The Role of Serotonin-2C Receptors in the Rat Circadian System

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TABLE OF CONTENTS

ABSTRACT

The suprachiasmatic nucleus receives dense serotonergic projections from the raphe nuclei and this input has been implicated in the modulation of circadian rhythms. This input appears to have many functions including the transmission of non-photic information during the day and the modulation of photic information at night. However, it has emerged that this input may also be involved in the transmission of light information with activation of 5-HT_{2C} receptors at night having a photo-mimetic effect. The studies described in this thesis aim to clarify the role of 5-HT_{2C} receptors in the control of circadian rhythms in the rat model and compare their actions to light.

The acute effects of 5-HT_{2C} receptor agonist administration on clock gene expression were investigated in the rat SCN. Systemic administration of the 5-$HT_{2A/2C}$ agonist DOI to rats during early night induced *c-fos*, *Per1* and *Per2* expression in a manner similar to light. This response was time of day dependent with maximal induction occurring in the early night, and no response during the day. The role of 5-HT_{2C} receptors in this response was confirmed with the use of the selective 5-HT_{2C} receptor agonist RO-60 0175.

The effect of 5-HT_{2C} receptor activation on the phase of expression of various circadian rhythms including temperature, melatonin and clock gene expression in the SCN and periphery was examined. Both DOI administration and light exposure at night phase delayed rhythms of melatonin and temperature. Similarly, the selective 5-HT_{2C} receptor agonist RO-60 0175 phase delayed rhythms of 6-sulphatoxymelatonin, a response which was antagonised by the 5-HT_{2C} receptor antagonist SB-242084. The expression of functional and clock genes within the pineal was also phase delayed following both light and 5-HT_{2C} receptor agonist administration. However, the phase of expression of clock genes within the

SCN or liver did not shift in response to either a single nocturnal light pulse or agonist administration.

To investigate the site of action of 5-HT_{2C} receptor agonists, rat SCN explants were maintained in culture allowing exposure of agonists to denervated tissue. The acute effect of DOI administration at various circadian times on *c-fos* and *Per1* expression was assessed. 5-HT_{2C} receptor activation significantly increased *Per1* expression when administered during early subjective night, but had no effect during either subjective day or late subjective night, similar to that observed *in vivo*.

Finally, the suitability of immortalised rat SCN cells for investigation of the intracellular actions of 5-HT_{2C} receptors in the circadian system was assessed. Using RT-PCR the expression of various serotonin receptors in the SCN2.2 cell line was compared with that observed in punches of adult rat SCN. The mRNA for 5-HT_{1B} and 5-HT_{2A} receptor was expressed in both the SCN2.2 cell line and the adult rat SCN. However, 5-HT_{2C} receptor mRNA along with 5-HT_3 receptor, 5-HT_{5A} receptor and 5-HT_7 receptor mRNA was expressed in the adult rat SCN tissue but not the SCN2.2 cells. These significant differences in serotonin receptor expression limit the usefulness of this cell line for further investigation.

Together these experiments further implicate 5-HT_{2C} receptors in the control of circadian rhythms. The role of these receptors appears limited to early night, with activation showing photo-mimetic responses. Furthermore, the location of action appears to be post-synaptic within the SCN, altering the core clock genes, which in turn phase delay various circadian rhythms.

DECLARATION

This work contains no material which has been accepted for the award for any other degree or diploma in any university or any other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I further grant my consent to the University of Adelaide to make this thesis available for loan and photocopying once accepted for the degree.

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PUBLICATIONS AND ABSTRACTS ARISING FROM THESE STUDIES

Publications:

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Varcoe, T.J. and Kennaway, D.J. (in preparation)

Activation of 5-HT_{2C} receptors acutely induces *Per* gene expression in the rat suprachiasmatic nucleus in vitro.

Abstracts:

Varcoe, T.J. and Kennaway, D.J. "5-HT_{2C} receptors and the transmission of photic information at night: site of action". Australasian Chronobiology Society International Conference, From Molecular Clocks to Human Health, Adelaide, Australia, 2007

Varcoe, T.J. and Kennaway, D.J. "The role of 5-HT_{2C} receptors in the rat circadian system". 1st Annual Meeting of the Australasian Chronobiology Society, Robe, South Australia, Australia, 2004.

Varcoe, T.J., Kennaway, D.J. and Voultsios, A. "The effects of 5-HT_{2C} receptor agonists on clock gene expression in the rat suprachiasmatic nucleus". Japan 1st World Congress of Chronobiology, Sapporo, Japan, 2003

Varcoe, T.J., Kennaway, D.J. and Voultsios, A. “Manipulating circadian rhythms with a serotonin agonist”. Australian Society for Medical Research. National Scientific Meeting, Adelaide, Australia, 2003.

Varcoe, T.J., Kennaway, D.J. and Voultsios, A. “Serotonin receptors and circadian rhythms”. Australian Society for Medical Research. South Australian Annual Scientific Meeting, Adelaide, Australia, 2003

TABLE OF FIGURES

ABBREVIATIONS

5,7-DHT	5,7-dihydroxytryptamine
5-HT	5-hydroxytryptamine (serotonin)
AA-NAT	Arylalkylamine N-acetyltransferase
AMPA	alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANOVA	Analysis of variance
ARNT	aryl hydrocarbon receptor nuclear translocator
AVP	Arginine vasopressin
aMT.6S	6-sulphatoxymelatonin
bHLH	basic helix loop helix
BLAST	Basic local alignment search tool
BMAL1	Brain muscle ARNT like protein 1
CaM	Calcium calmodulin
cAMP	cyclic adenosine monophosphate
CBP	CREB binding protein
cDNA	complementary deoxyribonucleic acid
cGMP	cyclic guanosine monophosphate
CK1ε	Casein kinase 1 epsilon
CLOCK	Circadian locomotor output cycles kaput
CNS	Central nervous system
CRE	cAMP response element
CREB	cAMP response element binding protein
CRY	Cryptochrome
CT	Circadian time
Ct	Cycle threshold
DAG	Diaminoglycerol

DAPI	4',6-diamidino-2-phenylindole
DBP	Albumin D-site binding protein
df	degrees of freedom
DMH	Dorsomedial hypothalamus
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
DOB	(-)-1-2, 5-dimethoxy-4-bromophenyl-2-aminopropane
DOI	1-(2,5-dimethoxy-4-iodophenyl)-propan-2-amine
DTT	Dithiothreitol
EPSC	Excitatory post synaptic current
FBS	Fetal bovine serum
GABA	Gamma-aminobutyric acid
GHT	Geniculohypothalmic tract
IGL	Intergeniculate leaflet
IP_3	Inositol tri-phosphate
MAP2	Microtubule associated protein 2
MAPK	Mitogen activated protein kinase
mCPBG	m-Chlorophenylbiguanidine
mRNA	messenger ribonucleic acid
MT1	Melatonin receptor 1
NAT	N-acetyltransferase
NeuN	Neuronal Nuclei
NMDA	N-methyl-D-aspartic acid
NOS	Nitrous oxide synthase
NPY	Neuropeptide Y
NSB	Non-specific binding
NSE	Neuron specific enolase

OC	Optic chiasm
PACAP	Pituitary adenylate cyclase-activating peptide
PAS	Per-ARNT-Sim
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
pCREB	phosphorylated cAMP response element binding protein
pCPA	p-Chlorophenylalanine
PDE	Phosphodiesterase
PER	Period
PGP	Protein gene product 9.5
PK2	Prokineticin2
PKC	Protein kinase C
PKG	Protein kinase G
PLC	Phospholipase C
PRC	Phase response curve
PVN	Paraventricular nucleus
RHT	Retinohypothalamic tract
RNA	Ribonucleic acid
RORα	retinoic acid-receptor-related orphan receptor alpha
RORE	retinoic acid-receptor-related orphan receptor response element
RT-PCR	Reverse transcription polymerase chain reaction
RyR	Ryanodine receptor
SAD	Seasonal affective disorder
s/c	subcutaneous
SCN	Suprachiasmatic nucleus
TBE	Tris-borate-EDTA
TGFα	Transforming growth factor alpha

TTX	Tetrodotoxin
VIP	Vasoactive intestinal peptide
ZT	zeitgeber time