Studies on the neuroendocrine role of serotonin

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1. INTRODUCTION

Serotonin (5-hydroxytrayptamine; 5-HT) is a neurotransmitter widely synthesised in the central nervous system (CNS) and is also found in gastrointestinal mucosa cells and blood platelets (Peroutka & Howell 1994). Serotonin is involved in the regulation of the central neuroendocrine system as well as in cognitive functions, mood and basal physiological functions (Van de Kar 1991). Dysfunction of the intra- and interneuronal 5-HT transmitter systems may result in impairment in coping with states of increased stress, cognitive dysfunction and eventually mental diseases (Graeff et al. 1996; Hensler 2003; Roth et al. 2004). Furthermore, the 5-HT system is involved in regulation of gastrointestinal function and in the development of diseases such as migraine, obesity and nausea (Meguid et al. 2000; Saxena 1995). In several of these pathological conditions disturbances of the neuroendocrine hormonal regulation is found (Gold et al. 1988; Holsboer et al. 1995; Holsboer & Barden 1996). Therefore, the study of serotonergic systems involvement in the regulation of the hypothalamic and pituitary gland hormone release can be seen as a tool to study both the basal and the more complex cerebral functions (Ruggiero et al. 1999). However, it is important to notice that changes in behaviour or pathological conditions are not always reflected in the levels of hormones (Zhang et al. 2000).

The hormonal secretion from the hypothalamus is influenced by peptides and neurotransmitters. Neurotransmitters released from neurons in the cerebral cortex, the thalamus, the limbic system and the brain stem regulate hypothalamic functions together with hormonal feedback from endocrine glands (Freeman et al. 2000; Carrasco & Van de Kar 2003). The hypothalamus synthesise regulatory neuropeptides (e.g. corticotrophin releasing hormone (CRH), arginine-vasopressin (AVP), thyrotrophic releasing hormone, growth hormone releasing hormone, somatostatin and gonadotropin releasing hormone) which together with classical neurotransmitters such as histamine, serotonin, catecholamine and dopamine regulates the secretion of hormones from the anterior and posterior pituitary gland (Reichlin 1998). These neurotransmitters interact in the regulation of these hormones (see chapter 5) (Jorgensen et al. 1996; Dryden et al. 1993; Aguilar et al. 1997). I found it essential to clarify the importance of 5-HT and its different receptors on the neuroendocrine system and stress related conditions. The hypothesis of the studies was that receptors other than the well-documented 5-HT_{1A} and 5-HT_2 , are involved in the regulation of pituitary gland hormones under basal conditions and stress stimulation. The studies were performed in male rats and focused exclusively on AVP, oxytocin, CRH, adrenocorticotropic hormone (ACTH) and prolactin (PRL).

The aim of this thesis was to investigate:

A. The involvement of 5-HT and 5-HT receptors in the regulation of:
   i. The gene expression of hypothalamic hormones
   ii. The hypothalamo-adenohypophysial system (prolactin and ACTH)
   iii. The neurohypophysial system (vasopressin and oxytocin)
B. The involvement of 5-HT and the 5-HT receptors in the stress-induced neuroendocrine responses
C. The relative importance of some distinctive central nuclei in the basal and stress-induced hormone secretion
D. The metabolism of 5-HT in the hypothalamus and the dorsal raphe nucleus

2. SEROTONIN IN THE CENTRAL NERVOUS SYSTEM

2.1 SYNTHESIS AND METABOLISM OF SEROTONIN

Serotonin was initially discovered as a vasoconstrictor substance in blood and later in blood vessel walls, platelets and in enterochromafine cells of the gastrointestinal system, the lungs and the heart (Rapport et al. 1948). Outside the CNS, 5-HT acts on autonomic smooth muscle cells, e.g. in blood vessels and the digestive tract (Zifa & Fillion 1992). More than 50 years ago the chemical structure of 5-HT was identified and it was synthesised (Twarog & Page 1953). Later, the function of 5-HT as a neurotransmitter in the CNS was proposed (Bogdanski et al. 1956) and 5-HT has been studied intensively since its identification in the pituitary gland (Hyyppa & Wurtman 1973).

In the CNS serotonin is synthesised in the perikarya of the neuron where tryptophan is hydroxylated to the 5-HT precursor 5-hydroxytryptophan (5-HTP) which is then decarboxylated to 5-HT (Hamon et al. 1982). To avoid immediate enzymatic oxidation to 5-hydroxy-indol acetic acid (5-HIAA) by monoamine oxidase, 5-HT is contained in neuronal vesicles until it is released into the synaptic cleft. Serotonin then activates either postsynaptic or presynaptic receptors or is reuptaken via the 5-HT transporter molecule into the neuron (Figure 1) (Hamon et al. 1982). The degradation processes are very fast due to a large surplus of monoamine oxidase. Therefore, concentrations of 5-HT in cerebral extra cellular space and in peripheral plasma are low, and do not reflect serotonergic activity (Page 1968).

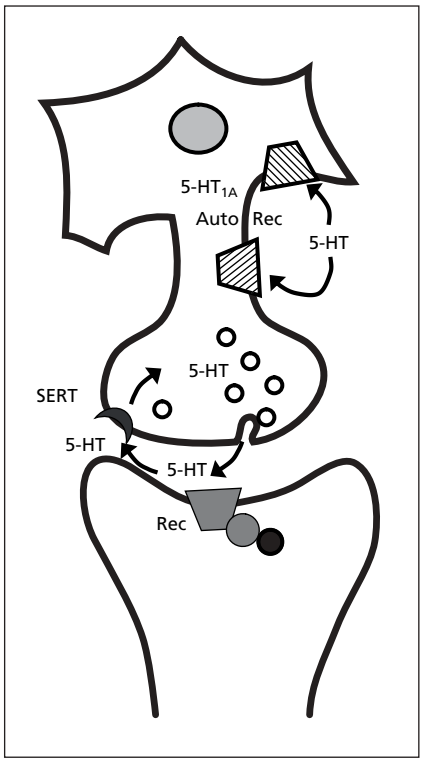

Figure 1. Schematic drawing of the 5-HT synaptic cleft with 5-HT vesicles in the presynaptic neuron, postsynaptic 5-HT receptor (shaded; G-protein coupled), 5-HT transporter for reuptake of 5-HT, dendritic and somatic 5-HT autoreceptors (dashed).

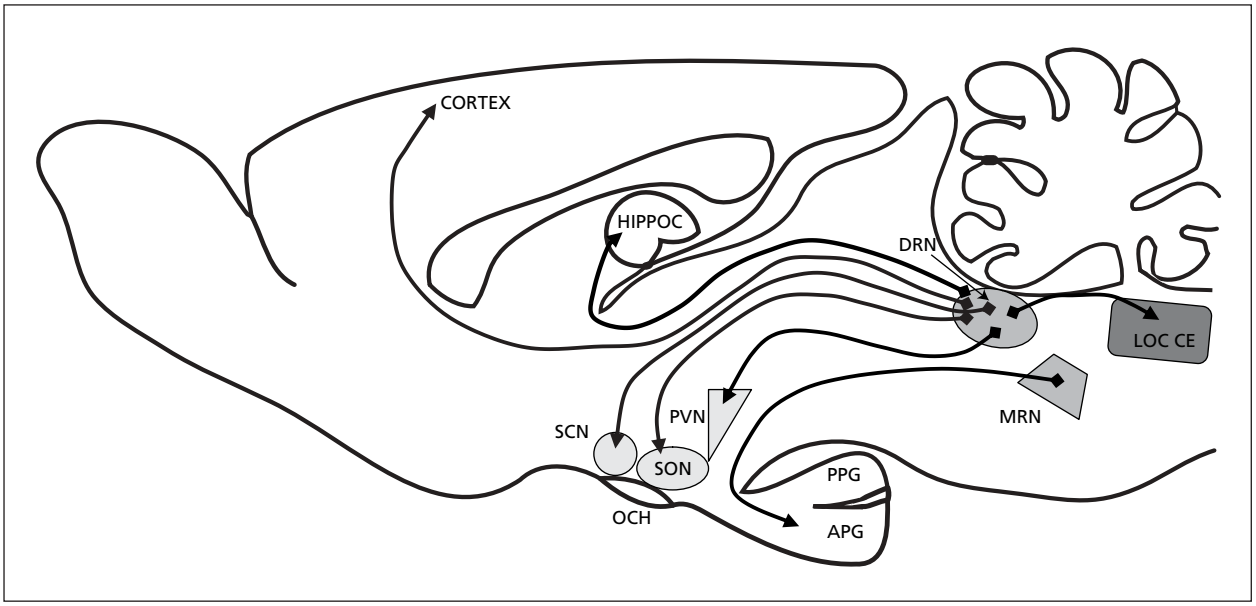

Figure 2. Sagital view of a rat brain with 5-HT neurons originating in the median and dorsal raphe nucleus (MRN; DRN) projecting to the locus cerolus (LOC CE), the cortex, hippocampus and the hypothalamic nuclei: paraventricular (PVN), suprachiasmatic (SCN), supraoptic (SON) and the anterior pituitary gland (APG).

2.2 SEROTONERGIC NEURONS IN THE BRAIN

Serotonergic cell bodies are located in the brain stem anatomically divided into nine groups, designated B1-B9, of whom the most important are the dorsal raphe (DRN, B7) and the median raphe nucleus (MRN, B8) (Dahlström & Fuxe 1964; Steinbusch & Nieuwenhuys 1983). Caudally in the midbrain the raphe magnus and the ventral lateral medulla are located (Dahlström & Fuxe 1964). Serotonergic neurons from these nuclei innervate the forebrain, whereas neurons from the MRN innervate the hippocampus and hypothalamus, and the DRN projects to the hypothalamus, caudate and putamen (Figure 2) (Steinbusch 1981; Jacobs & Azmitia 1992; Azmitia & Segal 1978; Dahlström & Fuxe 1964). High levels of immunoreactive 5-HT terminals are seen in the limbic system (hippocampus, amygdala, septum and venterolateral geniculate), the thalamus (periventricular nucleus), the hypothalamus (suprachiasmatic, arcuate and the mammilary nucleus) and in the substantia nigra (Azmitia 1987).

The hypothalamic paraventricular nucleus (PVN) receive a sparse input of serotonergic neurons, originating both in the DRN and the MRN and projecting especially to the parvocellular part of the PVN (Larsen et al. 1996; Sawchenko et al. 1983).

2.3 SEROTONERGIC RECEPTORS IN THE BRAIN

In the early 1950'ies Gaddum showed that 5-HT induced contraction of the small intestine was mediated through two different receptors, blocked by either morphine (M-receptors) or dibenzyline (D-receptors) (Gaddum & Picarelli 1957). In the CNS two distinct populations of 5-HT receptors, designated 5-HT_1 and 5-HT_2, could be labelled with radioligands on cerebral cortex membranes (Bennett & Aghajanian 1974; Peroutka & Snyder 1979). Subsequently, three subtypes of the 5-HT_1 receptor (Nelson et al. 1981; Pazos et al. 1984) together with the 5-HT_3 (Kilpatrick et al. 1987) and the 5-HT_4 receptor were identified in the brain (Dumuis et al. 1989; Bockaert et al. 1990). Based on radioligand binding studies and pharmacological experiments a classification into 5-$HT_{1\text{-like}}$, 5-HT_2 and 5-HT_3 receptors was proposed (Bradley et al. 1986). The original M- and D-receptor were reclassified as 5-HT_3 and 5-HT_2 receptors, respectively. With molecular biological technique the 5-HT_5 (Erlander et al. 1993; Matthes et al. 1993), 5-HT_6 (Monsma, Jr. et al. 1993) and 5-HT_7 receptors (Lovenberg et al. 1993; Ruat et al. 1993) were identified and characterised (Table 1). Confirmation of the classification system with addition of the new receptors was done based on struc-

Table 1. The serotonergic subreceptor system with primary type of receptor coupling, second messenger system, localisation and function.

Receptor	5-HT_{1A}	5-HT_{2A}	5-HT_3	5-HT_4	5-HT_{5A}	5-HT_6	5-HT_7
Type of rec.	G_i-protein	G_s-protein	Ion channel	G_q-protein	$G_{i/o}$-protein	G_s-protein	G_s-protein
Sec.mess.	inhibits adenylate cyclase	stimulates phospholipase	gated cat ion channel	stimulates adenylate cyclase	inhibits adenylate cyclase	stimulates adenylate cyclase	stimulates adenylate cyclase
Localisation	DRN limbic system	cortex hippocampus caudate nucleus	sparsely distribut. pons brain stem	widely distributed cortex hypothalamus	cortex hippocampus hypothalamus	striatum hippocampus cortex	limbic syst. suprachiasm. DRN
Function	mood, anxiety temp.regulation feeding , motor	sleep motor function behaviour	emesis reflex GI motility cardiovasc. system	memory control release of neurotransmitters	sleep motor function behaviour	control cholinerg function feeding?	mood, anxiety temp.regulation sleep pattern
	5-HT_{1B}	5-HT_{2C}			5-HT_{5B}		
Localisation	substantia nigra basal ganglia frontal cortex	hypothalamus limbic system basal ganglia			DRN CA1 hippocampus olfactory bulb		
Function	control release of neurotransmitters vascular function	penile erection regulation of CSF (?)			?		
References	(221, 145)	(230, 290)	(71, 160)	(33)	(160, 262)	(370)	(336)

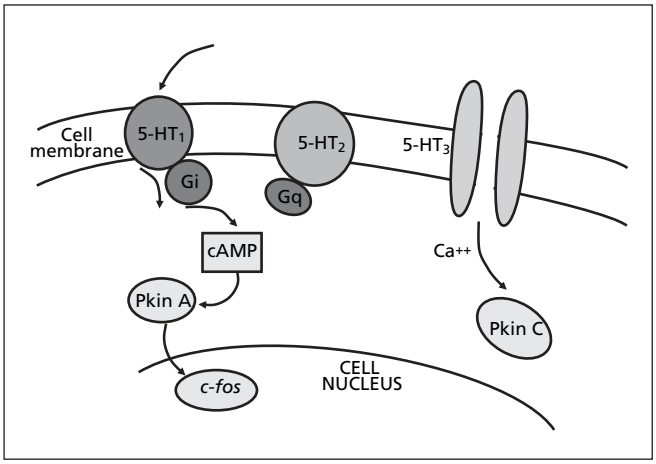

Figure 3. Schematic drawing of the G-protein coupled 5-HT receptor and the ion gated 5-HT_3 receptor in the cell membrane with their second messenger systems cyclic adenosine monophosphate (cAMP), protein kinase A and C (Pkin A) and the immediate early gene *c-fos* in the cell nucleus.

tural homology and functional similarities (Hartig 1989; Hoyer et al. 1994). Serotonergic receptors are primarily located postsynaptically, but 5-HT_{1A} and 5-HT_{1B} receptors are in addition located presynaptically as autoreceptors (Figure 1) (Boess & Martin 1994).

Seven 5-HT receptors with a total of 14 subtypes are yet identified. Six of these are heterotrimeric G-protein receptors with seven transmembrane α-helices (Hoyer et al. 2002). The 5-HT_1 receptors are G_i-protein coupled, which inhibits the second messenger adenylate cyclase, 5-HT_2 receptors stimulate phospholipase C (G_q-proteins) and 5-$HT_{4\text{-}7}$ receptors are coupled to G_s-proteins, stimulating adenyl cyclase (Figure 3) (Hoyer et al. 2002). The 5-HT_3 receptor is a ligand-gated ion channel, modulated via G-proteins and independent of adenylate cyclase (Costall & Naylor 2004). Local differences in the regulation of receptor sensitivity and abundance following prolonged drug administration or stress-induced changes are responsible for the differences in therapeutic effects or side effects of different 5-HT antagonists and agonists (Hoyer et al. 2002; Hensler 2003). Primary localisation and important functions of the 5-HT-receptors are indicated in Table 1. The investigated compounds used in the studies together with their abbreviations are listed in Table 2. In Table 3 and Table 4, the individual receptor affinities of the agonists and antagonists, respectively are listed.

3. EVALUATION OF EXPERIMENTAL METHODS

The methods used in the experiments in this thesis are developed and described by others and most of the methods are summarised in my articles I-X. The substances used in the pharmacological studies were administered centrally or peripherally. The effect of peripheral administration such as i.v., i.p., subcutaneous or intra-arterial may variate due to differences in absorbance from tissue compartments,

Table 2. List of compounds.

Abbreviation	Primary Receptor	Formal Chemical Name
5-HT	serotonin	5-hydroxytryptamine
5-HTP	serotonin precursor	5-hydroxy-d,l-tryptophan
Fluoxetine	5-HT reuptake inhib.	(±)-N-Methyl-3-phenyl-[(α,α,α-triflouro-p-tolyl)-oxy]-propylamine hydrochloride
8-OH-DPAT	5-HT_{1A+7} agonist	8-hydroxy-dipropylaminotetralin hydrobromide
5-CT	5-$HT_{1A+1B+5A+7}$ ago.	3-(2-amino ethyl)-1H-indol-5-carboxamide maleate
RU 24969	5-HT_{1B+1A} agonist	5-methoxy-3-[1,2,3,6-tetrahydro-4-pyridyl]-1H-indol
DOI	5-HT_{2A+2C} agonist	±1-2,5-dimethoxy-4-iodophenyl-2-aminopropane
mCPP	5-HT_{2C+2A} agonist	1(3-chlorophenyl) piperazine dihydrocholride
MK 212	5-HT_{2C} agonist	6-chloro-2-(1-piperazinyl) pyraxine hydrochloride
Sα-methyl-5-HT	5-$HT_{2A+2B+2C}$ ago.	S-α-methyl-serotonin, (±)-3-(2-amino propyl)-indol-5-ol-maleate
SR 57227	5-HT_3 agonist	1-(6-chloro-2-pyridinyl)-4-piperidinamine hydrochloride
m-CPBG	5-HT_3 agonist	1-(*m*-chlorophenyl)-biguanide hydrochloride
RS 67506	5-HT_4 agonist	1-(4-amino-5-chloro-2-methoxyphenyl)-3-[1-2-methylsulphonyl-amino-ethyl-4-piperidinyl]-1-propanone
WAY-100635	5-HT_{1A} antagonist	N-*tert*-butyl-3-(4-(2-methoxyphenyl) piperazine-1-yl-)2-phenyl-propionamide
Cyanopindolol	5-HT_{1A+1B} antag.	4-[3-Butylamino]-2-hydroxypopoxy]-1H-indole-2-carbonitrile
Metergoline	5-$HT_{2A+2C+6+7}$ antag.	[[(8β)-1,6-dimethylergolin-8-yl]-methyl]carbamic acid phenylmethyl ester
Metysergide	5-$HT_{1A+2A+2C+7}$ antag.	[8β(S)]-9,10-didehydro-N-[1-(hydromethyl)propyl]-1,6-demthylergoline-8-carboxamide
Flourobezoyl	5-HT_{2A} antagonist	4-(4-flourobenzoyl)-1-(4-phenylbutyl)-piperidine oxalate
Ketanserin	5-HT_{2A+2C} antag.	3-[2-[4-(4-fluorobenzoyl)-1-piperidinyl]ethyl]-2,4(1H,3H)-quinazolinedione tartrate
LY 53857	5-HT_{2C+2A} antagonist	6-methyl-1-(-methyl ethyl)-ergoline-8β-carboxylic acid 2-hydroxy-1-methyl propyl ester maleate
SB 242084	5-HT_{2C} antagonist	6-chloro-5-methyl-1-[6-(2-methylpyridin-3-yloxy) pyridin-3-yl carbomyl] indoline
Y-25130	5-HT_3 antagonist	N-(1-azabicyclo[2.2.2]oct-3-yl)-6-chloro-4-methyl-3-oxo-3,4-dihydro-2H-1,4-benzoxazine-8-carboxamide
Ondansetrone	5-HT_3 antagonist	1,2,3,9-tetrahydro-9-methyl-3[(2-methyl-1H-imidazol-1-yl)methyl]-4H-carbazol-4-one
ICS 205-930	5-HT_{3+4} antagonist	endo-8-methyl-8-axabiocylol[3.2.1]oct-3-ol indol-3-yl-carboxylate hydrochloride
RS 23597	5-HT_4 antagonist	3-(piperidin-1-yl)propyl 4-amino-5-chloro-2-methoxy benzoate
5,7-DHT	neurotoxin	5,7-dihydroxytryptamine creatinine sulfate

Table 3. Receptor affinities for the 5-HT agonists expressed as pKi values. The values are determined in several different techniques and are not directly comparable. Shaded areas indicate the primary receptor specificity of a given compund. Numbers in paranthesis indicate references.

Agonist	5-HT_{1A}	5-HT_{1B}	5-HT_{2A}	5-HT_{2C}	5-HT_3	5-HT_4	5-HT_{5A}	5-HT_7
5-HT	8.8 (282)	7.8 (282)	8.2 (282)	8.0 (282)	6.7 (195)	7.0 (159)	6.6 (243)	8.7 (326)
8-OH-DPAT	9.2 (254)	5.1 (254)	5.2 (254)	<5 (254)			7.0 (243)	7.5 (326)
5-CT	9.5 (159)	8.3 (159)	3.5 (159)	6.2 (157)		5.5 (159)	9.5 (243)	9.5 (326)
RU 24969	7.8 (159)	8.4 (159)	6.1 (328)	6.2 (328)			6.5 (243)	6.9 (326)
DOI	5.2 (357)	5.7 (357)	8.2 (357)	7.0 (323)			<6 (159)	4.6 (159)
Sα-5-HT*	6.6 (158)	5.5 (158)	7.3 (159)	7.3 (159)		5.8 (123)		
MK212	5.3 (157)	5.0	4.8	6.2				
mCPP	6.5 (157)	6.5	6.7	7.8				6.5 (326)
2-me-5-HT	5.8 (357)	6.1 (357)	<5.0 (158)	5.8 (158)	6.7 (130)	<4 (159)		
SR 57227					8.6 (324)			
*m*CPBG	<5 (148)		<5 (148)		8.8 (148)			
RS 67506	5.7 (92)		<6.0 (92)	5.7 (92)	5.6	8.8 (92)		

*) The affinity of Sα-5-HT at the 5-HT2B receptor = 8.4.

Table 4. Receptor affinities for the 5-HT antagonists expressed as pKi values. The values are determined in several different techniques and are not directly comparable. Shaded areas indicate the primary receptor specificity of a given antagonist. Numbers in paranthesis indicate references.

		5-HT$_{1A}$	5-HT$_{1B}$	5-HT$_{2A}$	5-HT$_{2C}$	5-HT$_{3}$	5-HT$_{4}$	5-HT$_{5A}$	5-HT$_{7}$
WAY-100635	5-HT$_{1A}$	8.9 (106)	<7.0	<7 (106)		<7	<7	-	-
NAN-190	5-HT$_{1A}$	8.9 (357)	6.2	6.6 (357)	6.2	5.9			
Cyanopindolol	5-HT$_{1A+7}$	8.3 (159)	8.3	4.5 (357)	4.4	-	-	-	<5.0 (159)
Metergoline	5-HT$_{2A+2C+6+7}$	7.6 (159)	7.2	8.5 (159)	10.6	-	-	<6.0 (159)	8.7
Methysergide	5-HT$_{1A+2A+2C+7}$	7.6 (157)	5.8	8.5 (157)	8.6	-	-	7.2 (243)	7.9
Ket	5-HT$_{2A+2C}$	5.9 (158)	5.9 (158)	8.7 (324)	7.2 (324)	<4 (159)		4.8 (243)	6.7 (326)
FBP	5-HT$_{2A}$			8.3 (147)					
LY 538457	5-HT$_{2C+2A}$	6. 4 (157)	5.5 (157)	7. 7 (324)	8. 3 (324)				
SB 242084	5-HT$_{2C}$			6.3 (360)	9.3 (360)				
Y-25130	5-HT$_{3}$	<5 (256)		<5 (256)	<5	8.5 (256)			
GR 38032F	5-HT$_{3}$					8.6 (159)	<<5 (256)		
ICS 205-930	5-HT$_{3+4}$			5.3 (157)	4.6	8.5 (196)	6.2 (159)		
RS 23597	5-HT$_{4}$	<5 (92)		5.2 (92)	5.2 (92)	5.7 (92)	8 (92)		

permeability and degradation. Central administration as i.c.v. or direct intranucleary infusion results in a rapid and brief response (Jorgensen et al. 2003b, IX). However, the PRL and ACTH responses to central infusion of 5-HT agonists were somewhat lower than upon systemically administration in the present experiments (Table 5 and Table 6; unpublished data) (Jorgensen et al. 1999, V; Jorgensen et al. 2001, VI; Jorgensen et al. 1993, III). On the other hand, localised central administration by a microdialysis probe has a prolonged effect due to the prolonged time of infusion (Neumann et al. 1993). Different effects of central versus peripheral administration may due to different involvement of anatomical structures, localisation of receptors, effects at the peripheral cardiovascular system and the gastrointestinal system probably inducing secondary effects in the CNS.

Studies of the hypothalamic regulation of hypophysial hormone release have been made by immunoneutralisation, in situ hybridisation and microdialysis in hypothalamic nuclei. Immunoneutralisation with a hormone specific antiserum was carried out in vivo and should theoretically eliminate all circulating CRH in the animal (van Oers & Tilders 1991), but insufficient neutralisation of CRH is a possible explanation for a residual effect of 5-HT compounds on the HPA-axis (Jorgensen et al. 2002a, VII; Kjaer et al. 1992). Stereotactical microdialysis reflects changes in central hormone release over time, in our experimental design, for up to 10 h in a single animal. The advantage, which also is the challenge of microdialysis, is the localised area of investigation, e.g. specific nuclei in the hypothalamus, and provides more reliable information about hormone release compared to in situ hybridization. Additional information can be supplied by dual simultaneously microdialysis, e.g. in the hypothalamus and in peripheral blood (Neumann et al. 1993). The operative implantation of the microdialysis guide cannula and the subsequent insertion of the probe may injure a part of the nuclei studied or other central structures affecting measurements (Benveniste & Huttemeier 1990). Measurement of gene expression of hormones by in situ hybridisation on coronal rat brain slices provides information about specific localisation of mRNA of several hormones fundamentally all over the brain. However, the amount of mRNA detected reflect initiation of hormone synthesis but can not uncritically be interpreted as release of hormone into the circulation, and is therefore only an indirect measure of response (McCabe et al. 1986). The methods used in the present thesis complement each other and the integrated information support a physiological pattern.

Table 5. Effect of i.c.v. infusion of 5-HT$_2$ agonists in combination with the relevant 5-HT antagonists on plasma level of ACTH or PRL. All doses are in nmol. Mean of 6-8 rats with SEM and expressed in pmol/l.

	ACTH	PRL
Saline	38,1±3,6	3,8±0,2
8-OH-DPAT (10 nmol)	68,7±9,1	35,2±6,1
RU 24969 (10)	68,3±5,0	5,5±0,8
DOI (10)	56,5±7,4	5,3±0,6
DOI (10) + LY53857 (50)	50,3±8,2	4,4±0,5
MK212 (10)	76,3±8,8[a]	7,0±0,3[a]
mCPP (10)	50,5±7,9	8,8±1,7[a]
mCPP (10) + LY53857 (50)	n.a.	6,3±1,9
Saline	38±3,6	3,8±0,2
DOI (10 nmol) + Saline	58±7,4	5,3±0,6
DOI + Flourobezoyl (1)	n.a.	3,4±0,4
DOI + Flourobezoyl (10)	n.a.	4,7±0,6
MK212 (10) + Saline	76±8,8[a]	7,8±0,4[a]
MK212 (10) + SB242084 (1)	n.a.	10,4±1,4
MK212 (10) + SB242084 (10)	n.a.	4,1±0,5*
MK212 (10) + SB242084 (100)	n.a.	3,8±0,5*

a) p<0.05 versus saline; *) p<0.05 compared to 5-HT agonist + saline.

Table 6. Effect of pretreatment with 5-HT$_3$ receptor antagonists before i.c.v. challenge 5-HT$_3$ receptor agonists on plasma ACTH or PRL. Data are means of 6-8 rats with SEM and expressed in pmol/l.

	ACTH	PRL
Saline	90±7	3,5±0,4
2-me-5-HT + Saline	145±17[a]	15,1±2[a]
2-me-5-HT + ICS	87±9**	6,5±0,9**
2-me-5-HT + Ondansetron	130±16	6,9±0,9*
Saline	38±4	3,8±0,2
SR 57227 + Saline	88±7[a]	11,8±2,1[a]
SR 57227 + Y-25130 (1)	68±10	13,9±1,7
SR 57227 + Y-25130 (10)	-	10,6±1,3
SR 57227 + Y-25130 (100)	-	5,4±1,1*

a) p<0.05 versus saline; *) p<0.05 and **) p< 0.01 compared to 5-HT agonist + saline.

4. STRESS

4.1 THE STRESS CONCEPT

Since the introduction of the term *alarm reaction* by Selye in 1936, using a broad non-specific definition (Selye 1936), several other definitions of stress has been proposed and discussed. A shift from a non-specific description towards a more differentiated and specific response definition has been suggested (Bohus et al. 1987). In this thesis with focus on rodent experiments, stress is defined as a state of threat to homeostasis, which normally is maintained via a set of physiological and behavioural adaptive responses – the general adaptation syndrome (Chrousos & Gold 1992). Stress and the derived adaptive responses affect the behavioural-, endocrine-, gastrointestinal and the immune system (Chrousos 1998). The primarily physiological adaptation mechanism (the stress response) to threatening conditions (stress) can cause pathophysiological conditions affecting the above mentioned systems and organs (Chrousos & Gold 1992).

4.2 EFFECTS OF STRESS ON THE NEUROENDOCRINE SYSTEM

There are several ways to categorise the different types of stress (Van de Kar et al. 1991; Carrasco & Van de Kar 2003; Summers 2001). In this context, stress that affects the neuroendocrine system in rats is categorised as (1) psychological (or emotional) stress such as fear, anxiety, novel environment and immobilisation (2) physical stres-

sors with a psychological component as ether vapour, endotoxin, hypoglycaemia, cold environment and restraint, or (3) cardiovascular stressors as haemorrhage, exercise, heat and dehydration (Carrasco & Van de Kar 2003). Stress is often regarded as a generalised and diffuse response. However, each type of stress can also be seen as a specific type of response, with individual involvement of neurotransmitters and hormones (Van de Kar et al. 1991). Physical and cardiovascular stress may also include a psychological component thus application of a single stress factor may contain several aspect of stress, e.g. swimming in ice-cold deep water include both physical, psychological and cardiovascular stress. In the rat, restraint stress applied by manually holding the animal on its back in supine position is both a physical and psychological stress factor (Husain et al. 1979).

The main activation route of the stress response is the hypothalamo-pituitary-adrenal (HPA-) axis and the symphato-adrenomedullary system, and their central components: the PVN with the parvocellular CRH and AVP neurons, the adrenocorticotropic cells in the anterior pituitary gland and the fasciculate zone of the adrenal cortex (Chrousos & Gold 1992).

Repeated or chronic stress exerts a negative influence on the majority of the physiological systems contributing to pathological conditions. Chronic stress have no effect on circulating ACTH in plasma (Anderson et al. 1993; Hashimoto et al. 1988; Chowdrey et al. 1995), but increases anterior pituitary gland levels of ACTH (Hashimoto et al. 1988), CRH mRNA in the PVN as measured by in situ hybridization (Imaki et al. 1991; Imaki et al. 1998; Prewitt & Herman 1997) and POMC mRNA in the anterior pituitary gland as measured by cytoplasmic dot hybridization (Hollt et al. 1986).

In this thesis stress was induced according to the following protocols. Restraint stress: holding the rat manual on its back for 5 min. Ether vapour stress: in a closed glass bowl filled with ether vapour for 5 min. Endotoxin (or lipopolysaccharide; LPS) stress: Intraperitoneal injection of a LPS suspension. Haemorrhage stress. Withdrawal of 3.0 ml of blood from the jugular vein over a period of 2 min. Dehydration stress: No access to water for 24 h. Cold-swim stress: the rat was placed in a deep open glass bowl filled with 2-4°C cold water for 3 min followed by a 2 min period for drying. Hypoglycaemic stress: Intraperitoneal injection of 3 IU of insulin. The idea of using different types of stress was to elucidate how general or specific the involvement of the serotonergic system was in the stress induced hormone response.

4.3 INVOLVEMENT OF 5-HT IN THE STRESS RESPONSE

Psychological stress activates the serotonergic neurons in the hippocampus and the amygdala through the cortical association areas and through ascending catecholaminergic neurons from the brain stem (Feldman & Weidenfeld 1998; Koob & Heinrichs 1999). Serotonergic and adrenergic neurons from the central nucleus of the amygdala projects to CRH neurons in the parvocellular PVN (Ruggiero et al. 1999).

Stress, in general, often results in changes of 5-HT metabolism (Culman et al. 1980; Chaouloff et al. 1989). The present finding of an increased content of 5-HT in the DRN after restraint stress but no changes in hypothalamic tissue, and no significant changes of 5-HT metabolism in either the hypothalamus or the DRN after swim-, ether vapour- or endotoxin stress (Jorgensen et al. 1998a, IV) is in accordance with some studies (Beaulieu et al. 1986; Dunn & Welch 1991; Culman et al. 1980; Saphier & Welch 1995), but in contrast to the findings of an increased 5-HT metabolism in the cortex and the hypothalamus or in the brainstem after restraint (De Souza & Van Loon 1986; Clement et al. 1993), foot shock stress (Dunn 1988) or endotoxin stress (Givalois et al. 1999) (Figure 4). These discrepancies may be due to variations in tissue preparation, duration and method of immobilization and analysis method of amine. In specific micro-dissected PVN's an increased metabolism was shown after restraint stress, contrary to our finding of no change in 5-HT activity in the hypothalamus (Garrido et al. 2002). Stereotactical cerebral microdialysis of extracellular 5-HT provide specific information in respect to localization, but a disadvantage is a relatively long duration of the sample period of at least 20 min due to the low sensitivity of the liquid chromatography and electrochemical detection (Rueter et al. 1997). Levels of 5-HT in the amygdala, the hippocampus and the prefrontal cortex are changed after forced swimming (Adell et al. 1997), in the PVN and the ventromedian nucleus after insulin-stress (Orosco & Nicolaidis 1994) and in the prefrontal cortex after conditioned fear stress (Yoshioka et al. 1995). Acute restraint stress increased the gene expression of the 5-HT_7 receptor in the CA1 hippocampal area (Yau et al. 2001) where as 5-HT_{1A} receptor mRNA was decreased (Lopez et al. 1999).

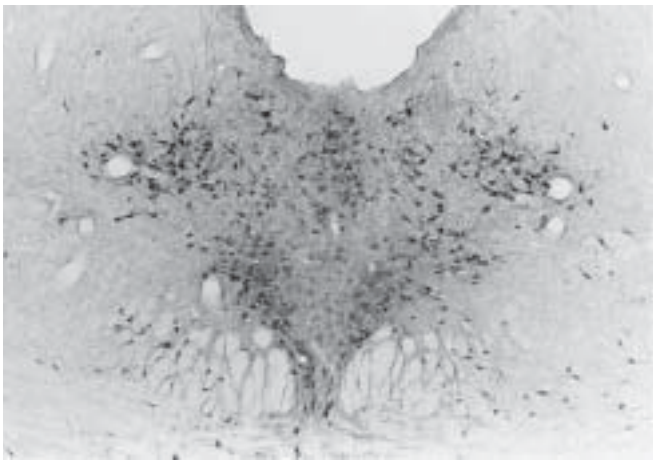

Figure 4. Photomicrograph of 5-HT immuno stained section of the rat brain through the dorsal raphe nucleus just below the IV ventricle.

Increased levels of circulating corticosteroids during acute stress situations affect 5-HT receptors. After adrenalectomy 5-HT_{1A} and 5-HT1B receptor mRNA and receptor binding density were increased in the C1-C4 hippocampal gyri, whereas there was no effect on these parameters for the 5-HT_{2C} receptor (Chalmers et al. 1993; Chalmers et al. 1994). However, chronic stress in general, does not seem to affect the serotonergic system. Various regimes of chronic stress from 5 to 21 days did not change neither gene expression of 5-HT_{1A} or 5-HT_{2A} receptor in the hippocampus (Ohi et al. 1989; Van Riel et al. 2003; Lopez et al. 1999; Holmes et al. 1995), 5-HT_{1A} receptor binding (Lanfumey et al. 1999) nor 5-HT_{1A} agonist induced ACTH response (Grippo et al. 2004). On the other hand, during chronic stress cortical 5-HT_{2A} and hippocampal 5-HT_{2C} receptors were upregulated or suppressed, respectively (Ossowska et al. 2002). In addition, repeated immobilisation stress has also been found to reduce metabolism of 5-HT both in the hippocampus and in the MRN and DRN (Clement et al. 1998).

5. NEUROENDOCRINE EFFECTS OF SEROTONIN

5.1 REGULATION OF PROLACTIN SECRETION

The secretion of prolactin (PRL) from the anterior pituitary gland is affected by multiple external stimuli, internal humoral and neural factors. Important physiological stimuli are suckling, stress, changes in female sexhormones, plasma osmolarity and glucocorticoids (Weiner et al. 1988). The internal factors, neurotransmitters and neuropeptides, are classified as PRL releasing- or inhibiting factors. Most important is dopamine, exerting a tonic inhibitory control, but 5-HT, histamine and TRH also contribute to the regulation of PRL secretion (Freeman et al. 2000; Samson et al. 2003). A specific PRL-releasing peptide has been identified and localized in the rat brain (Hinuma et al. 1998; Maruyama et al. 1999), but the existence of other yet unknown factors is still possible (Freeman et al. 2000).

5.1.1 5-HT neurons involved in prolactin secretion

Changes in PRL secretion upon challenging with 5-HT releasers, 5-HT precursors or treatment with neurotoxins were previously seen as an indirect evidence for the involvement of 5-HT in regulation of PRL secretion (Lawson & Gala 1975; Lawson & Gala 1976; Lu & Meites 1973). It was found that fenfluramine, which releases 5-HT from neuronal stores (Clineschmidt et al. 1976; Fuxe et al. 1975), increased plasma PRL levels (Fuller et al. 1981; Di Renzo et al. 1989; Van de Kar et al. 1985b). Generalized neurotoxic degeneration of 5-HT perikarya by intra-cerebro-ventricular (i.c.v.) infusion of the 5,7-dihydroxytryptamine (5,7-DHT) or inhibition of 5-HT synthesis by intraperitoneal (i.p.) injection of *p*-chlorophenylalanine decreased both basal- and suckling- induced PRL secretion (Gil Ad et al. 1976; Kordon et al. 1973; Caligaris & Taleisnik 1974; Clemens 1978). Administration of the 5-HT precursor 5-hydroxytryptophan (5-HTP) increased 5-HT synthesis and the content of 5-HT in the neurons (Jorgensen et al. 1998a, IV; Gartside et al. 1992a) and increased PRL levels in peripheral plasma (Lu & Meites 1973; Kato et al. 1974; Porter et al. 1971; Gala et al. 1978). This effect was potentiated by the 5-HT reuptake inhibitor fluoxetine, which had no effect by it self (Jorgensen et al. 1992b, II; Clemens et al. 1977; Cocchi et al. 1977; Lawson & Gala 1978). All the above mentioned studies substantiate the role of 5-HT and 5-HT neurons in the mediation of the PRL response. Serotonin does not seem to stimulate PRL secretion directly from the lactotrophe cells in the pituitary gland (Garthwaite & Hagen 1979; Lamberts & MacLeod 1978), even though it is reported that 5-HT releases PRL from incubated anterior pituitary gland cells (Meltzer et al. 1983; Balsa et al. 1998). Instead, the effect is exerted in the hypothalamus by serotonergic input from the raphe nuclei and mediated possibly through an action of a PRL releasing peptide (Freeman et al. 2000; Hinuma et al. 1998).

The relative importance of the DRN and MRN for 5-HT's involvement in PRL secretion is indicated by the reduced basal or stimulated PRL levels after radiofrequency or electrolytic lesion of the DRN (Fessler et al. 1984; Advis et al. 1979). Stereotactical knife lesion of 5-HT neurons between the DRN and the hypothalamus or lesion of the mediobasal hypothalamus abolished *p*-chloroamphetamine-induced PRL levels (Van de Kar et al. 1985a; Van de Kar et al. 1985a). Furthermore, localized stereotactical injections of 5,7-DHT in the DRN significantly blunted the PRL response to *p*-chlorophenylalanine, *p*-chloroamphetamine or to suckling whereas lesions in the MRN had no effect (Van de Kar & Bethea 1982; Barofsky et al. 1983). Likewise, localized lesion of the anterior hypothalamus blocked suckling-induced PRL secretion (Parisi et al. 1987).

The PVN has a 5-HT_2 receptor specific involvement in the serotonergic regulation of PRL secretion. Lesion of the PVN did not affect the PRL response to a 5-HT_{1A} agonist, whereas the response to 5-HT_2 agonists were markedly inhibited (Bagdy & Makara 1994; Bagdy & Makara 1995). Furthermore, the PRL response to either suckling- (Kiss et al. 1986), foot-shock- (Meyerhoff et al. 1987), restraint- or ether vapour-stress (Minamitani et al. 1987) or to *p*-chloroamphetamine (Rittenhouse et al. 1993) were inhibited or abolished after lesion of the PVN. On the other hand, stress-induced PRL secretion was not changed after super selective lesion of the parvocellular neurons in the PVN, indicating that magnocellular neurons may contribute to the regulation of PRL (Caldeira & Franci 2000). In conclusion, 5-HT neurons in general are required for the mediation of the PRL response especially the midbrain DRN and MRN and to less extend the hypothalamic PVN.

5.1.2 5-HT receptors involved in prolactin secretion

Systemically administration of 5-HT dose-dependently stimulated PRL secretion, either administered intra-arterial (Lawson & Gala 1978), i.v. (Jorgensen et al. 1993, III; Jorgensen et al. 1992b, II) or i.p. (Meltzer et al. 1983; Fessler et al. 1984). Central administration of 5-HT either direct into specific localisations in the brain (Willoughby et al. 1988) or more generally infused i.c.v. effectively increase plasma PRL dose-dependently with up to a 20-fold increment (Pilotte & Porter 1981; Krulich et al. 1979; di Sciullo et al. 1990; Kamberi et al. 1971).

The primary reports were contradictory on the involvement of the different 5-HT receptors in the PRL response, primarily due to low specificity of the 5-HT analogues used (Preziosi 1983; Jorgensen et al. 1999, V; Shen et al. 1993). Furthermore, the type of rat strain used in experiments is also important for the hormone response explaining variation in results (Aulakh et al. 1988). It was proposed that either the 5-HT_{1A} receptor (Carlsson & Eriksson 1986), the 5-HT_{1B} and the 5-HT_2 receptor (Van de Kar et al. 1989) or the 5-HT_2 receptor alone (Nash & Meltzer 1989; Gartside & Cowen 1990) was responsible for 5-HT-induced PRL secretion.

Initial studies found no effect of i.p., i.v. or i.c.v. administration of *5-HT_{1A} receptor* agonists on PRL secretion (Van de Kar et al. 1989; Di Renzo et al. 1989; Gartside et al. 1990). Subsequent experiments, where the relevant time to response was observed, did find effect in male Wistar rats after either s.c. (Van de Kar et al. 1998b), i.v. (Bagdy & Makara 1994; Jorgensen et al. 2001, VI; Jorgensen et al. 1993, III; Baumann & Rothman 1995), i.c.v. (Jorgensen et al. 2001, VI; Vicentic et al. 1998; di Sciullo et al. 1990) or intranucleary administration (Bluet Pajot et al. 1995) of 5-HT_{1A} agonists substantiating an involvement of this receptor in the serotonergic regulation of PRL secretion. Until recently, no selective 5-HT_{1A} receptor antagonist was available. We observed that the 5-HT_{1A} antagonist NAN-190 had no effect on the PRL response to either 8-OH-DPAT or 5-HT (Jorgensen et al. 2001, VI). Higher doses of NAN-190 even augmented the PRL responses, which may be due to its partial agonistic properties at the presynaptic 5-HT_{1A} autoreceptor in the DRN and its affinity for adrenergic receptors (Greuel & Glaser 1992; Routledge et al. 1995; Cowen et al. 1990). The newer selective 5-HT_{1A} antagonists LY-206130 and WAY 100-635 inhibited the PRL response to 8-OH-DPAT or 5-HT upon central administration as did the 5-HT_{1A+1B} antagonist cyanopindolol (Jorgensen et al. 2001, VI). Contrary to this, comparable experiments with systemically administration of low doses of WAY 100-635 did not find any effect on 8-OH-DPAT-induced PRL secretion, but identified an increase of basal PRL levels after 20-fold higher doses of WAY 100-635 than in our experiment (Groenink et al. 1996; Vicentic et al. 1998). Despite the divergence of the many reported results, it can be concluded that the 5-HT_{1A} receptor seems to be involved in the 5-HT-induced PRL release.

It is likely, that the *5-HT_{1B} receptor* also is involved in the PRL response as the non-selective 5-$HT_{1A+2A+2C+5A+7}$ antagonist methysergide inhibited, but did not abolish the PRL response to the non-specific 5-$HT_{1A+1B+5A+7}$ agonist 5-CT or the 5-HT_{1B+1A} agonist RU 24969 (Jorgensen et al. 1993, III). No other studies involving 5-CT on PRL secretion are identified, but one previous study failed to find effect of RU 24969 administered i.p. on plasma PRL levels 60 min after injection (Di Renzo et al. 1989), probably due to the short time response of PRL, which peaks 7-15 min after i.v. stimulation (Bluet Pajot et al. 1995; di Sciullo et al. 1990) and 15-30 min after i.p. stimulation (Van de Kar et al. 1989). The 5-HT agonist sumatriptan has low affinity for central 5-HT_{1B} receptors in rodents which compares to the 5-HT_{1Dbeta} receptor in humans (Hoyer et al. 1994). In humans, sumatriptan is found either to have no effect (Cleare et al. 1998; Mota et al. 1995) or to decrease basal PRL levels (Herdman et al. 1994; Rainero et al. 2001). No comparable experiments has been carried out in rodents. The lack of a specific 5-HT_{1B} antagonist makes it impossible to exclude an involvement of the 5-HT_{1B} receptor. However, based of the findings discussed above an involvement is not obvious.

An involvement of the *5-HT_2 receptor* was verified early (Lowy & Meltzer 1988; Van de Kar et al. 1989). Experiments carried out before the re-classification of 5-HT receptors can now add information for the elucidation of the involved subreceptors. E.g. our and others previous findings of an inhibiting effect of the antagonist ket-

anserin (5-HT_{2A+2C}) or LY 53857 (5-HT_{2C+2A}) on the PRL stimulating effect of 5-HT, DOI (5-HT_{2A+2C}), S-α-methyl-serotonin (5-$HT_{2A+2B+2C}$) or MK 212 (5-HT_{2B+2C}) show that the 5-HT_{2A} and the 5-HT_{2C} receptor are involved in this response (Jorgensen et al. 1992b, II; Jorgensen et al. 1993, III; Gartside & Cowen 1990).

Systemically administration of the 5-HT_{2A+2C} antagonist ritanserin completely prevented the PRL responses to DOI, quipazine or fenfluramine, indicating an involvement of the 5-HT_{2A} receptor (Di Renzo et al. 1989; Rittenhouse et al. 1993). On the other hand, there was no effect of LY 53857 on neither RU 24969-, DOI nor on 5-HT-induced PRL secretion when the compounds were administered i.c.v.(Rittenhouse et al. 1993) (Table 5; Jorgensen et al., unpublished observations). Centrally infusion of the specific 5-HT_{2C} antagonist SB 242084 inhibited the effect of MK 212, indicates an involvement of the 5-HT_{2C} antagonist receptor. The explanation for these differences in effect might be that the mediation of the PRL response to serotonergic stimulation is localized peripherally. From these data it can not be elucidated which of the 5-HT_2 receptors that is the most important mediating the PRL response, but at least the 5-HT_{2A} and the 5-HT_{2C} receptor are involved.

A possible involvement of the *5-HT_3 receptor* has been debated due to dissimilar results. Systemically infusion of the 5-HT_3 agonist 2-methyl-5-HT increased PRL secretion, but with less potency than 5-HT itself, which has almost the same affinity for the 5-HT_3 receptor (Levy et al. 1993; Jorgensen et al. 1993, III). The 5-HT_{3+4} antagonist tropisetrone (ICS 205-930) and the 5-HT_3 antagonist ondansetrone (GR38032F) inhibited PRL secretion both to systemically and centrally administered 5-HT, 5-HTP/fluoxetine and to 5-HT agonists (Table 6, unpublished observations) (Jorgensen et al. 1993, III). Furthermore, the PRL response to SR 57227 (5-HT_3 agonist) was dose dependently inhibited by the corresponding antagonist Y-25130 (Table 5). In accordance with our findings, the 5-HT_3 antagonist MDL 72222 and ICS 205-930 attenuated the PRL response to 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM) (Aulakh et al. 1994). Contrary to this, there were no effect of ondansetrone on 5-HT agonist-induced PRL response in female rats (Lacau-Mengido et al. 1996; Levy et al. 1993). In conclusion, both peripheral and central 5-HT_3 receptors are involved in the serotonergic induced PRL response.

The involvement of the *5-HT_4 receptor* is not clarified. As tropisetrone in addition to its affinity for the 5-HT_3 receptor also possesses some affinity for 5-HT_4 receptors although 100-fold lower, theoretically it can be possible that the 5-HT_4 receptor is involved in the serotonergic induced PRL response. In a pilot study we found that the 5-HT_4 agonist RS 67506 dose-dependently stimulated PRL secretion upon systemically administration, whereas central infusion had no effect (Table 7).

Any involvement of the *5-HT_5, 5-HT_6 or 5-HT_7 receptor* is not yet clarified. We have not investigated this subject with specific agonists for the 5-HT_5 or the 5-HT_7 receptor, and no studies are published. However, as combined administration of the cyanopindolol (5-HT_{1A+1B}) and LY 53857 (5-HT_{2A+2C}) only partly inhibited the PRL response to 5-CT, which in addition to the 5-HT_1 receptor possesses high affinity for both 5-HT_5 and 5-HT_7 receptors, it seems that some of the PRL response to 5-CT might be mediated via these two receptors (Table 8).

The involment of the 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C} and the 5-HT_3 receptor in the serotonergic induced PRL response is well documented and the 5-HT_{1B}, 5-HT_{5A} and the 5-HT_7 receptor is possibly involved.

5.1.3 Stress-induced PRL secretion

PRL secretion is stimulated by stress such as ether vapour, restraint/immobilisation, forced swimming, foot shock stress and the conditioned fear response (Neill 1970; Shin 1979; Krulich 1975; Collu et al. 1979; Kawakami et al. 1979; Knigge et al. 1988a; Demarest et al. 1985) (Rittenhouse et al. 1992; Paris et al. 1987; Van de Kar et al. 1984; Rittenhouse et al. 1992).

Table 7. Effect of pretreatment with the 5-HT_4 receptor antagonists ICS 205-930 or RS 23597 before i.c.v. challenge-infusion of the 5-HT4 receptor agonist RS 67506 on plasma ACTH or PRL. Data are means of 6-8 rats with SEM and expressed in pmol/l.

	ACTH	PRL
Saline	32±4,1	1,7±0,3
RS 67506 (0.2 mg/kg i.p.)	29±2,1	4,8±0,8
RS 67506 (1.0 mg/kg i.p.)	28,5±2,4	8,5±2,7[a]
RS 67506 (5 mg/kg i.p.)	29,1±3,1	10,6±2,6[b]
Saline	64±5,1	1,6±0,2
RS 67506 (4 nmol i.c.v.)	53,2±6,7	2,5±0,6
RS 67506 (20 nmol i.c.v.)	80,2±15	1,7±0,3
RS 67506 (100 nmol i.c.v.)	60±8,8	2,0±0,7
RS 67506 + RS 23597 (0.5 mg/kg i.p)	77,1±9	2,9±0,5
RS 67506 + RS 23597 (2 mg/kg i.p)	69,3±12	2,1±0,2
RS 67506 + ICS 205-930 (0.5 mg/kg i.p)	61,0±7,1	1,9±0,3

a) p<0.05 and b) p< 0.01 versus saline.

Table 8. Effect of (I) pretreatment with the 5-HT_{1A} antagonist WAY 100-635 before i.c.v infusion of the 5-HT1A agonist 8-OH-DPAT or (II) i.c.v. pretreatment with 5-HT antagonists with different receptor characteristics (metergoline (MG), cyanopindolol (CY) or pindolol (PI) before infusion of the 5-$HT_{1A+1B+5A+7}$ agonist 5-CT on plasma ACTH or PRL. Data are means of 6-8 rats with SEM and expressed in pmol/l. Antagonists and agonists were infused i.c.v.at 20 min and15 before sampling, respectively.

	ACTH	PRL
Saline	38,1±3,6	3,8±0,22
8-OH-DPAT (10 nmol)	136±11[a]	22,8±5,8[a]
8-OH-DPAT + WAY 100-635 (1 nmol)	110±15	17,5±2,0
8-OH-DPAT + WAY 100-635 (10 nmol)	44±8*	9,7±2,0
8-OH-DPAT + WAY 100-635 (100 nmol)	44±8*	7,7±1,9
8-OH-DPAT + Cyanopindolol (50 nmol)	87±6*	4,5±0,4
Saline	53±4,4	4,5±0,7
5-CT (10 nmol)	207±16[a]	52±7[a]
5-CT + Metergoline (50 nmol)	104±3,3*	42±9,1
5-CT + Cyanopindolol (50 nmol)	133±10*	29±6,5

a) p<0.05 versus saline; *) p<0.05 compared to 5-HT agonist + saline.

Table 9. Effect of intraperitoneal pretreatment with the 5-HT receptor antagonists WAY 100-635, LY 53857, ketanserin or ICS 205-930 before 3 min of cold swim stress (CSW) in 4ºC, deep water on plasma ACTH or PRL. Doses are indicated in mg/kg. Data are expressed in pmol/l as means of 6-8 rats with SEM.

	ACTH	PRL
Saline	12±1	2,8±0,4
Cold Swim stress	47±6[a]	9,9±3,2[a]
CSW + WAY 100-635 (2.5)	56±6	9,9±1,8
CSW + methysergide (2.5)	78±7	4,2±1,0*
CSW + ketanserin (2.0)	53±5	11,5±2,2
CSW + LY 53857 (2.5)	62±5	6,9±1,7
CSW + ICS 205-930 (0.5)	58±8	10,8±1,5

a) p<0.05 versus saline; *) p<0.05 compared to cold swim stress + saline.

An involvement of 5-HT in the mediation of the stress response to PRL was supported by the findings of a stimulation and inhibition of fluoxetine and buspirone on stress-induced PRL responses, respectively (Krulich 1975; Urban et al. 1986). The specific *5-HT_{1A} receptor* antagonist WAY 100-635 had no effect on either restraint-, ether vapour-, emotional- or cold swim stress-induced PRL secretion (Table 9, and unpublished observations) (Jorgensen et al. 2001, VI; Groenink et al. 1996). The *5-HT_2 receptor* seems definitely to be involved, since we found that ketanserin and LY 53857 inhibited the PRL response to restraint- and ether vapour stress (Jorgensen et al. 1992a, I), which has been confirmed by others (Ramalho et al. 1995). The non-specific antagonist methysergide inhibited and LY 53857 had a non-significant tendency to decrease the PRL response to cold swim stress, indicating a possible involvement of 5-HT in cold swim stress-induced PRL secretion (Table 9). Likewise, the *5-HT_3 receptor* antagonists tropisetrone and ondansetrone inhibited

Table 10. Primary 5-HT receptors involved in the hypothalamic and pituitary hormone secretion under basal or stress conditions. Darker and lighter areas indicate major or minor involvement, respectively. Numbers indicate literature references.

		5-HT$_{1A}$	5-HT$_{1B}$	5-HT$_{2A}$	5-HT$_{2C}$	5-HT$_3$	5-HT$_4$	5-HT$_{5A}$	5-HT$_7$
Hormone	PRL	176	?	185 18	185 18	185 229	÷	?	?
	ACTH	181 176	181	181	181	÷ 181			181
	CRH mRNA	177	177	177	177	÷ 177			177
	AVP	186 22	?	186 280	186 53	÷ 186	186	186	186
	OT	186	?	186 345	186 45	÷	186	186	186
	AVP mRNA	÷ 175		175	175				
	OT mRNA	175		175	175	175			
Stress	PRL	÷ 176	?	183	183	183			
	ACTH	178	?	178	178	÷ 178			
	AVP	÷ 182		182	182	182	182		
	OT	182		182	182	÷ 182			

the PRL response to restraint and ether vapour (Jorgensen et al. 1992a, I). Ondansetrone had an U-shaped dose-response curve as seen with tropisetrone the response to 5-HT-induced PRL secretion (Jorgensen et al. 1992b, II; Nonaka 1999). In conclusion, these findings and the literature indicate that both 5-HT$_{2A}$, 5-HT$_{2C}$ and 5-HT$_3$ receptors are involved in stress-induced PRL secretion whereas the involvement of the 5-HT$_{1A}$ receptor is unlikely.

5.1.4 Conclusion

Serotonergic compounds and 5-HT stimulate the secretion of PRL from the anterior pituitary gland. The DRN is essential and for the major part the PVN is involved in the mediation of the PRL response, as the majority of studies report a reduced PRL response to at least 5-HT$_2$ agonists after specific lesion of the PVN. We found that 5-HT$_{1A}$, 5-HT$_{2A}$, 5-HT$_{2C}$ and as a novelty at the time of investigation, that 5-HT$_3$ receptors is involved in the regulation of both the basal and the stress-induced PRL response. The 5-HT$_{1B}$ receptor is likely involved, but it cannot be clarified on the present data. A possible involvement of the 5-HT$_5$ or the 5-HT$_7$ receptor can not be clarified from these experiments. For some receptors the route of administration, the strain of rat, and the dose and time schedule for administration are important factors for the 5-HT induced PRL response.

5.2 REGULATION OF THE HPA-AXIS

5.2.1 CRH and ACTH

CRH is synthesised in neurons which originates in the dorsomedial parvocellular part of the PVN (Palkovits 1987). The majority of CRH neurons descend to the external zone of the median eminence, and a minor part colocalised with AVP descend to the neurohypophysis (Swanson et al. 1983). CRH neurons also have projections to other hypothalamic and extrahypothalamic structures such as the brain stem, cortex, amygdala and septum (Swanson et al. 1983; Palkovits 1987; Sawchenko & Swanson 1983).

The release of CRH is regulated by a circadian rhythm located in the hypothalamic suprachiasmatic nucleus, corresponding with the PVN (Kalsbeek et al. 2003; Buijs et al. 1998). To maintain homeostasis CRH is in addition regulated by several neurotransmitters. Acetylcholine, 5-HT and neuropeptide Y have stimulating effect, while GABA, substance P and opioid peptides have inhibitory effect (Stratakis & Chrousos 1995; Calogero 1995; Carrasco & Van de Kar 2003).

Hypothalamic CRH stimulates the corticotrophe cells of the anterior pituitary gland to synthesis POMC, the precursor for ACTH and β-lipotropine and is in this way a central parameter in the stress response (Osborne et al. 1979; Vale et al. 1981). CRH also have effect on the sympatoadrenergic system (Dunn & Berridge 1987), the immune system and several behavioural functions (De Souza 1995; Dieterich et al. 1997).

The effects of CRH is exerted by binding to G-protein coupled receptors, CRH$_1$ – CRH$_3$ (Perrin et al. 1993; Lovenberg et al. 1995) distributed heterogeneously through out the rat brain (Aguilera et al. 1987; Liposits et al. 1987; Fuxe et al. 1985). The three CRH receptors are expressed in different extent at various areas indicating difference in function. The CRH$_1$ receptor mediates the ACTH response and is involved in the stress response (Luthin et al. 1996; Chalmers et al. 1996) (Samgin et al. 1998). The CRH2α receptor is not involved in stress response, but act as a target for CRH in an ultra short-loop feedback system (Chalmers et al. 1995; Mansi et al. 1996).

ACTH exerts its effect on the adrenocortical cells, binding to Gs protein receptors stimulating cAMP to activate protein kinase and inducing mitochondrial steroidogenesis, hence the production of corticosterone (rat) or cortisol (human). Regulation of ACTH secretion is primarily mediated via CRH neurons in the hypothalamus (Mezey et al. 1987; Antoni 1986). In addition, AVP is an important ACTH secreting peptide (Rivier & Vale 1983) and catecholamines, acetylcholine, histamine, neuropeptide Y, interleukine-1β and angiotensin II stimulates ACTH secretion via an effect on CRH, whereas in the same way GABA and β-endorphin have an inhibitory effect (Calogero 1995; Mezey et al. 1987) (Table 10).

5.2.2 5-HT neurons involved in the activation of the HPA-axis

Serotonergic neurons originating in the B7-B9 cell group of the MRN and DRN (Sawchenko et al. 1983), projects to the PVN of the hypothalamus where some of them interact with CRH neurons (Liposits et al. 1987; Larsen et al. 1996). CRH neurons and receptors are found in the DRN, indicating an involvement of CRH on excitation of 5-HT neurons in the raphe nuclei (Chalmers et al. 1995; Day et al. 2004). However, the effect of CRH on 5-HT is differentiated via the CRH$_1$ and CRH$_2$ receptors (Pernar et al. 2004). Systemic administration of CRH$_1$ antagonists reduced 5-HT and 5-HIAA in hippocampal dialysates in basal or stressed rats (Isogawa et al. 2000; Oshima et al. 2003) and modulated 5-HT neuron discharge in the DRN (Kirby et al. 2000). In previously stressed rats CRH infusion decreased both 5-HT and 5-HIAA in the MRN or DRN (Summers et al. 2003). Together, these findings indicate that CRH regulates the 5-HT neuronal system from the DRN (Figure 5).

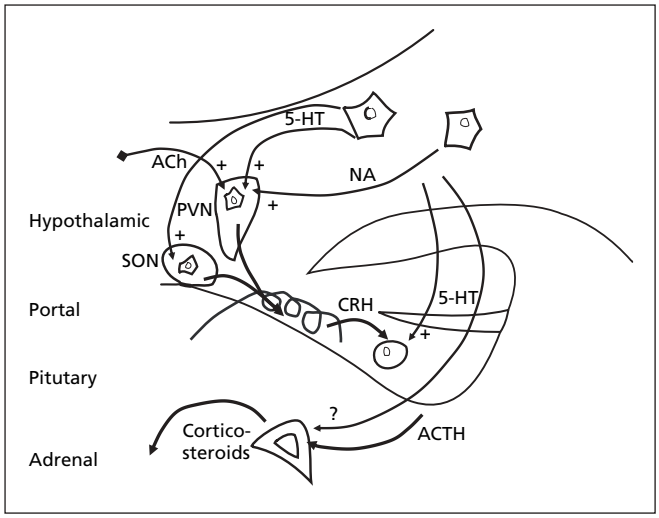

Figure 5. Schematic drawing of the hypothalamus and the pituitary gland in the rat showing the 5-HT neuronal projections from the dorsal raphe nucleus together with other aminergic connections to the hypothalamus. The four levels of interference with the hormonal system are indicated: hypothalamic, pituitary portal vessels in the median eminence, the pituitary gland and the adrenal cortex.

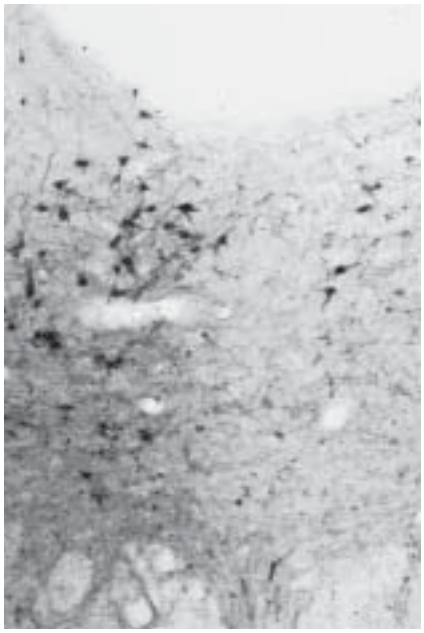

Figure 6. Photomicrograph of 5-HT immuno stained section of the rat brain through the dorsal raphe nucleus just below the IV ventricle (upper part) with right unilateral lesion of 5-HT neurons with the neurotoxic agent 5,7-DHT.

Data from lesion- or stimulation experiments on the involvement of the DRN are contradictory. Lesion of the DRN with 5,7-DHT did not inhibit 5-HT-induced corticosterone release whereas electric stimulation of the DRN increased ACTH in plasma (Van de Kar et al. 1982; Feldman & Weidenfeld 2004). We found that injection of 5,7-DHT either in the DRN, the PVN or i.c.v. partly inhibited stress-induced ACTH release (Figure 6) (Jorgensen et al. 1998a, IV). The importance of the PVN is also shown in lesion experiments which abolished the *p*-chloroamphetamine induced ACTH response or the stress-induced corticosterone response (Van de Kar et al. 1985a; Richardson Morton et al. 1990). Microinjection of 5-HT_{1A} agonists into the PVN increased plasma ACTH and dose-dependently decreased the amount of hypothalamic CRH (Pan & Gilbert 1992). Surgical lesion of the PVN abolished the ACTH response to the 5-HT_{1A} agonist ipsapirone (Bagdy 1996b). Administration of the 5-HT releaser fenfluramine induced an extensive neuronal activation, indicated by an up-regulation of the gene expression of Fos in the parvocellular PVN where c-5-HT_{2A}*fos* is expressed in CRH-immunoreactive neurons (Richard et al. 1992; Laflamme et al. 1996). In accordance with this, our experiments showed that an increased level of endogenous 5-HT, achieved by injection of 5-HTP/fluoxetine, or direct stimulation with 5-HT agonists activated the HPA-axis shown as an increased level of CRH mRNA in the PVN (Jorgensen et al. 2002a, VII).

The amygdala also seems to be involved in the regulation of CRH together with the PVN. Serotonergic neurons from the raphe nuclei projects to the amygdala and lesion herein inhibited the ACTH response to stimulation in the DRN (Weidenfeld et al. 2002b). Furthermore, the ACTH response to electric stimulation or injection of 5-HT agonists in the amygdala was blocked by neurotoxic lesion of the PVN (Feldman & Weidenfeld 1998; Feldman et al. 2000).

Immunoneutralisation of CRH by prior administration of anti CRH-antiserum (abCRH) inhibited the gene expression of POMC and the plasma ACTH response to 5-HT or 5-HT agonists (Jorgensen et al. 2002a, VII; Calogero et al. 1990). This indicate that CRH has a major role in the mediation of the 5-HT induced ACTH response, but also that 5-HT might stimulate the release of ACTH from the anterior pituitary gland via other pathways than through the hypothalamic CRH neurons, e.g. either directly at the corticotrophs or via interneurons in other brain areas.

The above mentioned findings indicate involvement of 5-HT neurons in the DRN and in the PVN in the mediation of the serotonergic induced activation of the HPA-axis, and in addition the amygdala possibly also contribute to this activation.

5.2.3 5-HT receptors involved in stimulation of CRH and ACTH

Neuronal 5-HT is involved in the mediation and regulation of the responses from all levels of the HPA-axis, including regulation of receptors, gene expression and release of hormones and in addition, 5-HT also interact with other neurotransmitters in this regulation (Mezey et al. 1987; Weiner & Ganong 1978; Jorgensen et al. 1996; Carrasco & Van de Kar 2003). The involvement of the different 5-HT receptors has been investigated on the possible synthesis of CRH and the secretion of ACTH.

5.2.3.1 Effect of 5-HT stimulation on CRH

Serotonin and 5-HT agonist were found to stimulate the release of CRH from explanted hypothalamic glands in vitro (Jones et al. 1976; Holmes et al. 1982; Nakagami et al. 1986; Buckingham & Hodges 1977), and fluoxetine increased the content of CRH in hypophysial portal plasma and of ACTH in peripheral plasma indicating a stimulating effect of 5-HT on CRH in vivo (Gibbs & Vale 1983).

With the use of in situ hybridisation with CRH oligopeptides on hypothalamic slices I investigated the relative importance of the 5-HT receptors on the induction of CRH and POMC synthesis measured as gene expression in relevant locations.

Serotonergic stimulation had no influence on gene expression of POMC in the intermediate pituitary gland (Jorgensen et al. 2002a, VII). This can be due to a higher basal level of POMC in the intermediate lobe, reducing the overall effect of 5-HT (Knigge et al. 1995) or to differentiated regulation of the two lobes (Garcia-Garcia et al. 1997). An involvement of the *5-HT_{1A} receptor* in the regulation of CRH was indicated by the effect of i.c.v. injection of 8-OH-DPAT on CRH mRNA and further substantiated by the finding of a depletion of CRH from the PVN, while increasing the level of ACTH in peripheral plasma after PVN injection of the 5-HT_{1A} agonist 8-OH-DPAT (Pan & Gilbert 1992). Infusion of a *5-HT_{2A} receptor* agonist induced a higher response of CRH mRNA and ACTH than a 5-HT_{2C} agonist (Jorgensen et al. 2002a, VII).

The importance of the 5-HT_{2A} receptor was supported by increased c-*fos* immunoreactivity in the PVN CRH neurons after injection of DOI (5-HT_{2A+2C} agonist) (Van de Kar et al. 2001). In accordance with our findings, a recent study identified an induction of c-*fos* in the PVN in response to 8-OH-DPAT or DOI (Mikkelsen et al. 2004). I did not find effect of stimulation of the 5-HT_3 receptor

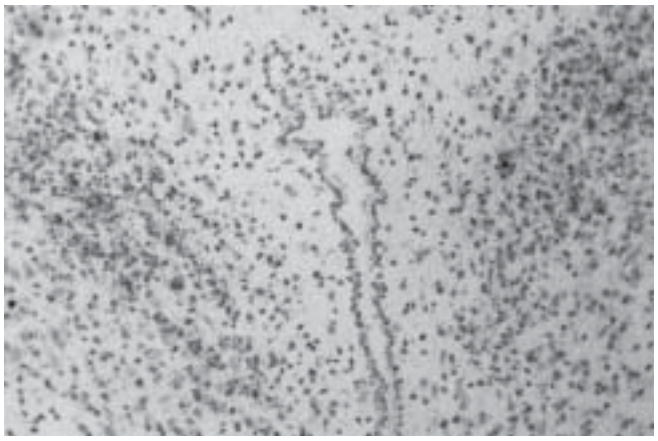

Figure 7. Photomicrograph of emulsion-dipped slice with in situ hybridization of mRNA expression of CRH in the rat brain paraventricular nucleus of the hypothalamus after intracerebroventricular infusion of the 5-HT agonist 8-OH-DPAT at -270 min.

on CRH or POMC mRNA. Microinjection of 5-HT in the PVN stimulated CRH mRNA in the PVN, POMC mRNA in the anterior pituitary gland and ACTH in peripheral plasma (Kageyama et al. 1998). Contrary to my findings they concluded that the effect was mediated through 5-HT_{1A} and 5-HT_3, but not 5-HT_2 receptors. However, this conclusion was drawn on the basis of the effect of 5-HT antagonists on ACTH in plasma, which may not completely reflect the effect of 5-HT in the PVN.

Therefore, the effect of 5-HT on the level of CRH mRNA in the PVN and POMC mRNA in the anterior pituitary lobe is mediated via the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A} and 5-HT_{2C} receptors, whereas the 5-HT_3 receptor do not seem to be involved (Figure 7) (Jorgensen et al. 2002a, VII).

The acute effect of 5-HT on CRH regulation, does not seem to apply to chronic serotonergic stimulation, since systemically administration of 8-OH-DPAT for 3 weeks increased CRH concentration in the hippocampus and the amygdala, whereas there was no effect on CRH in the median eminence (Owens et al. 1990). An explanation for the lack of effect might be down regulation of CRH receptors and of CRH synthesis.

5.2.3.2 Effect of 5-HT stimulation on ACTH

The above discussed indirect stimulation of ACTH via CRH neurons in the hypothalamus is the most important route for the 5-HT-induced ACTH secretion. A direct stimulation of the corticotrophs is also possible, since 5-HT agonists stimulated ACTH secretion directly from the pituitary gland in vitro (Spinedi & Negro Vilar 1983; Balsa et al. 1998; Calogero et al. 1993) an effect which was inhibited by 5-HT_2 antagonists (Jorgensen 1999), and since systemically infusion of 5-HT, which do not cross the blood-brain barrier (Bulat & Supek 1967), stimulated ACTH secretion in vivo (Gartside & Cowen 1990; Jorgensen et al. 1999, V). Furthermore, 5-HT_{1A}- or 5-HT_2 agonists stimulated ACTH and corticosterone in rats with transectioned pituitary stalk, indicating a direct effect of 5-HT on ACTH secretion (Calogero et al. 1990). Early studies indicated that the stimulating effect on ACTH primarily was mediated via the 5-HT_{1A} receptor (Gilbert et al. 1988; di Sciullo et al. 1990; Pan & Gilbert 1992; Koenig et al. 1987) and the 5-HT_2 receptor (Bagdy 1996a; Rittenhouse et al. 1994).

Ranked in order of potency, the primary 5-HT_{1A} agonists 8-OH-DPAT and 5-CT dose-dependently stimulated ACTH as did the 5-HT_2 agonist DOI, MK-212, mCPP and S-α-methyl-5-HT, whereas the 5-HT_3 agonist 2-methyl-5-HT did not (Jorgensen et al. 1999, V). Subsequently, additional experiments have shown that neither the 5-HT_3 agonists' mCPBG and SR-57227 nor the 5-HT_4 agonist RS 67506 had any effect on plasma ACTH (Table 6).

Concommitant administration of two agonists with different receptor specificity in submaximal doses potentiated the ACTH response to values much higher than the maximal response achieved by a high dose of a single agonist (Jorgensen et al. 1999, V). This indicate that large hormone reserves seem to be present in the pituitary gland and that stimulation of one type of 5-HT receptor do not induce activation of the whole population of CRH neurons or corticotrophe cells, and therefore do not induce a maximal hormone response. It has been reported that early pretreatment with a 5-HT_{2A+2C} agonist 2-4 h before a challenge injection of a 5-HT_{1A} agonist decreased the effect on the HPA-axis of the later injected substance, indicating either a desensization of the 5-HT_{1A} receptor or that the two 5-HT agonists interact on the same hypothalamic CRH neurons (Zhang et al. 2001; Mikkelsen et al. 2004). In addition, repetitive administration of 5-HT agonists of the same receptor type resulted in an attenuated ACTH response (Ross et al. 1992; Mazzola-Pomietto et al. 1996).

The importance of the different 5-HT receptors for the mediation of the ACTH response was studied by different combinations of agonists and antagonists. There is now substantial evidence for the involvement of the *5-HT_{1A} receptor* in the 5-HT-induced response as found in studies with 8-OH-DPAT (Rittenhouse et al. 1994; Gartside et al. 1992b; Vicentic et al. 1998; Van de Kar et al. 1998b) or other 5-HT_{1A} agonist (Koenig et al. 1988; Owens et al. 1990; Critchley et al. 1994; Pan & Gilbert 1992; Bagdy 1996b). The effect of 8-OH-DPAT was inhibited by the 5-HT_{1A} antagonist WAY 100-635 (Jorgensen et al. 2001, VI). The 5-HT_{1B} receptor might also be involved, as the 5-HT_{1B+1A} agonist RU 24969 had a stimulating effect, all though the evidence is weak (Jorgensen et al. 1999, V; Van de Kar et al. 1994).

The *5-HT_2 receptor* is indisputably involved (Jorgensen et al. 1999, V), but discrimination between the relatively importance of the three 5-HT_2 receptors was initially difficult due to the lack of receptor specific compounds (Calogero et al. 1990; Koenig et al. 1987; King et al. 1989). DOI (primarily 5-HT_{2A} agonist), MK-212 (5-HT_{2B+2C} agonist) and mCPP (primarily 5-HT_{2C} agonist) stimulated the HPA-axis (King et al. 1989; Jorgensen et al. 1999, V; Gartside et al. 1992b). It has been necessary to combine 5-HT drugs with lower specificity and interpret the results to clarify the involvement of different 5-HT receptors (Rittenhouse et al. 1994). The development of more specific antagonists and agonists has more precisely identified an involvement of the 5-HT_{2A} receptor (Van de Kar et al. 2001; Zhang et al. 2002). Since the 5-HT_{2B} receptor is not present in the rat brain it can be excluded.

In our studies, there was no significant effect of *5-HT_3 receptor* compounds on 5-HT-induced ACTH responses. The minor inhibiting effect of ICS was initially proposed to be due to an effect on the 5-HT_4 receptor (Jorgensen et al. 1999, V), but recent experiments did not find convincing dose-response effect of either 5-HT_4 agonists or antagonists on plasma ACTH (Table 6; unpublished observations) .

Our studies led to the proposal of an involvement of the 5-HT_5 and especially the 5-HT_7 receptor since 5-CT, which in addition to its affinity for 5-HT_1 receptors also possesses high affinity for 5-HT_5 and 5-HT_7 receptors, stimulated ACTH secretion (Table 7). In addition, this response was only partly inhibited by 5-HT_1 and 5-HT_2 antagonists, but this has not been confirmed by others (Jorgensen et al. 1999, V).

5.2.4 Stress-induced ACTH secretion

The HPA-axis is, together with the sympatho-adrenalmedullary system, one of the main components in the stress response. Different kind of stressors increase one or several of the hormones of the HPA-axis (Carrasco & Van de Kar 2003; Chaouloff 2000; Stratakis & Chrousos 1995). The most investigated mode of stress in rats, restraint stress, is found to stimulate CRH in plasma (Hashimoto et al. 1989), hypothalamic CRH mRNA (Harbuz et al. 1991), PVN CRH1

receptor (Imaki et al. 2001), ACTH in plasma (Gibbs 1984; Haas & George 1988; Hashimoto et al. 1989; Kjaer et al. 1992; Jorgensen et al. 1998a, IV) and corticosterone (Harbuz et al. 1993; Kant et al. 1986). Other paradigms of stress such as ether vapour, endotoxin (lipopolysaccharide; LPS), swim stress, electric foot shock and cold stress increased several parts of the HPA-axis (Hashimoto et al. 1989; Gibbs 1984; Assenmacher et al. 1995; Conde et al. 1998; Rivier et al. 1989; Jorgensen et al. 1998a, IV; Givalois et al. 1999; Yasuda & Greer 1978; Raghupathi & McGonigle 1997).

Abundant evidence substantiate the involvement of 5-HT in the mediation of the majority of the neuroendocrine effects of the stress response. Depletion of central stores of 5-HT by pretreatment with *p*-chlorophenylalanine inhibited the ether vapour stress- or LPS-induced ACTH response (Ixart et al. 1985). Neurotoxic lesion by injection of 5,7-DHT into either the PVN, the DRN or i.c.v. reduced the ACTH response to restraint stress by approximately 50% (Jorgensen et al. 1998a, IV). The role of 5-HT seems to be differentiated in various locations, since 5,7-DHT infusion before restraint stress further increased the level of POMC mRNA in the anterior pituitary gland but abolished it in the intermediate pituitary gland (Garcia-Garcia et al. 1997).

The 5-HT_{1A}, 5-HT_2 and the 5-HT_4 receptors seem to be involved in the restraint-, ether vapour- or LPS-induced ACTH response as antagonists with affinity for these receptors inhibited the ACTH responses (Jorgensen et al. 1998a, IV). *The 5-HT_{1A}* receptor antagonist WAY 100-635 partly inhibited the ACTH responses to restraint and LPS, whereas there was no effect on the conditioned emotional response (Groenink et al. 1996). Several studies show that stress alter the expression of the 5-HT_{1A} receptor in the hippocampus, further supporting the involvement of the 5-HT_{1A} receptor in stress-induced neuroendocrine changes (Mendelson & McEwen 1991; Watanabe et al. 1993; Raghupathi & McGonigle 1997). Studies with selective 5-HT_{1B} antagonists has not been performed.

The *5-HT_{2A} receptor* seem to be more important than the 5-HT_{2C} receptor, since ketanserin (5-HT_{2A} antagonist) inhibited the ACTH responses to restraint- and ether vapour stress, whereas LY 53857 (5-HT_{2C} antagonist) did not (Jorgensen et al. 1998a, IV). In agreement with this finding is the demonstration of an inhibition of the ACTH response to photic stress by ketanserin (Feldman et al. 1998). The *5-HT_3 receptor* does not seem to be involved in the ACTH response to restraint, ether vapour or endotoxin stress, since ondansetrone did not inhibit the repsonse (Jorgensen et al. 1998a, IV). On the other hand, it has been found that the 5-HT_3 receptor is involved in the mediation of the corticosterone response to acoustic stress (Saphier et al. 1995). The inhibiting effect of ICS might be mediated via the *5-HT_4 receptor*. Serotonin does not seem to be involved in the ACTH response due to swim stress since none of the antagonists had any effect on the ACTH response, a finding which is comparable to other studies, where there in addition to the absence of effect of cold swim stress also were lack of effect on hypoglycaemia- and conditioned emotional response stress (Table 9) (Fuller & Snoddy 1977; Paris et al. 1987).

5.2.5 Conclusion

Serotonergic neurons from the MRN and DRN projects to the PVN where they are in close contact with CRH neurons. These three nuclei are important for the 5-HT mediated responses but not essential, as lesion of 5-HT neurons in either the PVN or DRN reduced but not abolished the ACTH response to stress. The serotonergic system stimulates the HPA-axis both at hypothalamic and at pituitary gland level with increased levels of CRH mRNA in the PVN, POMC mRNA in the anterior pituitary lobe, CRH in pituitary portal plasma, ACTH and corticosterone in plasma. Serotonin stimulates ACTH secretion in vitro from the anterior pituitary gland. The effect of 5-HT is mediated mainly through the 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors. Furthermore, the 5-HT_{1B} receptor seems to be involved and an involvement of the 5-HT_{5A} and 5-HT_7 receptor is possible. An involvement of the 5-HT_4 receptor cannot be excluded, but the 5-HT_3 receptor does not seem to be involved in the serotonergic regulation of the HPA-axis. In respect to restraint- and endotoxin stress the same receptors seem to be involved, whereas in respect to ether vapour stress a more specific differentiation between the subtypes of the 5-HT_1 and the 5-HT_2 receptors has not be clarified. The 5-HT_3 or 5-HT_4 receptor does not seem to be involved in the ACTH response to ether vapour stress. The serotonergic system is not involved in the ACTH response to swim stress.

As seen in the experiments in this thesis and in the literature reviewed, 5-HT is involved in the basal and stress-induced regulation of hypothalamic and pituitary gland hormones in the rat. Furthermore, the finding of colocalisation of CRH-immunoreactive terminals and CRH_2 receptors on the neuronal soma and dendrites of serotonergic raphe nuclei substantiate an involvement of 5-HT in stress-related conditions (Ruggiero et al. 1999; Day et al. 2004). The data presented above and the studies mentioned in the discussion are primarily based on experiments in rodents and can not directly be applied to a clinical effect in humans. In humans, alterations in circulating levels and gene expression of hypothalamic and pituitary gland hormones and down regulation of the HPA-axis can be found in psychiatric diseases, as chronic activation of the HPA-axis is seen in melancholic depression (Holsboer et al. 1987), anorexia nervosa, panic disorder, obsessive-compulsive disorder, chronic alcoholism and excessive exercise (Gold et al. 1988; Stratakis & Chrousos 1995).

5.3 REGULATION OF VASOPRESSIN AND OXYTOCIN SECRETION

Vasopressin (AVP) and oxytocin (OT) are synthesised in the magnocellular neurosecretory cells in the PVN and supraoptic nucleus (SON) of the hypothalamus, transported via axons to the posterior pituitary gland (the neurohypophysis) where they are stored in axon terminals until they are released by relevant stimulation (Reichlin 1998). AVP acts at the vaspressin-2 (V2) receptors on the renal tubuli inhibiting diuresis. OT acts on OT receptors primarily contracting the lactiferous ducts and the uterus (Reichlin 1998). A minor part of AVP is synthesised in the parvocellular neurons of the PVN projecting to the external lamina of the median eminence and secreted into pituitary portal capillaries as a part of the hypothalamo-pituitary axis. The parvocellular AVP is contributing to the regulation of ACTH from the anterior pituitary gland (Rivier & Vale 1983) via the vasopressin V1 receptors (Antoni et al. 1984; Aguilera & Rabadan-Diehl 2000). In general, magnocellular AVP is stimulated by osmotic changes, while parvocellular AVP is stimulated by stress. Changes in osmotic parameters are identified in magnocellular AVP neurons in the SON and PVN (Bourque 1989). In addition, the SON AVP neurons receive afferent inputs from the subfornical organ and the organum vasculosum of the lamina terminalis (OVLT) which lack an effective blood-brain barrier (McKinley et al. 2004; Leng et al. 1992).

Volume related stimuli sensitized in the baroreceptors are transmitted to the hypothalamus by noradrenergic neurons from the locus ceruleus and tractus solitarius (Reeves et al. 1998; Sawchenko & Swanson 1983). Norepinephine, 5-HT and histamine have stimulating effect on AVP and OT secretion (Kjaer et al. 1994a; Falke 1991), whereas dopamine and γ-aminobutyric acid might have inhibiting effect (Kovacs et al. 2004). Furthermore, peptides as cholecystokinin, gastrin, vasoactive intestinal polypeptide seem also to be involved in the regulation of neurohypophysial hormones and endogenous opioids may exhibit a tonic inhibitory control of OT secretion (Falke 1991).

5.3.1 5-HT neurons involved in stimulation of AVP and oxytocin

The serotonergic input to the magnocellular part of the PVN and to the SON was found to be relatively limited, but their anatomical distribution was very distinctive with a plexus of networking AVP and 5-HT neurons (Sawchenko et al. 1983; Ferris et al. 1997; Larsen

et al. 1996). An indirect evidence for the involvement of 5-HT in the regulation of AVP was provided 20 years ago, as pretreatment with *p*-chlorophenylalanine or i.c.v. infusion of 5,7-DHT reduced the AVP response to dehydration by 60% (Iovino & Steardo 1985) and the 5-HT releasing agent fenfluramine dose-dependently increased plasma AVP (Iovino & Steardo 1985). However, 5-HT did not stimulate AVP secretion in vitro from hypothalamic fragments and neither did the 5-HT releaser 1-(m-triflourobethylphenyl)-piperazine have any effect on hypothalamic content of AVP, even though plasma AVP was increased (Hashimoto et al. 1982; Hillhouse 1989).

Systemic injection of 5-HTP/fluoxetine activated 5-HT neurons and stimulated gene expression of OT in the PVN, whereas neither AVP nor OT in the PVN were affected (Jorgensen et al. 2003a, X). A differentiated response on the neurohypophysial hormones was also identified for histamine, which induced an increase in OT mRNA in both PVN and SON but no change of AVP mRNA (Kjaer et al. 1994b; Kjaer et al. 1998). In accordance with the limited effect of 5-HT on AVP, it was found that fenfluramine did not enhance neuronal AVP c-*fos* expression in the hypothalamus (Javed et al. 1999; Mikkelsen et al. 1999). Norepinephrine has the same differences in its regulation of AVP and OT, as there was no effect on AVP hnRNA after PVN injection of norepinephrine (Itoi et al. 1999). Therefore, it seems that AVP and OT are differently regulated by neurotransmitters.

Lesion of 5-HT neurons in the PVN by localized injection of ibotenic acid reduced basal and stimulated levels of plasma OT, while there was no change in these responses after lesion in the SON, indicating that serotonergic neurons in the PVN but not the SON is involved in the regulation of OT secretion (Van de Kar et al. 1995). Furthermore, bilateral i.c.v. infusion of 5,7-DHT reduced pituitary gland content of both AVP and OT by 30% measured by RIA on extracted homogenized pituitary gland tissue (Saydoff et al. 1993). We found that lesion of 5-HT neurons in either the PVN or the DRN by direct injection of 5,7-DHT reduced the restraint-stress induced AVP response by 40% or increased the response with 50%, respectively (unpublished observations, (Jorgensen et al. 1998b)).

Direct stimulation by stereotactically intranuclear infusion of 5-HT in the PVN released extracellular AVP, as measured by microdialysis in the PVN (Jorgensen et al. 2003a, X). Localized release of AVP has also been measured after physiological stimuli as osmotic- (Ludwig et al. 1994; Landgraf & Ludwig 1991), and swim stress (Wotjak et al. 1998), but also emotional stress such as social defeat (Ebner et al. 2005). The physiological effect of AVP released intranucleary from the neuronal soma and dendrites seems to be related to the processes of learning and memory (Engelmann et al. 1996; Wotjak et al. 1998), adequate behaviour and coping strategies (Wigger et al. 2004; Wotjak et al. 1996; Engelmann et al. 2000). Central release of OT was detected in the SON after osmotic stimulation and lactation (Neumann et al. 1993), and in the PVN after emotional stress even if it did not release OT in peripheral plasma (Engelmann et al. 1999; Nishioka et al. 1998; Bosch et al. 2004).

Serotonergic-induced secretion of neurohypophysial hormones might to some extend be mediated by CRH. After neutralisation of circulating CRH by a specific anti CRH-antiserum we found that the AVP and OT responses to systemically injected 5-HTP/fluoxetine was reduced by 50-60% (Jorgensen et al. 2003a, X).

5.3.2 5-HT receptors involved in stimulation of AVP

Studies performed in the beginning of the 1990'ies showed that 5-HT_1 and 5-HT_2 receptors were important in the serotonergic regulation of AVP (Van de Kar 1991). I have re-evaluated the previous results using the new receptor classification supply new information (Hoyer et al. 1994). Serotonergic involvement in the mediation of activation of AVP neurons and AVP secretion seems primarily to be mediated via 5-HT_2 and 5-HT_4 receptors. Experiments with a battery of more or less selective or specific 5-HT agonists and antagonists were performed to evaluate the involvement of subreceptors and the latest identified 5-HT receptors.

The *5-HT_{1A} receptor* does not seem to be involved either in hypothalamic AVP gene expression or in peripheral AVP secretion as centrally infused 8-OH-DPAT had no effect and as the 5-HT_{1A+1B} antagonist cyanopindolol had no effect on the AVP stimulating effect of 5-CT (Jorgensen et al. 2003b, IX).

Agonists' specific for the *5-HT_2 receptor* stimulated AVP mRNA in the PVN measured by in situ hybridization, with no effect in the SON or of other 5-HT agonists (Jorgensen et al. 2003a, X). The stimulating effect on AVP of centrally infused 5-HT was abolished by the 5-HT_{2C} antagonist LY 53857 (Jorgensen et al. 2003b, IX), a finding in accordance with others (Pergola et al. 1993; Saydoff et al. 1996). The 5-HT_{2A} agonist DOI had either a much less stimulating effect or no effect at all on AVP secretion than other 5-HT_2 agonists, and DOI-induced responses were not inhibited by the specific 5-HT_{2A} antagonist flourobenzoyl piperadine xalate (Jorgensen et al. 2003b, IX; Bagdy et al. 1992). Furthermore, DOI failed to stimulate Fos-immunoreactivity in AVP neurons (Van de Kar et al. 2001). The primary 5-HT_{2C} agonists MK-212 and m-CPP potently stimulated AVP and these responses were inhibited by the primary 5-HT_{2C} antagonists as ritanserine, LY 53857 and SB 242084 but not by the 5-HT_{2A} antagonist ketanserin (Jorgensen et al. 2003b, IX; Brownfield et al. 1988; Bagdy et al. 1992). Thus the 5-HT_{2C} receptor is crucial in AVP secretion, whereas the 5-HT_{2A} receptor seems of less importance.

Involvement of the *5-HT_3 receptor* is not obvious since SR 57277 stimulated AVP secretion whereas m-CPBG had no effect (Jorgensen et al. 2003b, IX). The combined 5-HT_{3+4} antagonist ICS partly inhibited the AVP response to SR 57277, whereas the selective 5-HT_3 antagonist Y-25130 did not. An involvement of the *5-HT_4 receptor* seems more likely, since ICS also have affinity at this receptor, and since the selective 5-HT_4 antagonist markedly inhibited the AVP response to 5-HT and to some extend inhibited the response to the selective 5-HT_4 agonist RS 67506. We have not identified other studies involving 5-HT_3 or 5-HT_4 antagonists in rats, but in man the 5-HT_3 receptor was found to be involved in hypoglycaemia- and nausea induced AVP secretion (Volpi et al. 1998; Barreca et al. 1996).

An involvement of the *5-HT_{5A} and 5-HT_7 receptor* might be possible since 5-CT, which has high and equal affinity for these receptors, increased AVP secretion contrary to the 5-HT_{1A} receptor agonist 8-OH-DPAT. Furthermore, the AVP response to 5-CT was inhibited by the combination of metergoline and methysergide but not by selective 5-HT_{1A} or 5-HT_{1B} antagonists. No other studies have investigated the involvement of these receptors in the neurohypophysial hormone response.

5.3.3 5-HT receptors involved in stimulation of oxytocin

I.c.v. infusion of 8-OH-DPAT or agonists with affinity for 5-HT_{1B}, 5-HT_{2A}, or 5-HT_{2C} receptors increased OT mRNA in the SON and the PVN, and in addition a 5-HT_3 agonist stimulated OT mRNA in the PVN (Jorgensen et al. 2003a, X). In accordance with some of these findings, activation of the 5-HT_{2A} receptor stimulated Fos-immunoreactivity in OT neurons in the SON and PVN (Van de Kar et al. 2001).

The plasma OT responses to the *5-HT_{1A} receptor* agonists 5-CT and 8-OH-DPAT were inhibited by specific antagonists, indicating an important effect of this receptor, in accordance with others findings (Jorgensen et al. 2003b, IX; Bagdy et al. 1992; Uvnas-Moberg et al. 1996; Li et al. 1994; Van de Kar et al. 1998a; Vicentic et al. 1998).

Unlike the AVP responses, the *5-HT_2 receptor* agonists DOI (5-HT_{2A}) and MK 212 (5-HT_{2C}) had almost equal effect on OT secretion (Jorgensen et al. 2003b, IX; Van de Kar et al. 2001). However, only the 5-HT_{2C} receptor-induced response was inhibited by the relevant antagonist, indicating that the 5-HT_{2C} receptor is more important than the 5-HT_{2A} receptor (Jorgensen et al. 2003b, IX).

The 5-HT_3 receptor is unlikely to be involved in the 5-HT induced

OT secretion, since the specific antagonist Y-25130 had no effect on the OT response to SR 57277 and since another 5-HT_3 agonist m-CPBG had no effect on OT secretion (Jorgensen et al. 2003b, IX; Uvnas-Moberg et al. 1996). The specific *5-HT_4 antagonist* RS 23597 inhibited the OT response to 5-HT and to the 5-HT_4 agonist RS 67506, indicating an involvement of this receptor (Jorgensen et al. 2003b, IX). Activation of OT neurons and stimulation of OT secretion involve the 5-HT_{1A} receptor, the 5-HT_2 receptor, the 5-HT_4 receptor and to less extend the 5-HT_3 receptor.

5.3.4 Stress-induced secretion of AVP and oxytocin

Beside the normal physiologically induced secretion of neurohypophysial hormones they were found to be stimulated by stress such as haemorrhage or water-deprivation (Kasting 1988; Reeves et al. 1998; Jorgensen et al. 2002b, VIII). In addition, non-osmotic stress was capable of increasing AVP or OT levels in plasma (Gibbs 1986).

Initially only OT was considered a stress-hormone (Lang et al. 1983; Hashimoto et al. 1989; Jezova et al. 1995), but later the involvement of AVP in the stress response has been documented (Engelmann et al. 1998; Jorgensen et al. 1998b). Even the theory of AVP as a primary result of physical stress (hypovolemia or haemorrhage) (Fyhrquist et al. 1981; Baylis & Robertson 1980), contrary to psychological stress (immobilization, ether-vapour or forced swimming) (Yagi & Onaka 1993; Keil & Severs 1977; Lang et al. 1983), was modified as it was shown that repeated psychological stress increased the level of extracellular AVP and AVP mRNA in the PVN (Wotjak et al. 1996; Ma et al. 1999). In addition, AVP is a weak stimulator of ACTH secretion and potentates the stress-induced effect of CRH on ACTH secretion (Antoni et al. 1984; Plotsky 1988), mediated via an upregulation of the CRH1 receptor (Aguilera et al. 2001). Despite this, there are discrepancies between the responses to different stress factors in respect to AVP, OT and ACTH (Jezova et al. 1995; Gibbs 1986; Jorgensen et al. 2002b, VIII; Jorgensen et al. 1998a, IV; Gibbs 1984; Hashimoto et al. 1989).

Restraint stress has in several studies shown to increase both ACTH and OT but not AVP secretion (Hashimoto et al. 1989; Gibbs 1984). Contrary to this, we and other groups have documented that manual restraint stress (which may be considered more as physical stress contrary to passive immobilization in a plastic tube which is psychological stress) increased AVP (Jorgensen et al. 2002b, VIII; Kasting 1988; Husain et al. 1979). Comparison of three different states of progressive immobilizations stress (1) in a Plexiglas restrainer, (2) manual restraint and (3) body compression showed a progressive increment in plasma AVP (Husain et al. 1979). Furthermore, acute manually restraint increased the gene expression of AVP mRNA in the medial parvocellular PVN (Aubry et al. 1999; Bartanusz et al. 1993), whereas AVP synthesis and AVP mRNA in the PVN was unchanged after immobilization in a Plexiglas tube for 6 h or 1 h, respectively (Franco-Bourland 1998; Pinnock & Herbert 2001). Chronic stress induced as short daily periods of immobilization slightly enhanced CRH immunoreactive neurons, but markedly increased the number of AVP containing CRH cell bodies (de Goeij et al. 1992; Aubry et al. 1999). This and other reports indicate that in the PVN the response to chronic stress is primarily regulated by AVP rather than CRH (Aguilera 1994).

Five minutes of *ether vapour stress* did not increase either AVP or OT in our study (Jorgensen et al. 2002b, VIII), whereas others found an increase of both hormones (Gibbs 1984). However, the detection of this effect might be dependent on the timing of measurement since the increased level of AVP after 2 min of ether vapour stress disappeared within 3 min (Hashimoto et al. 1989).

Endotoxin and foot shock stress increased AVP and OT in peripheral plasma (Kasting 1988; Husain et al. 1979), hypoglycaemia increased AVP in pituitary portal plasma (Plotsky et al. 1985) but not in peripheral plasma (Jorgensen et al. 2002b, VIII; Kasting 1988; Kjaer et al. 1995a) and hyper- or hypothermia and exercise increased OT but not AVP in plasma (Kasting 1988).

I found that *cold swim stress* increased ACTH and OT but not AVP in peripheral plasma (Jorgensen et al. 2002b, VIII), which is in accordance with other findings (Lang et al. 1983; Husain et al. 1979). Even though, swim stress augmented central release of both AVP and OT from the SON, the PVN and the suprachiasmatic nucleus (Wotjak et al. 1998; Engelmann et al. 1998; Wotjak et al. 2001).

Lesion of noradrenergic neurons affects some aspects of stress-induced AVP and OT responses (Carter & Lightman 1987; Onaka & Yagi 1998) and inhibition of histamine synthesis or histamine receptors decreased the dehydration induced AVP responses or central and peripheral OT responses (Kjaer et al. 1994a; Kjaer et al. 1995b). As both histamine, norepinephrine and 5-HT is involved in basal release of AVP and OT (Kjaer et al. 1994a; Iovino & Steardo 1985), it was investigated if 5-HT is involved in stress-induced AVP secretion. Pretreatment with 5-HT antagonists before exposure to stress revealed that at least 5-HT_2, 5-HT_3 and 5-HT_4 receptors are involved in the restraint stress-induced AVP secretion, whereas the OT response involved the 5-HT_{1A} and 5-HT_2 receptors (Jorgensen et al. 2002b, VIII). Unfortunately, there are no comparable experiments, but the receptors involved in the hormonal responses to restraint stress are comparable to the 5-HT agonists who increased basal hormone responses (Jorgensen et al. 2003b, IX). Serotonin is not involved in osmotic or dehydration-induced AVP secretion, since 5-HT compounds had no effect on dehydration (Jorgensen et al. 2002b, VIII) or osmotic-induced AVP secretion (Faull et al. 1993), but at least the 5-HT_2 receptor is involved in the OT response to dehydration (Jorgensen et al. 2002b, VIII). Likewise, 5-HT_2 receptors are involved in the OT response to haemorrhage but the potent induction of AVP secretion does not seem to involve 5-HT (Jorgensen et al. 2002b, VIII). Swim stress-induced release of OT is probably not mediated via 5-HT receptors as there where no effect of pretreatment with 5-HT antagonists.

5.3.5 Conclusion

Serotonergic neurons from the MRN and DRN projecting to the PVN of the hypothalamus are important for the basal and stress-induced secretion of AVP and OT. Serotonin is an important neurotransmitter in stimulation of neurohypophysial hormone gene expression and in the regulation of hormonal release. Central administration of 5-HT releases AVP into the extracellular tissue in the PVN. Serotonin agonists with affinity for the 5-HT_{2A+2C} receptors increase the level of AVP mRNA in the PVN but not in the SON. The secretion of AVP into peripheral plasma primarily involves stimulation of the 5-HT_{2C}, 5-HT_4 and the 5-HT_7 receptor whereas the 5-HT_{2A}, the 5-HT_3 and the 5-HT_{5A} receptor is less important.

Serotonin activates the gene expression of OT in the SON and PVN and this effect is at least mediated via 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, and 5-HT_{2C} receptors. The secretion of OT is primarily mediated via 5-HT_{1A}, 5-HT_{2C} and 5-HT_4 receptors, and in addition the 5-HT_{1B}, the 5-HT_{2A}, the 5-HT_{5A} and the 5-HT_7 receptors probably are involved, whereas the 5-HT_3 receptor has minor importance.

The various stress procedures used in our and others experiments have different effect on AVP and OT secretion, indicating a different regulation of the two neurohypophysial hormones. In general, these findings indicate that both physical and psychological stress procedures activate the hypothalamic-neurohypophysial system, which may not necessarily be reflected in elevated peripheral hormone levels. Serotonin 5-HT_{2A+2C} receptors are involved in the stress-induced AVP and OT responses, and possibly the 5-HT_3 or 5-HT_4 receptor is in addition involved in restraint stress-induced AVP release and the 5-HT_{1A} receptor is involved in the OT response to restraint stress.

6. INTERACTION BETWEEN 5-HT AND OTHER NEUROTRANSMITTERS

Hormone release from the hypothalamus and the pituitary gland is mediated by a combination of monaminergic neurotransmitters, amino acids transmitters and neuropeptides. The finding of an in-

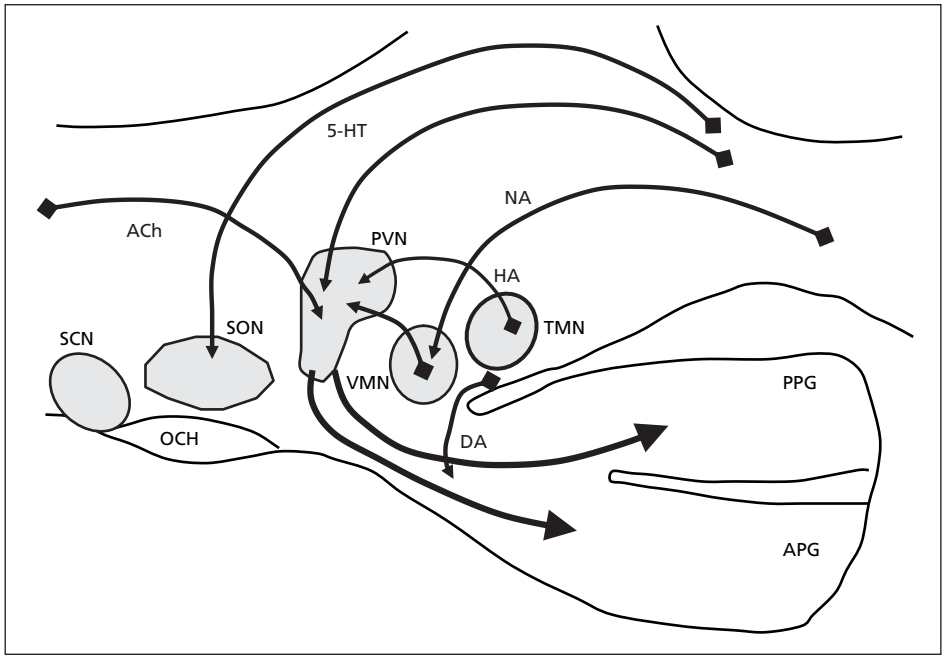

Figure 8. Schematic drawing of the hypothalamic region of the rat brain showing the interactions between the serotonergic (5-HT), the catecholaminergic (NA), dopaminergic (DA), histaminergic (HA) and the acethylcolinergic (ACh) systems on the different nuclei of the hypothalamus: paraventricular (PVN), suprachiasmatic (SCN), supraoptic (SON), tuberomammilary (TMN), venteromedial (VMN). OCH = optic chiasm; APG = anterior pituitary gland; PPG = posterior pituitary gland.

fluence of histamine on hypothalamic 5-HT release (Tuomisto & Tuomisto 1980; Pilc & Nowak 1979) lead to investigations of the possible interaction between 5-HT and histamine in the regulation of PRL secretion (Knigge et al. 1988b). Later we found that histamine H_1 or H_2 receptor antagonists inhibited the 5-HT induced PRL but not ACTH secretion, and likewise a 5-HT_{1+2} or a 5-HT_{2C+2A} antagonist inhibited histamine induced PRL but not ACTH secretion (Jorgensen et al. 1996). Furthermore, stereotactical lesion of histaminergic perikarya in the posterior hypothalamus reduced the PRL response to 5-HTP/fluoxetine (Knigge et al. 1999). Likewise, we found that histamine interact with the catecholaminergic system in the regulation of PRL but not ACTH (Willems et al. 1999). An interaction between the glutamate and 5-HT system does also exist, since blockade of *N*-methyl-D-aspartate (NMDA) receptors with selective glutamate ionotropic receptor antagonists inhibited the PRL response to 5-HTP or DOI (5-HT_{2A+2C} agonist) (Aguilar et al. 1997) and the ACTH response to 8-OH-DPAT (5-HT_{1A} agonist), respectively (Feldman & Weidenfeld 2004). Furthermore, NMDA antagonists reversed the attenuated ACTH response induced by repetitive 8-OH-DPAT or mCPP injections (Ross et al. 1992; Mazzola-Pomietto et al. 1996). Noradrenaline and 5-HT interact in the regulation of the HPA-axis, since the ACTH responses to intranuclear injection of 8-OH-DPAT in the PVN or to the α-1 receptor agonist phenyladrenaline were inhibited by hypothalamic depletion of either noradrenaline by means of the neurotoxin 6-OH-dopamine or 5-HT by the neurotoxin 5,7-DHT, respectively (Weidenfeld et al. 2002a). In conclusion, both the histaminergic, catecholaminergic, glutaminergic, cholinergic and dopaminergic system interact with the serotonergic system in their regulation of the release of hypothalamic and pituitary gland hormones, possibly via neuronal connections in the hypothalamic PVN (Figure 8).

7. FUTURE PERSPECTIVES AND CLINICAL IMPLICATIONS

The data presented above and the studies mentioned in the discussions are primarily based on experiments in rodents and cannot without limitations be generalized to a clinical effect in humans. However, knowledge about basal mechanisms in serotonergic signaling in rodents is essential for understanding and development of new drugs for treatment of pathological conditions in these systems, since the rat brain is a very useful model and results can often be applied for studies in man.

Alterations in circulating levels and gene expression of hypothalamic and pituitary hormones and down regulation of the HPA-axis are found in especially psychiatric diseases, as chronic activation of the HPA-axis is seen in melancholic depression (Holsboer et al. 1987), anorexia nervosa, panic disorder, obsessive-compulsive disorder, chronic active alcoholism and excessive exercise (Gold et al. 1988; Stratakis & Chrousos 1995). As reported in the literature reviewed 5-HT is involved in the basal and stress-induced regulation of several hypothalamic and pituitary hormones in humans. Furthermore, the finding of co-localization of CRH-immunoreactive terminals and CRH2 receptors on the neuronal soma and dendrites of serotonergic raphe nuclei substantiate an involvement of 5-HT in stress-related diseases (Ruggiero et al. 1999; Day et al. 2004). Together with recent findings this indicates an involvement of 5-HT in the pathophysiology of these diseases and possible therapeutic implications as 5-HT drugs may regulate and modify disturbances in the HPA-axis (Bohus et al. 1987; Graeff et al. 1996).

The data presented in this thesis indicate that the role of the 5-HT_5 and the 5-HT_7 receptors, which are located in the amygdala, the hippocampus and the suprachiasmatic nucleus (Gustafson et al. 1996) is important and these receptors should be further studied. Serotonin and agonists with affinity for the 5-HT_7 receptor induce phase shifts in SCN cells, indicating an effect on circadian rhythms. This effect should be studied in animal models. Furthermore, the involvement of 5-HT_4, 5-HT_{5A} and 5-HT_7 receptor in the hormonal stress responses, should be elucidated more intensively. Development and characterisation of 5-HT antagonists with higher affinity for the 5-HT_5 and the 5-HT_7 receptors would be valuable. The clinical value of such stress-experiments with such substances could be interesting, since 5-HT_5 and 5-HT_7 receptors have influence on anxiety, psychosis, depression and circadian rhythms (Vanhoenacker et al. 2000; Roth et al. 1994).

Several small clinical trials indicate that physical exercise relieves depressive symptoms. Preliminary in situ experiments in rodents indicate that gene expression of 5-HT transporter protein and 5-HT_{1B} receptor is changed in response to physical exercise. Further studies with other receptors and localisations should be performed.

The interaction of 5-HT with leptin, neuropeptide Y, α-melanocyte stimulating hormone, norepinephrine and histamine should be studied in respect to changes in neuroendocrine functions. E.g. these neurotransmitters and some hypothalamic peptides are involved in the central regulation of food-intake. Possible genetic differences in 5-HT receptors and 5-HT transporter as responsible for abnormal sensation of hunger and satiety can be studied with gene expression of 5-HT receptors in different animal strains or SPECT

scan in objects with either low or high energy intake pattern (Dunlop et al. 2005).

8. CONCLUDING REMARKS

These studies together with comparison to the literature have described the role of 5-HT in important areas of the neuroendocrine regulation. With respect to the aims the following conclusions can be made:

A. The involvement of 5-HT and the 5-HT receptors in regulation of:
i. the gene expression of hypothalamic hormones

The 5-HT-induced increase in gene expression of CRH in the PVN and POMC in the anterior pituitary lobe is mediated via 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A} and 5-HT_{2C} receptors, but not the 5-HT_3 receptor. 5-HT_{2A+2C} agonists increase the level of AVP mRNA in the PVN but not in the SON, whereas OT mRNA is increased both in the SON and the PVN. This effect is in addition to the 5-HT_2 receptors also mediated via 5-HT_{1A+1B} receptors.

ii. the hypothalamo-adenohypophysial system (prolactin and ACTH)

The PRL and ACTH response to 5-HT stimulation is mediated via 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors. An involvement of 5-HT_{1B}, 5-HT_5 or 5-HT_7 receptors is possible. As a novelty at the time of investigation, the 5-HT_3 receptor was found to be involved in the secretion of PRL, but not ACTH secretion, which in contrast may involve the 5-HT_4 receptor.

iii. the neurohypophysial system (vasopressin and oxytocin)

Central infusion of 5-HT releases AVP into the extracellular tissue in the PVN. The peripheral secretion of AVP primarily involves 5-HT_{2C}, 5-HT_4 and 5-HT_7 receptors. The secretion of OT is primarily mediated via 5-HT_{1A}, 5-HT_{2C} and 5-HT_4 receptors and probably also 5-HT_{1B}, 5-HT_{2A}, 5-HT_{5A} and 5-HT_7 receptors.

B. The relative importance of some distinctive central nuclei in the basal and stress-induced hormone secretion

The DRN is essential and for the major part the PVN is also involved in the 5-HT-induced PRL response, whereas the MRN, the DRN and the PVN probably are equal in the mediation of the HPA-axis response. Likewise 5-HT neurons from the MRN and DRN projecting to the PVN of the hypothalamus are important basal and stress-induced secretion of AVP and OT.

C. The involvement of 5-HT and the 5-HT receptors in the stress-induced neuroendocrine response

Neuroendocrine responses to stress are dependent on the type of stress, how the stress is induced, the experimental time schedule and the specific hormone of interest, therefore generalisations cannot be made. Stress-induced PRL responses is mediated via 5-HT_{2A}, 5-HT_{2C} and 5-HT_3 receptors, whereas an involvement of 5-HT_{1A} receptors is doubtful. ACTH secretion due to stress is mediated via 5-HT_{1A}, 5-HT_2 and 5-HT_4 and possibly also 5-HT_{5A} and 5-HT_7 receptors. The AVP response to restraint stress involve 5-HT2 and possibly 5-HT_3 or 5-HT_4 receptors. The 5-HT_{1A} receptor is important in restraint induced OT secretion and 5-HT_2 receptors are involved in the OT responses to dehydration and haemorrhage whereas 5-HT does not seem to be involved in the AVP response to these stressors.

D. The stress-induced changes in metabolism of 5-HT in the hypothalamus and the dorsal raphe nucleus

Restraint stress increases the content of 5-HT in the DRN but not in hypothalamic tissue, and there are no significant changes of 5-HT metabolism in either the hypothalamus or DRN after swim-, ether vapour- or endotoxin stress, however very different results are found in the literature.

Serotonin is deeply involved in the regulation of hypothalamic and pituitary gland hormonal secretion. The effect of 5-HT on these hormones is primarily mediated via neurons originating in the MRN and DRN projecting to the PVN or the SON, where 5-HT exerts its effect on CRH, AVP or oxytocin neurons.

9. SUMMARY

The aim of the thesis was to investigate in male Wistar rats, the involvement of serotonin (5-HT) and 5-HT receptors in the regulation of the gene expression of hypothalamic hormones and in the secretion of the pituitary gland hormones prolactin (PRL), adrenocorticotropic hormone (ACTH), vasopressin (AVP) and oxytocin in basal and stress conditions. Furthermore, to study the significance of some distinctive central nuclei in these processes, and the metabolism of 5-HT in the hypothalamus and the dorsal raphe nucleus (DRN). The experiments were focused on (1) determination of involved neurons and nuclei (2) the hypothalamic level and (3) the pituitary gland level of regulation. The studies were typically performed in vivo but some studies were performed in vitro.

Stereotactically neurotoxic lesion with 5,7-dihydroxy-5-HT in the dorsal raphe nucleus (DRN) or the hypothalamic paraventricular nucleus (PVN) reduced the ACTH and AVP response to stress, indicating an importance of these structures for this response. In situ hybridization on rat brain slices with oligopeptides showed an increase of corticotropin releasing hormone (CRH) mRNA in the PVN and proopiomelanocortin in the anterior pituitary lobe upon stimulation of the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A} and 5-HT_{2C} receptors. Stimulation of 5-HT_{2A+2C} receptors increased AVP mRNA in the PVN but not in the supraoptic nucleus (SON), whereas the level of oxytocin (OT) mRNA was increased both in the SON and the PVN and this effect was in addition mediated via 5-HT_{1A+1B} receptors. Serotonin infused directly into the PVN by microdialysis stimulated local release of AVP. CRH was found to have a major role but not a complete responsibility in the 5-HT-induced release of ACTH, since immunoneutralisation of CRH inhibited the POMC gene expression and the ACTH response and since 5-HT and 5-HT antagonists were able to modulate the ACTH release from anterior pituitary gland cells in vitro.

Through the years of investigation, the classification of the 7 main groups of 5-HT receptors (5-HT_1 – 5-HT_7) has changed due to molecular biological characterisation of the receptors and new receptors have been identified. With a battery of 5-HT agonists and antagonists several pharmacological experiments were performed with systemically or central administration of compounds and radioimmuno assay of plasma for pituitary gland hormone levels. Specific substances were not available for all 5-HT receptors and subreceptors thus some conclusions are a based on combination of experiments. The 5-HT induced PRL response is mediated via 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C} and 5-HT_3 receptors. In addition an involvement of 5-HT_{1B}, 5-HT_5 or 5-HT_7 receptors seem possible. The ACTH response to 5-HT is mediated via 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A} and 5-HT_{2C} receptors and an involvement of the 5-HT_4, 5-HT_5 and 5-HT_7 receptors is proposed. Peripheral secretion of AVP upon stimulation with 5-HT is mediated via 5-HT_{2C}, 5-HT_4 and 5-HT_7 receptors but not 5-HT_{1A} receptors. The secretion of OT is primarily mediated via 5-HT_{1A}, 5-HT_{2C} and 5-HT_4 receptors and probably also 5-HT_{1B}, 5-HT_{2A}, 5-HT_{5A} and 5-HT_7 receptors.

Physical and psychological stress activates hippocampal and hypothalamic 5-HT neurons. In contrast to other stress factors, restraint stress increase the content of 5-HT in the DRN but do not increase the metabolism of 5-HT and do not induces changes in hypothalamic levels of 5-HT. Large variations are found in the literature with different kinds of stress, different measurements and different time schedules. Restraint or ether stress induced secretion of PRL involves 5-HT_2 and 5-HT_3 receptors, whereas the ACTH secretion is mediated via 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors. In the present study restraint stress increased AVP secretion, but opposite findings has reported possibly due to differences in the stress procedure. The 5-HT_2, 5-HT_3 and 5-HT_4 receptor is involved in the

AVP response to restraint whereas the OT response involves the 5-HT_{1A} and the 5-HT_2 receptor. The 5-HT_2 receptor is involved in the OT response to dehydration or haemorrhage, whereas the AVP responses to these stressors probably do not involve 5-HT.

It can be concluded that 5-HT is involved in basal and stress-induced regulation of PRL, ACTH, AVP and oxytocin mainly via the 5-HT_{2A+2C} receptors but other receptors are also important but differs from hormone to hormone. Serotonin affect the secretion of CRH and ACTH both at the hypothalamic, pituitary portal and pituitary gland level, and possibly also at the adrenal level.

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