N', N'-tetra-acetic acid
EPSP	excitatory postsynaptic potential
fET	frog adrenaline transporter
FITC	fluorescein isothiocyanate
GABA	gamma-amino butyric acid
GAD	glutamic acid decarboxylase
GAD67	isoform of GAD
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
GAT-1	GABA transporter

GLT	glial glutamate transporter
GLYT2	glycine transporter
GR	glucocorticoid receptor
GRE	glucocorticoid response element
GST	glutathione-S-transferase
h	hour(s)
HEPES	*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid
hNET	human noradrenaline transporter
i.c.v	intracerebroventricular
i.m.	intramuscular
i.p.	intraperitoneal
i.t.	intrathecal
i.v.	intravenous
I_A	transient potassium current
IgG	immunoglobulin G
I_K	sustained potassium current
IML	intermediolateral cell column
IPSP	inhibitory postsynaptic potential
K_a	affinity constant
K_m	Michaelis constant
LC	locus coeruleus
min	minute(s)
MR	mineralocorticoid receptor
mRNA	messenger ribonucleic acid
MW	molecular weight
NA	nucleus ambiguus
NAD	nicotinamide adenine dinucleotide
NET	noradrenaline transporter
NET-IR	NET-immunoreactivity
NMDA	N-methyl-D-aspartate
NPY	neuropeptide Y
NSE	neuron-specific enolase
nt	nucleotides

NTS	nucleus of the solitary tract
O_2	oxygen
ODN	oligodeoxyribonucleotide (or oligonucleotide)
PAG	midbrain periaqueductal gray
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
PNMT	phenylethanolamine N-methyltransferase
R_N	membrane resistance
rpm	revolutions per minute
R_s	access resistance
RT	reverse transcription
RVL	rostral ventrolateral medulla
s	second(s)
SPNs	sympathetic preganglionic neurons
SRF	subretrofacial nucleus
STN	spinal trigeminal nucleus
TEA	tetraethylammonium chloride
TH	tyrosine hydroxylase
TH-IR	TH-immunoreactivity
T_m	melting temperature
Tris	tris[hydroxymethyl]-aminomethane
TRITC	tetramethyl-rhodamine isothiocyanate
TTX	tetrodotoxin
U	units of activity
V_h	holding potential
XIIn	hypoglossal nucleus
°C	degrees Celsius