Role of neuropeptides in behavioural and neuroendocrine changes mediating hypothalamo-pituitary-adrenal axis in rats

PhD thesis

Éva Bíró MD

Department of Pathophysiology
Albert Szent-Györgyi Medical University,
Szeged

1998

Contents

List of publications related to the subject of the thesis

Full papers

I. Biró É., Sarnyai Z., Szabó G., Telegdy G.: The role of endogenous CRH in the mediation of the behavioural and neuroendocrine response to cholecystokinin octapeptide sulphate esther (CCK) in rats. Neuroendocrinology 57: 340-345, 1993.

II. Biró É., Penke B., Telegdy G.: Role of different neurotransmitter systems in the cholecystokinin octapeptide-induced anxiogenic response in rats. Neuropeptides 31(3): 281-285, 1997.

III. Biró É., Tóth G., Telegdy G.: Involvement of neurotransmitters in the "anxiolytic-like" action of atrial natriuretic peptide in rats. Neuropeptides 29: 215-220, 1995.

IV. Biró É., Gardi J., Vecsernyés M., Julesz J., Tóth G., Telegdy Gy.: The effects of atrial natriuretic peptide (ANP_{1-28}) on corticotropin releasing factor in brain of rats. Life Sci 59, 16: 1351-1356, 1996.

V. Biró É., Tóth G., Telegdy G.: Effect of receptor blockers on brain natriuretic peptide and C-type natriuretic peptide caused anxiolytic state in rats. Neuropeptides 30(1): 59-65, 1996.

VI. Gardi J., **Biró É.,** Vecsernyés M., Julesz J., Nyári T., Tóth G., Telegdy Gy.: The effects of brain and C-type natriuretic peptides on corticotropin-releasing factor in brain of rats, Life Sci 60, 23: 2111-2117, 1997.

Quotable abstracts

VII. Gardi J., **Biró É.,** Vecsernyés M., Julesz J., Nyári T., Tóth G., Telegdy Gy.: The effects of brain and C-type natriuretic peptides on corticotropin-releasing factor in brain of rats. Life Sci, Vol 60, No 23, pp2111-2117, 1997.

VIII. Biró É., Sarnyai Z., Penke B., Telegdy G.: The role of endogenous CRH in the mediation of the neuroendocrine and behavioural response to CCK in rats. Abstracts of Second International Meeting of European Neuropeptide Club, "Neuropeptides in normal and pathologic function", Vol 22, No 1, p9, 1992.

IX. Biró É., Telegdy G.: "Anxiolytic-like" effects of atrial natriuretic factor and it's neuromodulation. Journal of Psychopharmacology, BAP/EBPS Joint Meeting Abstract Book, A-271, 1992.

X. Biró É., Telegdy G.: The role of different neurotransmitter systems in anxiogenic response to centrally administered cholecystokinin (CCK-8) in rats. Suppl. to Eur J Neuroscience No5, p1228, 1992.

XI. Biró É. Tóth G., Telegdy G.: The role of atrial natriuretic peptide (ANP) in basal and stress induced neuroendocrine and behavioural responses in rats. J Endocrinol. Invest.16, (Suppl.1 to No 8.), C27, 1993.

XII. Biró É., Gardi J., Vecsernyés M., Julesz J., Tóth G., Telegdy G.: The effect of atrial-natriuretic peptide (ANP) on corticotropin releasing-factor content in different brain regions of rats. Eur J Endocrinol, Vol 130 (Suppl.2), p3070, 1994.

XIII. Telegdy G., **Biró É.**: Anxiolytic action of atrial natriuretic peptide family in rats. Involvement of transmitters. 7th Meeting of European Neuroendocrine Association, 1995.

XIV. Gardi J., **Biró É.**, Vecsernyés M., Julesz J., Tóth G., Telegdy G.: The effects of brain-natriuretic peptide (BNP) and C-type natriuretic peptide (CNP) on corticotropin-releasing factor (CRF) in different brain regions of rats. Behav Pharmacol., Vol.7 (Suppl.1.), 1996.

1. Introduction

1.1. Short overview on systems involved in response to stressors

The stability of milieu interieur was first described by Claude Bernard and extended by Walter Cannon. He coined that term homeostasis is denoting the condition of relative consistency of the internal environment according to the classic view. The hypothalamic-pituitary-adrenal axis (HPA) and the autonomic nervous system are recognised as primary effector systems which serve to minimize excursions from the homeostatic state and to restore a relative equilibrium in response to disturbances (Selye et al., 1946 and Cannon et al., 1929).

Many of the physiological adjustments accompanying sympathetic activation complement those brought about by glucocorticoid secretion. Most stressors are first encoded into neurochemical messages and are then processed into the central nervous system. These signals are relayed to the hypothalamus via multiple, distributed pathways, where the signals are transformed into a hypopysiotropic message by specialised neurosecretory cells in the paraventricular nuclei. The hypophysiotropic message generated is multifactorial in composition and may include corticotropin-releasing factor (CRF), arginine vasopressin and possibly oxytocin, epinephrine and angiotensine II. The intercellular messengers diffuse from capillaries into the pituitary to act on corticotropes expressing appropriate membrane receptors to stimulate the secretion of adrenocorticotrop hormone (ACTH), which in turn acts on adrenocortical cells to initiate synthesis and secretion of glucocorticoids. The adrenal steroids, in addition to their diverse functions dump further secretory activity of the HPA at pituitary and central sites of action (Keller-Wood et al., 1984). The HPA represent a classical closed loop negative feedback system with circulating glucocorticoids as the controlled variable. Numerous neuronal afferents to the CRF and arginine vasopressin (AVP) containing pericarya in the parvicellular paraventricular nucleus permit rapid adaptation to interoceptive and exteroceptive stimuli, allowing for an emergency override in response to unexpected stressors.

1.2. CRF and HPA axis

CRF is generally thought to be the primary activator of the HPA axis trough physical and emotional stresses, initiating biological responses to stress within the brain, including the effects on the autonomic nervous system. Since investigators in La Jolla, namely Vale, Riviere, Brown, Ficher and Koob recognised that many of the effects of CRF resembled those observed in stress it suggested the possibility that CRF may be an endogenous mediator of stress. Studies published by Rivier and coworkers in 1984 with specific CRF antagonist - alpha helical CRF (ahCRF) or antisera to CRF (Ono et al., 1985; Rivier et al., 1982) indicate that CRF is the major factor causing elevations of the plasma ACTH under basal and stressful situations. Immunoreactive CRF has been found to be altered in various brain areas following both acute and chronic stress (Chapell et al., 1986).

CRF injected centrally can potentiate the effects of exposure to novel, aversive environment as determined in several tests of anxiety, namely in an open filed test, in operant conflict test, in a social interaction test, in an acustic startle test, in a defensive withdrawal test and in an elevated plus-maze test (Dunn et al., 1990) and can be blocked by centrally administered ahCRF. Because CRF antagonists were able to attenuate or reverse the effects of various stressors, CRF appears to be a mediator of these responses. Because the responses to intracerebrally administered CRF cover the entire spectrum of responses observed in stress, it is possible to postulate that the secretion of brain CRF may be both necessary and sufficient to define stress.

There is good body of evidence that activation of noradrenergic systems in stress is the primary mechanism for the release of CRF from neurones in the paraventricular nucleus (PVN), possibly through α_1-receptor, although undoubtedly other neurotransmitter systems (eg. acetylcholine, serotonin and γ amino butyric acid) are also involved (Tuomisto et al., 1985).

Behavioural and physiological responses to intracerebroventricularly (i.c.v.) administered CRF can be divided into high-dose and low-dose effects. The high-dose effects include the activation of the sympathetic nervous system as well as of the adrenal medulla and of cerebral catecholamines. These effects include the decreased feeding and sexual behaviour, the decreased locomotor activity in a novel

environment, the increased locomotor activity in a familiar environment, the anxiogenic effects in the Geller-Seifter conflict test and the exacerbated acoustic startle response. On the other hand, the enhancement of locomotor behaviour in a novel environment, the decreased social interaction and exploratory behaviour in the multicompartment chamber, the defense withdrawal and the increased feeding and shock induced fighting are observed at considerably lower doses. It is possible that these two sets of dose effects of CRF might represent two distinct degrees of stress. The low-dose effects may correspond to mild to moderate activations of noradrenergic systems such as may be associated with arousal and induce a state of mild anxiety (cf. review article of Dunn and Berridge, 1990).

It is possible that CRF-containing neurones in the PVN have collaterals reaching to widespread areas of the brain or an interesting alternative would be the innervation of CRF-containing cells by the network of catecholaminergic terminals in widespread areas of the brain, including the cortex but only further detailed research on the inputs to CRF-containing neurones can resolve this issue. The noradrenergic activation caused by high doses of CRF may provoke release of endogenous CRF, forming a positive feedback loop, escalating the release of both catecholamines and CRF. In the absence of exogenous CRF, a similar state may perhaps be achieved by prolonged or intense activation of noradrenergic system, such the amounts of CRF released could be sufficient to activate catecholaminergic systems, and thus provide a positive feedback, stimulating the release of still more CRF (Plotsky et al., 1989). Such a situation may be akin to panic.

A number of observations have suggested that CRF functions are abnormal in depressed patients. Nemeroff has reported that the cerebrospinal fluid (CSF) concentrations of CRF were significantly elevated over normal in depressed patients. Such a result is consistent with the long standing observation of elevated plasma cortisol and an insenzitivity to dexamethasone (Nemeroff et al., 1988). Moreover, same authors have published that depressed suicide victims have been found to exhibit decreased number of CRF-binding sites in the prefrontal cortex. This relationship suggest that the secretion of brain CRF is elevated in depression and that this elevation leads to a desensitisation of CRF receptors.

CRF hypersecretion may also play a role in the pathophysiology of anorexia nervosa as was found in case of many patients who exhibit depressive symptoms. CRF dysregulation appears to occur in case of posttraumatic stress disorder, Alzheimer's disease, Parkison's disease and progressive supranuclear palsy and amyiotropic lateral sclerosis (cf. review article of Owens and Nemeroff, 1991).

1.3. CCK and HPA axis

Cholecystokinin (CCK) was one of the first gastrointestinal hormones to be discovered in the mammalian brain (Barden et al., 1981), especially in the lymbic system, an area involved in the control of emotion and anxiety. A considerable amount of evidence suggests that CCK octapeptide sulphate ester (CCK 8) has a wide range of central actions as a neurotransmitter or neuromodulator affecting activity of the central nervous system (Crawly et al., 1989). CCK appears to modulate actions of several classic neurotransmitters, such as dopamine, serotonin (5-HT), endogenous opioids, γ amino butyric acid (GABA) and excitatory amino acids (Harro et al., 1993). Stress leads to elevated CCK-levels in discrete hypothalamic regions (Siegel et al., 1987). Accordingly, CCK B receptors are found in high concentrations in regions thought to be involved in the control of affective behaviour.

It has been recognized for several years that treatment with CCK 8 can depress the exploratory activity of rodents in the elevated plus maze test (Ravard et al., 1990). The anxiogenic potential of CCK in animals was first proposed by Fekete and co-workers, who reported that injection of CCK 8 into the central nucleus of the amygdala enhances behavioural arousal and fear motivation of rats (Fekete et al., 1984). CCK peptides also increase defensive burying in rats (Csonka et al., 1988).

Considerable in vivo and in vitro evidence supports a direct excitatory action of CCK 8 on adenohypophyseal pro-opiomelanocortin-containing cells, leading to the release of ACTH and ß-endorphin and to increased corticosteroid levels (Itoh et al., 1979).

The possibility that CCK might act on neurotransmitters was shown in earlier experiments done in our institution, which demonstrated that CCK altered the

turnover of dopamine, norepinephrine and serotonin in different brain areas (Fekete et al., 1981 and 1982). Peripheral injection of CCK has increased the release of noradrenaline, dopamine and serotonin from hypothalamic nuclei (Kendrik et al., 1991). Crawley and her collegues in 1988 have shown that CCK potentiate dopamine mediated behaviours.

1.4. Natriuretic peptides and HPA axis

The family of natriuretic peptides contains the atrial (ANP), brain (BNP) and C-type (CNP) natriuretic peptides. These peptides are localized in different brain areas which are critically involved in the regulation of neuroendocrine and some other central nervous system functions.

Numerous studies indicated that ANP can inhibit secretion of corticotropin (Hattori et al., 1986 and Wittert et al., 1993) and other proopiomelanocortin-derived peptides, such as β-lipotropin, β-endorphin, α melanocyte-stimulating hormone (α-MSH), as well as prolactin, growth hormone and thyroid-stimulating hormone, which are released during stress (Franci et al., 1992). Evidence in literature could be found that ANP inhibits the release of CRF from the hypothalamus in vitro (Ibanez-Santos et al., 1990 and Takao et al., 1988). In addition to above mentioned it is known that ANP has an inhibitory effect on the release of vasopressin, the most potent cofactor of CRF on pituitary ACTH secretion (Bardeleben et al., 1985 and Gillies et a., 1982). In isolated perifused rat anterior pituitary cells atriopeptin 1-28 and 5-28 potently suppressed the corticotropin secretion elicited by the 41-residue CRF and VP at concentrations which are in the range of those in hypophyseal portal vessel blood (Fink et al., 1991).

Only a few data have been reported on the central nervous system (CNS) actions of other members of natriuretic peptide family. Centrally administered BNP antagonized the central endothelin-induced pressor response, plasma cathecholamine and ACTH secretion (Makino et al., 1990).

Polymerase chain reaction (PCR) and in situ hybridization analysis demonstrated that ANP and CNP messenger ribonucleic acid (mRNA) are

coexpressed (and potentially colocalized) in several hypothalamic loci, suggesting an interaction in the regulation of the reproductive, adrenocortical and neurohypophyseal axes (Herrman et al., 1993).

1.5. Aims of the study

The aim of the experiments described below was to investigate different neuropeptides in commonly used experimental animal setting examining anxiety as well as to describe possible neurotransmission of these anxiogenic/anxiolytic effects and to eventually correlate our findings with changes in endogenous CRF levels in several hypothalamic and extrahypothalamic brain regions after central administration of these neuropeptides. Based on the quite substantial literature of neuropeptides we identified some of them with special importance which might be the candidates for further investigation in number of psychiatric illnesses, including affective disorders and anxiety disorders. As others in their previous work, we in our studies have tried to describe possible involvement of quite different systems, namely classic neurotransmitters and endogenous CRF which is also known to act as a neurotransmitter within the central nervous system.

We made attemption to find experimental evidences to a well established hypothesis that CRF is the predominant chemical messenger by which the CNS controls the activity of the pituitary-adrenal axis and which is therefore ultimately responsible for orchestrating the endocrine response to stress.

The bulk of research has been conducted with CCK peptides and much data has been accumulated over the past decade supporting the hypothesis that CCK plays a role in neurobiology of anxiety and panic attacks. In our first experimental setting we investigated a possible involvement of endogenous CRF and different neurotransmitter systems in the anxiogenic and pituitary-adrenal axis activating effects of cholecystokinin octapeptide sulphate ester in rats.

Based on findings in the literature of natriuretic peptides we have tried to test effects of different doses of ANP in a well established behavioural model of anxiogenic/anxiolytic state. Since central CRFergic system, as it is widely accepted,

plays a critical role in anxiety and other behavioural stress responses attempts were made to find experimental evidences of correlation of these two peptides. Although changes in activity of the brain CRFergic system could not be considered as the answer regarding the transmission of the effects of ANP we investigated the involvement of classic neurotransmitter systems in the mediation of peptides' action. In other words we tried to answer how can ANP influence the neurotransmitter metabolism or the action of neurotransmitters.

To investigate the other members of natriuretic peptide family BNP and CNP were also tested in elevated plus maze paradigm to assess their effect on anxiety state of rats. Besides examining the effects of central application of various doses of both peptides on the CRF-LI levels in different brain regions we tried to establish involvement of neurotransmitters in the BNP and CNP induced anxiolytic action.

2. Materials and Methods

2.1. Animals

Adult male Wistar rats (LATI Gödöllő, Hungary) weighing 200-250 g were used throughout the experiments. They were housed 5 per cage at a constant room temperature, with 12-hour light/12-hour dark cycles. All animals had access to commercially available food and tap water ad libitum. Rats were handled daily (5-10 minutes) for 6 consecutive days before the experiments, in order to accustom them to presence of humans and to minimize the effect of nonspecific stress. The studies were approved by the Ethical Committee for the Protection of Animals in Research of Albert Szent-Györgyi Medical University.

Surgery

Under pentobarbital anesthesia (Nembutal, CEVA, Paris France; 35mg/kg, i.p.), the animals had an indwelling cannula implanted into the right lateral brain ventricule 7 days before the experiment. The stainless steel cannula, with an external diameter of 0.7 mm, was stereotaxically inserted into the ventricule at a point 1.0 mm

posterior, 1.5 mm lateral and 3.5 mm ventricular according to the atlas of Pellagrino and co-workers. After confirmation of the outflow of cerebrospinal fluid from the upper end of the cannula, it was fixed to the skull with screws and dental cement. After termination of the experiment, the location of the cannula was checked via the injection of methylene blue solution. The dye was distributed not only in the lateral ventricle but also in the third ventricule in every case. Only data from animals with accurate placement were considered to further investigations.

2.2. Peptides and drugs

CCK 8

CCK 8 (synthetized by B.Penke) was dissolved in 0.9% saline and injected by microsyringe in a 1 μg dose in 2 μl volume to conscious, freely moving rats. The control animals received the same volume of artificial CSF.

CRF antiserum, ahCRF

The CRF antiserum was obtained from a rabbit immunized with CRF coupled to bovine serum albumin. The affinity-purified CRF antibody did not cross-react with fragments of human $CRF_{(1-20)}$ and $CRF_{(6-33)}$. Cross reactivity with $CRF_{(21-41)}$ was 2.1%. Different solutions (1:10, 1:20 and 1:100) of CRF antiserum, kindly donated by P.Vecsei, Department of Pharmacology, University of Heidelberg and normal rabbit serum for control animals were injected i.c.v. in 2 μl volume 24 hours prior to CCK 8 administration. Different doses of ahCRF (0.001-1.0 μg) in 2 μl volume were given i.c.v. 30 minutes before CCK treatment while control animals received the same volume of artificial CSF.

ANP

ANP_{1-28} was purchased from Bachem (CA, USA) or was synthetized by G.Tóth. There was no difference between chemical parameters and physiological effects of these peptides originating from different sources. It was dissolved in 0.9% saline and was injected i.c.v. in a volume of 2 μl in six doses (50, 100, 150, 200, 500

and 1000 ng per animal) to conscious, freely moving rats 30 minutes before the start of the experiment. The control animals received the same volume of artificial CSF.

BNP and CNP

Rat BNP and CNP were purchased from Bachem (CA, USA) or were synthetized by G.Tóth. There was no difference between chemical parameteres and physiologic effects of these peptides originating from different sources. They were dissolved in 0.9% saline and injected i.c.v. in a volume of 2 μl in different doses (25, 50, 100, 200, 400 and 1000 ng per animal) to conscious, freely-moving rats. The control animals received the same volume of artificial CSF.

Neurotransmitter antagonists

Neurotransmitter receptor blockers were injected i.p. 30 minutes before peptide or vehicle administration as follows: 5 μg/kg haloperidol (G.Richter Budapest); 2 mg/kg phenoxybenzamine hydrochloride (Smith Klein and French, UK); 10 mg/kg propranolol hydrochloride (Imp. Chem. Indust. Ltd, UK); 2 mg/kg atropine sulphate (EGYS, Budapest); 1mg/kg bicuculline methiodide (Sigma, USA); 5 mg/kg methysergide hydrogenmaleinate (Sandoz, D); 0.1 mg/kg naloxone hydrochloride (Endo Lab. Inc., USA). Control animals received the same volume of 0.9% saline.

2.3. Behavioural study

The elevated plus maze was first used and described by Pellow and coworkers, which become one of the standard tests to assess the anxiolytic/anxiogenic properties of peptides and drugs. The method is based on an approach avoidance conflict generated by a fear of open, brightly-lit areas and a drive to explore a new environment. In other words, the idea of the test is that open arms are more fear provoking and that the ratio of time spent in open vs. closed arms or the ratio of the entries into open vs. closed arms reflects the relative "safety" of closed arms as compared with the relative "danger" of open arms. This paradigm has an advantage over other conflict tests that it requires only the spontaneous activity of animals. The elevated plus maze apparatus was constructed to be elevated above the floor (50 cm),

and it consists of two open and two closed arms with each arm (10 x 50 cm) positioned at a 90 degree angle to the adjacent arm. ahCRF and CRF antiserum were administered i.c.v. 24 hour and 30 minutes before neuropeptide treatment. Neurotransmitter receptor blockers were injected i.p. 30 minutes before the central administration of peptide or arteficial CSF. Behavioural testing started 30 minutes after the injection of neuropeptides. The animals were placed on the center of maze, facing one of the open arms. During a five minutes test period, the behaviour of the rat was recorded by a trained observer, blind to the treatment conditions, sitting 1 m from the center of the maze. Between each trial, the plus maze was wiped clean with a damp cloth. Behavioural testing was conducted in a quiet room with constant illumination. Animals were transported to the experimental room a night before the behavioural test to eliminate the stressor effects of transportation and the new environment. Recordings were made of the numbers of entries into open and closed arms, and the times spent in open and closed arms. Entry into an open arm was defined as entry of all four feet into that arm. These scores were converted into percentage (open/open + closed) values. Anxiolytic compaounds increase the percentage time spent in and frequency of entries into the open arms. Anxiogenic substances have the opposite effect. Total entries into arms provided a measure of overall activity. Anxiety-related behaviour (grooming, defecation) was observed also. Each animal was tested only once in the plus maze apparatus.

2.4. Determination of plasma corticostrone

For testing adrenal response and behavioural changes two separate groups of animals were used. 30 minutes after the peptide treatment, animals were decapitated and the trunk blood was collected in a heparinized glass tube and the plasma corticosterone content was assayed fluorimetrically according to Zenker and Bernstein.

2.5. Determination of CRF-LI

2.5.1. Preparation of samples

Animals were sacrificed in a separate room 30 minutes after i.c.v. injection of artificial CSF and of different doses of peptides (ANP, BNP and CNP). After decapitation, the brains were quickly removed and various brain regions were isolated from both hemispheres on ice by modification of the technique of Glowinsky and Iversen. The hypothalamus was defined as the tissue within 3 mm of the ventral surface of the brain. The anterior extreme was the optic chiasm; the posterior extreme were the mamillary bodies; the lateral extremes were the lateral hypothalamic sulci. The hippocampus was removed as a whole. The amygdaloid complex was dissected form below surface. The anterior and posterior borders of the amygdaloid complex were defined by the genu of the corpus callosum and the optic chiasm. The basal forebrain area (tuberculum, nucleus accumbens and septal area) was removed form the anterior-ventral part of the telencephalon as a trapezoid from tissue sliced by razor cuts. This complex was bordered by the frontal cortex anteriorly, and by a razor cut at the level of the anterior commisure posteriorly. The dorsal border of the basal forebrain complex was the corpus callosum and the lateral borders were the internal walls of the lateral ventricules laterally to the ventral surface of the anterior telencephalon. The frontal cortex was obtained by a razor cut at the level of the genu of the corpus callosum.

2.5.2. Radioimmunassay

For the radioimmunassay (RIA) to determine the CRF like immunoreactivity (CRF-LI), each area was homogenized with ultrasound (Soniprep 150 MSE, GB) in HCl (100 mM) containing 1 mM ascorbic acid, and an aliquot was sampled for protein measurement. The residual homogenates were centrifuged at 6,000 g for 20 minutes, at 4°C, and aliquots were taken and lyophylized for RIA. The CRF tracer was prepared by using a modified iodogen method in order to minimalize damage to the iodinated peptide. The labelled material was purified via two steps of reverse-phase high performance liquid chromatography, a gradient HPLC system being applied in the second step. The specific radio-activity of the purified tracer was 1700-1900 Ci/mmol. The freeze-dried residues were redissolved in a 1 ml assay buffer (50 mM phosphate, pH 7.4, containing 0.25% human serum albumin and 0.1% Triton X-100) and 200 ml aliquots were subjected to RIA. The assay standard was a synthetic

h/rCRF preparation (Bachem, Budendorf, Switzerland). The procedure involved a standards with antiserum (100 ml, working dilution 1:10,000) which was followed by a 24-hour incubation with CRF tracer (100 ml, 10.000 cpm). The immunologically bound and free fractions were separated with a second antibody (raised in sheep against whole rabbit IgG in the Laboratory of Endocrine Unit of First Department of Medicine, Albert Szent-Györgyi Medical University, Szeged and subsequently by polyethylenglycol 6000 precipitation (FERAK Laborit Gmbh, Berlin, Germany) by the DAB/PEG method. The lower limit of assay detection was 7-8 pg/tube. The intra and interassay coefficients of variation were 4.0 and 13.8%, respectively. CRF-LI in the brain extracts subjected to HPLC has been shown to chromatograph with synthetic r/h CRF. The CRF-LI is expressed in pg/mg protein.

2.6. Statistical analysis

All data were presented as mean ± S.E.M. Data were analysed by one-way ANOVA followed by Dunnett's, Duncan's or Tukey's tests. A probability level of 0.05 was accepted as indicating a statistically significant difference.

3. Results

3.1. Role of endogenous Corticotropin-Releasing Factor in mediation of neuroendocrine and behavioural responses to cholecystokinin octapeptide sulphate ester

In our first experimental setting we were able to prevent anxiogenic effect of centrally administered CCK 8 with pretreatment with different dilutions of CRF antiserum as indicated in figure 1. I.c.v. administered CCK 8 induced 'anxiogenic like' response in the rats scored in the plus maze apparatus which was blocked by 1:10 and 1:20 dilutions of CRF antiserum administered 24 hours prior CCK 8 treatment. Both the decrease of the time spent in the open arms as well as decrease of the number of entries into the open arms were inhibited by these doses of CRF antiserum while 1:100 dilution has not such effect.

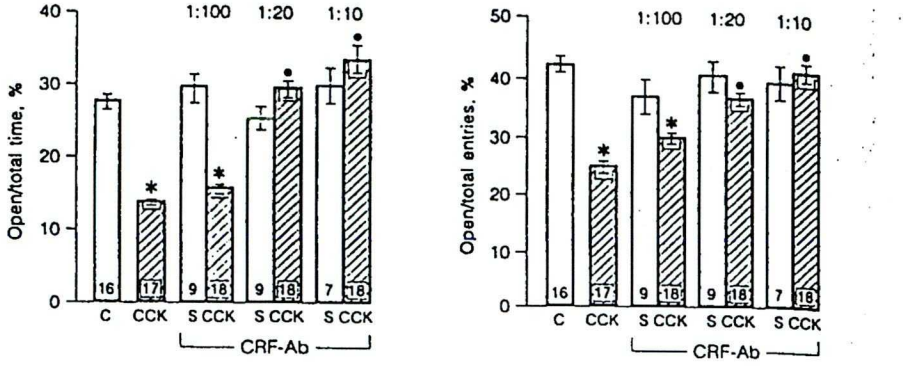

Figure 1. Histobars represent the mean ± SEM percentage of time spent in the open arms and the percentage number of entries into the open arms. Numbers of animals in each group are shown in the bars. C = control rats; S = saline treated rats; CRF-Ab = CRF antibody; CCK= cholecystokinin sulphate ester

* $p < 0.05$ vs. control group; • $p < 0.05$ vs. CCK 8.

In the same experimental setting rats were pretreated with different doses of synthetic CRF antagonist ahCRF as was presented in figure 2. CCK 8 induced stress response was prevented by central administration of 0.01, 0.1 and 1.0 μg ahCRF while 0.001 μg dose was found ineffective. None of the doses of CRF antiserum and ahCRF alone affected the exploratory behaviour of animals tested.

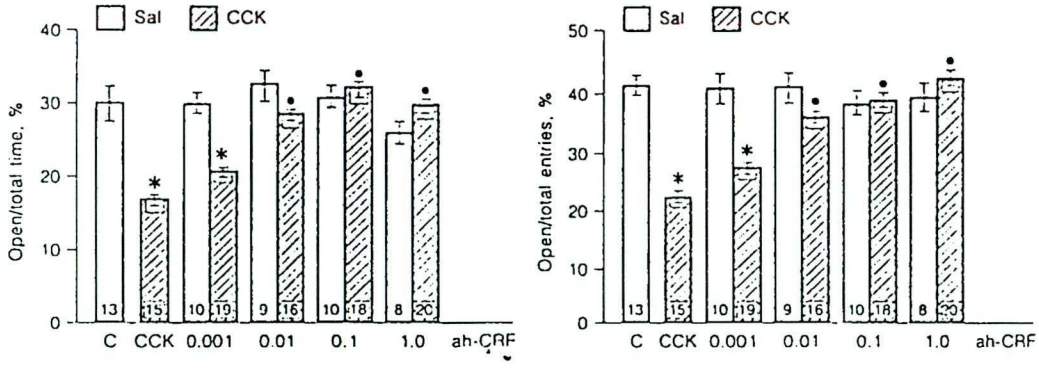

Figure 2. Histobars represent the mean ± SEM percentage of the time spent in the open arms and the percentage number of entries into the open arms. Numbers of

animals in each group are shown in the bars. C = control animals; S = saline treated rats; ah-CRF = alpha helical CRF (synthetic CRF antagonist); CCK= cholecystokinin sulphate ester

* $p < 0.05$ vs. control group; • $p < 0.05$ vs. CCK 8.

Not only behavioural changes were tested during this experiment but pituitary-adrenal axis activating effects of CCK 8. Figure 3 indicates effects of different doses of CRF antiserum on CCK 8-stimulated neuroendocrine response, namely pretreatment with 1:10 and 1:20 dilutions of CRF antiserum 24 hours prior to peptide administration significantly prevented the plasma corticosterone elevation, while pretreatment with 1:100 dilution has not such an effect.

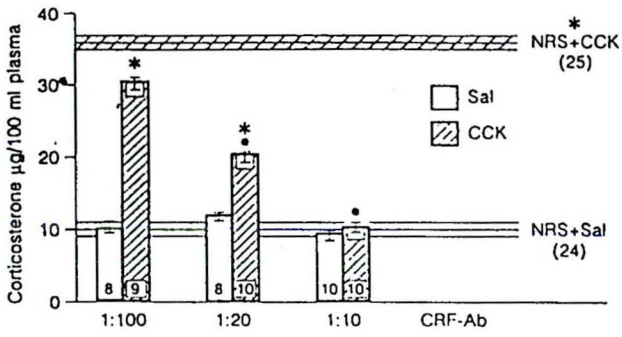

Figure 3. Results are expressed as mean ± SEM plasma concentrations of corticosterone. Numbers of animals in each group are shown in histobars. NRS = normal rabbit serum; SAL = saline treated rats; CRF-Ab = CRF antibody; CCK= cholecystokinin sulphate ester

* $p < 0.05$ vs. control group; • $p < 0.05$ vs. CCK 8.

Figure 4 represents the effects of different dilutions of synthetic CRF antagonist ah-CRF on CCK 8-induced plasma corticosterone elevation, namely pretreatment with 0.01, 0.1 and 1.0 µg of ahCRF prevented this well known effect of CCK 8, while 0.001 µg was ineffective.

None of the doses of CRF antiserum and ahCRF alone modified the plasma corticosterone level in the saline treated control rats.

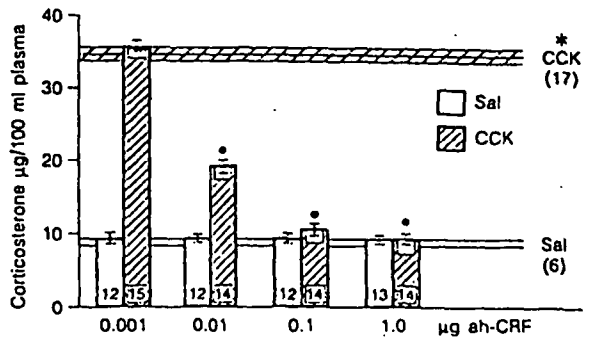

Figure 4. Results are expressed as mean ± SEM plasma concentrations of corticosterone. Numbers of animals in each group are shown in histobars. CCK = cholecystokinin sulphate ester; SAL = saline treated rats; ah-CRF = alpha helical CRF;

* $p < 0.05$ vs. control group; • $p < 0.05$ vs. CCK 8.

3.2. *Role of different neurotransmitter systems in the cholecystokinin octapeptide induced anxiogenic response*

1 µg of CCK 8 administered i.c.v. significantly decrease percentage of the time spent in the open arms and the percentage number of entries into the open arms. These anxiogenic behavioural changes were blocked by peripheral (intraperitoneal) pretreatment of dopaminergic, muscarinergic cholinergic and opiate receptor blockers, or in other words 10 µg/kg haloperidol and 2 mg/kg atropine and 0.1 mg/kg naloxone prevented the reduction of the time spent in the open arms of the elevated plus maze and also antagonised the decrease in number of entries into the open arms. On the other hand pretreatment with α and β adrenoreceptor blockers (2 mg/kg phenoxybenzamine and 10 mg/kg propranolol), GABA (5 mg/kg bicuculline) and 5-HT (5 mg/kg methysergide) receptor blockers were unable to modulate the anxiogenic effect of CCK 8 in the elevated plus maze test of anxiety. Table 1 summarizes data on effects of these neurotransmitter blockers on CCK 8 induced 'anxiogenic-like' state.

Groups	Number of animals	Open/total time %	Open/total entries %
Haloperidol (10μg/kg i.p.)			
Control (saline)	9	24.28±1.93	35.87±3.34
Saline+CCK-8 (1μg)	7	10.19±1.97*	23.24±4.75*
Haloperidol+saline	7	22.24±5.85	47.13±4.85
Haloperidol+CCK-8 (1μg)	12	22.43±2.96	46.11±3.62
Phenoxybenzamine (2mg/kg i.p.)			
Control (saline)	7	27.56±2.56	44.05±4.80
Saline+CCK-8 (1μg)	8	15.23±1.13*	23.38±2.84*
Phenoxybenzamine+saline	8	21.02±3.34	35.00±1.09
Phenoxybenzamine+CCK-8 (1μg)	10	14.51±1.40*	25.33±3.21*
Propranolol (10 mg/kg i.p.)			
Control (saline)	7	26.59±2.03	44.05±4.80
Saline+CCK-8 (1μg)	8	14.14±1.61*	23.80±2.04*
Propranolol+saline	10	19.87±3.44	36.22±3.25
Propranolol+CCK-8 (1μg)	10	17.56±1.78*	38.38±2.81
Atropine (2 mg/ kg i.p.)			
Control (saline)	8	27.58±1.34	42.54±2.42
Saline+CCK-8 (1μg)	9	16.31±1.12*	28.95±1.78*
Atropine+saline	6	24.06±5.16	33.89±4.33
Atropine+CCK-8 (1μg)	7	23.83±2.04	35.51±2.77
Methysergide (5mg/kg i.p.)			
Control saline	6	26.36±1.86	40.97±4.36
Saline+CCK-8 (1μg)	6	15.21±2.11*	19.89±3.17*
Methysergide+saline	10	27.14±3.70	46.99±3.89
Methysergide+CCK-8 (1μg)	8	17.04±1.06*	29.79±3.75*
Bicuculline (5mg/kg i.p.)			
Control (saline)	8	26.25±2.37	41.67±4.79
Saline+CCK-8 (1μg)	7	16.05±1.41*	21.13±2.95*
Bicuculline+saline	9	23.88±1.20	34.81±2.16
Bicuculline+CCK-8 (1μg)	9	16.02±0.83*	23.73±1.72*
Naloxone (0.1 mg/ kg i.p.)			
Control (saline)	32	22.49±1.89	35.03±2.11
Saline+CCK-8 (1μg)	35	10.69±1.52*	23.55±2.61*
Naloxone+saline	30	28.39±3.40	36.24±2.50
Naloxone+CCK-8 (1μg)	31	22.91±2.62	35.05±2.94

Table 1. Results are presented as means ± SEM. * $p < 0.05$ (ANOVA, Tukey's test) vs control group.

3.3. ANP induced behavioural effects tested in elevated plus maze paradigm

Figure 5 shows the effect of i.c.v. administration of ANP_{1-28} in doses of 50, 100, 150, 200, 500 and 1000 ng to rats tested in the elevated plus-maze paradigm. It can be seen that doses of 100, 150 and 200 ng of ANP_{1-28} significantly increased percentage of the time spent in the open arms as compared with saline treated control rats. Better said these doses abolished the normal preference of the closed arms of the maze which is consistent with an 'anxiolytic-like' effect. The doses of 50, 500 and 1000 ng ANP_{1-28} were ineffective. From this dose escalation 200 ng of ANP_{1-28} was chosen to be used in further experiments.

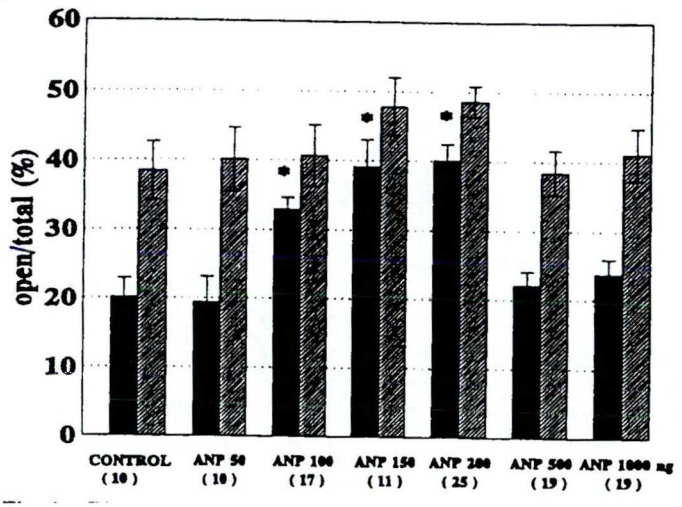

Figure 5. Results are presented as means ± SEM percentage of the time spent in the open arms. * $p < 0.05$ (ANOVA, Tukey's test) vs control group. Numbers in brackets represent the number of animals used.

3.4. Role of different neurotransmitter systems in the Atrial Natriuretic Peptide induced 'anxiolytic-like' state

Figure 6 demonstrates the effect of different neurotransmitter systems in centrally admisnistered ANP_{1-28} induced 'anxiolytic-like' state. Intraperitoneal pretreatment with dopaminergic blocker (10 μg/kg haloperidol), an α and β adrenoreceptor blockers (2 mg/kg phenoxybenzamine and 10 mg/kg propranolol)

were found to significantly antagonize the 'anxiolytic-like' effect of ANP_{1-28}. In the contrast, muscarinergic cholinergic blocker (2 mg/kg atropine), a GABA receptor antagonist (1 mg/kg bicuculline), a 5-HT receptor antagonist (5 mg/kg methysergide) and an opiate antagonists (0.1 mg/kg naloxone) were not able to modulate the 'anxiolytic like' activity of the peptide.

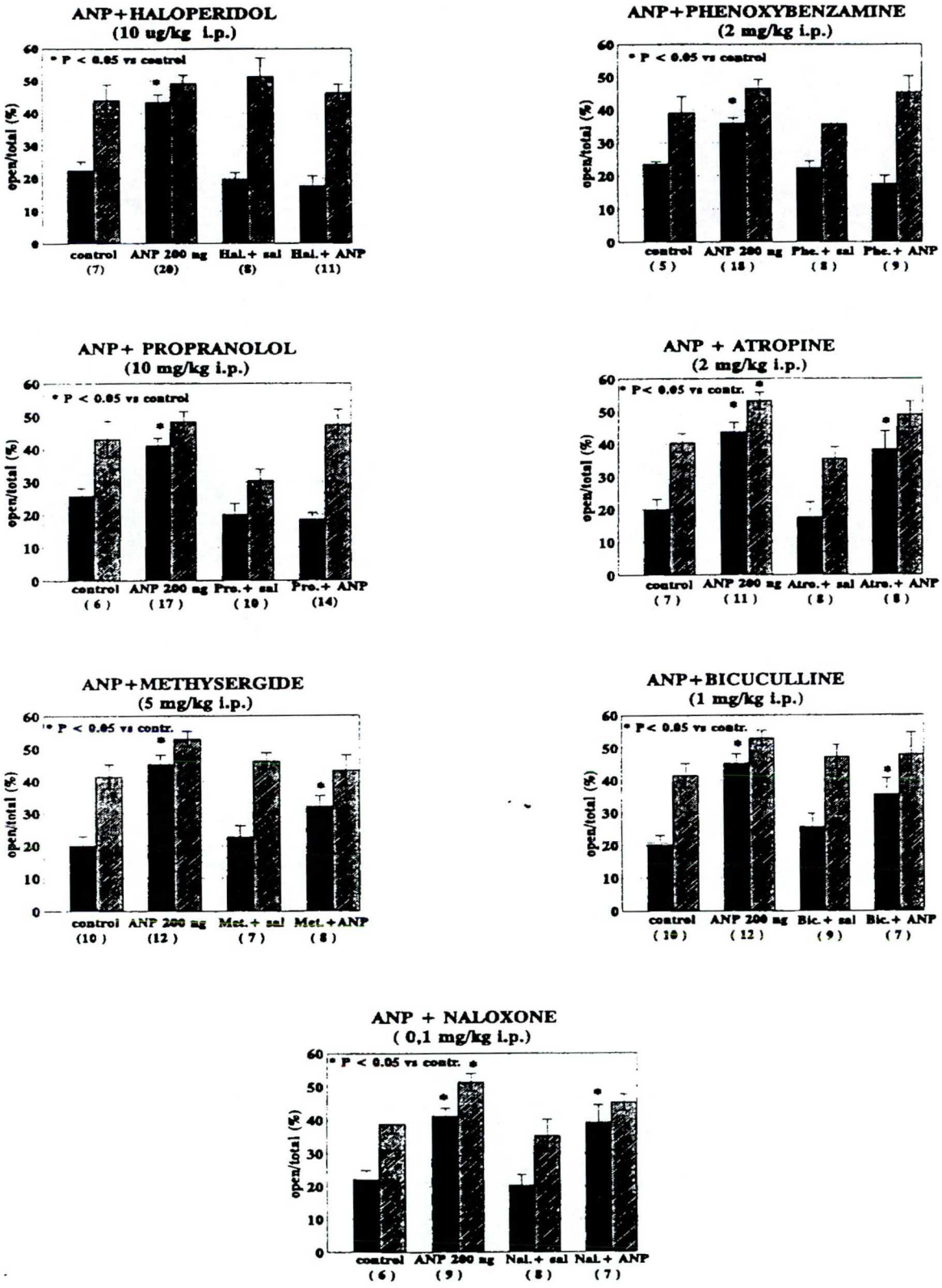

Figure 6. Results are presented as means ± S EM percentage of the time spent in the open arms of the maze. * $p < 0.05$ (ANOVA, Tukey's test) vs control group. Numbers in brackets represent the number of animals used.

3.5. Effects of Atrial Natriuretic Peptide on Corticotropin Releasing Factor in the brain

A set of experiments were done in order to investigate the possible alterations in the CRF-LI in different regions of the brain in rats following central administration of different doses of ANP_{1-28}. Table 2 indicates the effects of 100, 200 and 400 ng of ANP_{1-28} at immunoreactive CRF content of hypothalamus, basal forebrain, hippocampus, frontal cortex and amygdala. CRF-LI determined by RIA method was significantly increased in the hypothalamus, the hippocampus and the frontal cortex. In the amygdala there was a marked but non-significant CRF-LI enhancement. On the other hand CRF-LI decreased in the basal forebrain after administration of 200 ng of ANP_{1-28}.

Brain region	Control	100 ng ANP	200 ng ANP	400 ng ANP
Hypothalamus	1052 ± 348	2611 ± 580 *	3868 ± 671 *	2321 ± 178 *
Basal forebrain	208 ± 37	230 ± 76	109 ± 11 *	354 ±53 *
Hippocampus	18 ± 7	30 ± 14	83 ± 14 *	82 ± 28 *
Frontal cortex	21 ± 5	36 ± 3	86 ± 11 *	62 ± 8 *
Amygdala	732 ± 273	1100 ± 310	972 ± 151	1069 ± 241

Table 2. Each group contains 5-7 animals. * $p < 0.05$ vs. control group by ANOVA, followed by Dunnett's test.

3.6. BNP induced behavioural effects in elevated plus maze paradigm

Different doses of i.c.v. administered BNP were tested and table 3 shows the effect of 50, 100, 200, 400 and 1000 ng BNP on exploratory behaviour of rats. 100, 200 and 400 ng doses of BNP were found to significantly increase the time spent in the open arms while such statistically significant increase in the frequency of entries into the open arms was not observed. The doses of 50 and 1000 ng were found

ineffective. 200 ng of BNP was selected for further experiments where the possible involvement of different neurotransmitters was investigated.

Groups	No. of animals	Open/ total time %	Open/ total entries %
Control (saline)	7	27,56±2,56	44,05±4,8
50 ng BNP	10	21,81±6,17	41,02±4,66
100 ng BNP	10	50,24±5,18 [a]	49,62±3,04
200 ng BNP	11	60,56±8,49 [a]	46,71±6,02
400 ng BNP	10	66,27±6,06 [a]	54,31±4,48
1000 ng BNP	10	36,71±9,56	43,73±7,35

Table 3. Results are presented as means ± SEM. [a] $p < 0.05$ (ANOVA, Tukey's test) vs control group.

3.7. Role of different neurotransmitter systems in the Brain Natriuretic Peptide induced 'anxiolytic-like' state

Table 4, which summarizes the behavioural changes induced by blockade of different neurotransmitter systems, indicates that β-adrenergic and muscarinegic cholinergic systems are involved in 'anxiolytic-like' action of BNP. Namely 10 mg/kg propranolol and 2 mg/kg atropine preteatment were able to block the increase of percentage time spent in open arms as well as the percentage number of entries into the open arms of the elevated plus-maze apparatus. Pretreatment with other receptor blockers was found to be without any statistically significant effect, namely preteatment with 10 μg/kg haloperidol (dopaminergic blocker), 2 mg/kg phenoxybenzamine (α-adrenergic blocker), 5 mg/kg methysergide (5-HT receptor blocker), 1 mg/kg bicuculline (GABA-ergic blocker) and 0.1 mg/kg naloxone (endogenous opioid blocker) were not able to modify the increase in percentage time spent in the open arms as well as had no effect on the percentage number of entries into the open arms.

Groups	No. of animals	Open/ total time %	Open/ total entries %
Haloperidol (10μg/ kg i.p.)			
Control (saline)	8	24,64±1,03	33,07±3,07
Saline+BNP (200ng i.c.v.)	10	63,67±9,23 [a]	47,33±6,88
Haloperidol+saline	10	32,88±8,76	39,5±6,70
Haloperidol+BNP	9	64,24±8,91 [a]	47,96±5,68
Phenoxybenzamine (2mg/ kg i.p.)			
Control (saline)	7	27,56±2,56	44,05±4,8
Saline+BNP (200ng i.c.v.)	9	43,63±4,54 [a]	42,7±3,84
Phenoxybenzamine+saline	8	27,51±8,27	39,29±5,55
Phenoxybenzamine+BNP	10	54,94±8,09 [a]	52,92±5,99
Propranolol (10mg/ kg i.p.)			
Control (saline)	10	20,76±3,31	31,92±2,79
Saline+BNP (200ng i.c.v.)	10	42,96±7,74 [a]	49,95±6,06 [a]
Propranolol+saline	8	26,07±5,54	34,88±2,62
Propranolol+BNP	10	25,02±3,60	39,5±3,08
Atropine (2mg/ kg i.p.)			
Control (saline)	7	20,07±3,11	40,41±2,85
Saline+BNP (200ng i.c.v.)	8	51,58±6,65 [a]	49,71±6,00 [a]
Atropine+saline	8	17,85±4,45	36,00±3,66
Atropine+BNP	10	20,76±3,31	31,92±2,79
Methysergide (5mg/ kg i.p.)			
Control (saline)	8	24,67±4,03	34,32±3,67
Saline+BNP (200ng i.c.v.)	9	63,04±8,92 [a]	44,26±6,88
Methysergide+saline	6	24,14±6,83	40,08±5,22
Methysergide+BNP	7	59,96±10,00[a]	48,16±8,75
Bicuculline (1mg/ kg i.p.)			
Control (saline)	10	22,2±2,98	43,45±3,94
Saline+BNP (200ng i.c.v.)	9	48,05±3,94 [a]	44,55±3,55
Bicuculline+saline	10	28,22±8,95	36,87±5,69
Bicuculline+BNP	8	47,19±5,51[a]	35,3±4,38 [a]
Naloxone (0,1mg/ kg i.p.)			
Control (saline)	8	24,86±5,91	30,57±3,27
Saline+BNP (200ng i.c.v.)	8	51,88±6,65 [a]	49,71±6,00[a]
Naloxone+saline	9	21,29±3,65	36,16±6,31
Naloxone+BNP	9	46,32±7,13 [a]	42,77±6,8

Table 4. Results are presented as means ± SEM. [a] $p < 0.05$ (ANOVA, Tukey's test) vs control group.

3.8. CNP induced behavioural effects in elevated plus maze paradigm

Table 5 lists the behavioural effects of i.c.v. injected C-type natriuretic peptide in the same standard test of anxiety. It has found that 100, 200 and 400 ng doses of CNP significantly increased the percentage time spent in the open arms. Doses of 25, 50 and 1000 ng were found ineffective. A statistically significant increase of the percentage number of entries into the open arms was not observed at any dose.

Groups	No of animals	Open/total time %	Open/total entries %
Control (saline)	7	29.53 ± 2.48	48.21 ± 5.74
25 ng CNP	7	26.65 ± 2.68	35. 48 ± 3.86
50 ng CNP	9	38.98 ± 3.90	41.88 ± 5.65
100 ng CNP	9	54.51 ± 7.46 [a]	46.16 ± 4.68
200 ng CNP	9	66.69 ± 3.48 [a]	54.31 ± 4.48
400 ng CNP	9	58.30 ± 6.35 [a]	54.29 ± 5.53
1000 ng CNP	9	31.45 ± 3.71	41.81 ± 3.50

Table 5. Results are presented as means ± SEM. [a] $p < 0.05$ (ANOVA, Tukey's test) vs control group.

3.9. Role of different neurotransmitter systems in the CNP induced 'anxiolytic-like' state

Table 6 summarizes the effect of different neurotransmitter blockers of CNP induced 'anxiolytic-like' behaviour scored in the elevated plus-maze paradigm. An involvement of dopaminergic, α and β-adrenergic systems was demonstrated, namely increase of the percentage time spent in the open arms of the maze was blocked by pretreatment with 10 μg/kg haloperidol, and 2 mg/kg phenoxybenzamine as well as by 10 mg/kg propranolol administration. Percentage number of entries into the open arms of the maze were not affected by pretreatment with any receptor blockers. The involvement of other neurotransmitter systems have not been confirmed in these experiments, namely 2 mg/kg atropine (muscarinergic cholinergic blocker), 5 mg/kg methysergide (5-HT blocker), 1 mg/kg bicuculline (GABA-ergic blocker), 0.1 mg/kg naloxone (endogenous opioid receptor blocker) have no effect neither on increase of the percentage time spent in the open arms nor on the percentage number of entries into the open arms of the maze.

Groups	Number of animals	Open/total time %	Open/total entries %
Haloperidol (10μg/kg i.p.)			
Control (saline)	6	23.20±2.89	35.56±3.98
Saline+CNP (200 ng i.c.v.)	7	50.14±4.13[a]	56.19±6.24
Haloperidol+saline	6	28.30±4.13	41.67±4.77
Haloperidol+CNP	7	35.64±6.45	50.00±5.59
Phenoxybenzamine (2mg/kg i.p.)			
Control (saline)	6	24.94±1.97	47.92±4.20
Saline+CNP (200 ng i.c.v.)	8	44.85±5.97[a]	41.49±3.43
Phenoxybenzamine+saline	7	29.87±3.04	55.17±6.49
Phenoxybenzamine+CNP	7	29.52±3.12[a]	49.52±4.01
Propranolol (10 mg/kg i.p.)			
Control (saline)	6	24.81±1.78	40.20±4.75
CNP (200 ng i.c.v.)	8	44.41±3.06[a]	47.44±3.72[a]
Propanolol+ saline	7	25.44±3.99	35.66±7.32
Propanolol+CNP	9	25.38±1.90	47.59±5.25
Atropine (2 mg/ kg i.p.)			
Control (saline)	7	22.32±2.43	41.77±4.47
Saline+CNP (200 ng i.c.v.)	7	52.72±6.32[a]	47.72±5.04[a]
Atropine+saline	8	19.50±2.73	40.73±5.30
Atropine+CNP	8	52.16±2.16[a]	37.17±2.18
Methyergide (5mg/kg i.p.)			
Control saline	7	20.92±2.14	41.43±3.84
Saline+CNP (200 ng i.c.v.)	8	50.96±2.73[a]	43.58±3.81
Methysergide+saline	7	19.03±0.78	43.55±4.68
Methysergide+CNP	7	50.75±3.38[a]	48.62±4.76
Bicuculline (5mg/kg i.p.)			
Control (saline)	7	20.29±3.85	38.50±4.58
Saline+CNP (200 ng i.c.v.)	8	44.69±5.13[a]	44.11±2.58
Bicuculline+saline	8	22.00±3.15	45.89±4.46
Bicuculline+CNP	7	50.35±2.16[a]	43.81±5.22
Naloxone (0.1 mg/ kg i.p.)			
Control (saline)	6	21.13±2.87	40.24±2.82
Saline+CNP (200 ng i.c.v.)	8	47.89±2.74[a]	45.77±2.63[a]
Naloxone+saline	7	23.67±3.16	41.30±4.39
Naloxone+CNP	7	45.87±3.81[a]	43.79±4.56

Table 6. Results are presented as means ±SEM. [a] $p < 0.05$ (ANOVA, Tukey's test) vs control group.

3.10. Effects of Brain and C-type Natriuretic Peptides on Corticotropin-Releasing Factor in brain

Alterations in the CRF-LI in different regions of the brain after central injection of different doses of BNP are shown below in figure 7. After i.c.v. administration of a high dose (400 ng) of BNP, the CRF-LI increased significantly in the hypothalamus ($F=3.07$, $p<0.05$) and amydala ($F=11.55$, $p<0.001$), while a tendency towards an increase was found in the hippocampus ($F=2.23$, $p=0.12$).

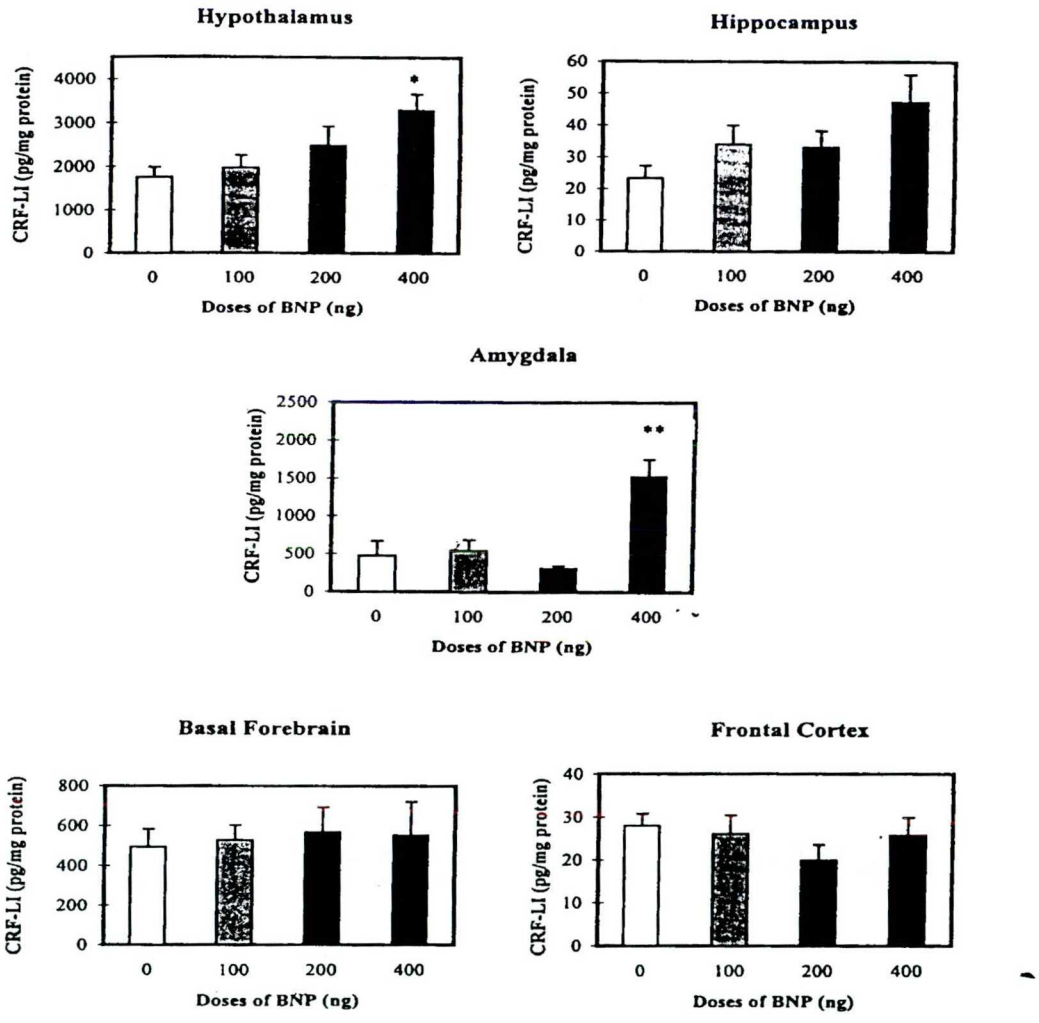

Figure 7 . Effects of i.c.v. administered BNP on CRF-LI (pg/mg protein ± S.E.M.) in various brain areas. Each group contains 5-8 animals.

*$p<0.05$ vs. control group, **$p<0.001$ vs. control group

Alterations of central CRFergic system after central administration of CNP is shown in figure 8. In the hypothalamus the CRF-LI decreased after a high dose (400 ng) of CNP ($F=3.9$, $p<0.05$). The CRF-LI increased in the basal forebrain after a low dose (100 ng) of CNP ($F=3.13$, $p<0.05$). At all other doses the CRF-LI contents of the investigated brain regions did not exhibit any marked alteration.

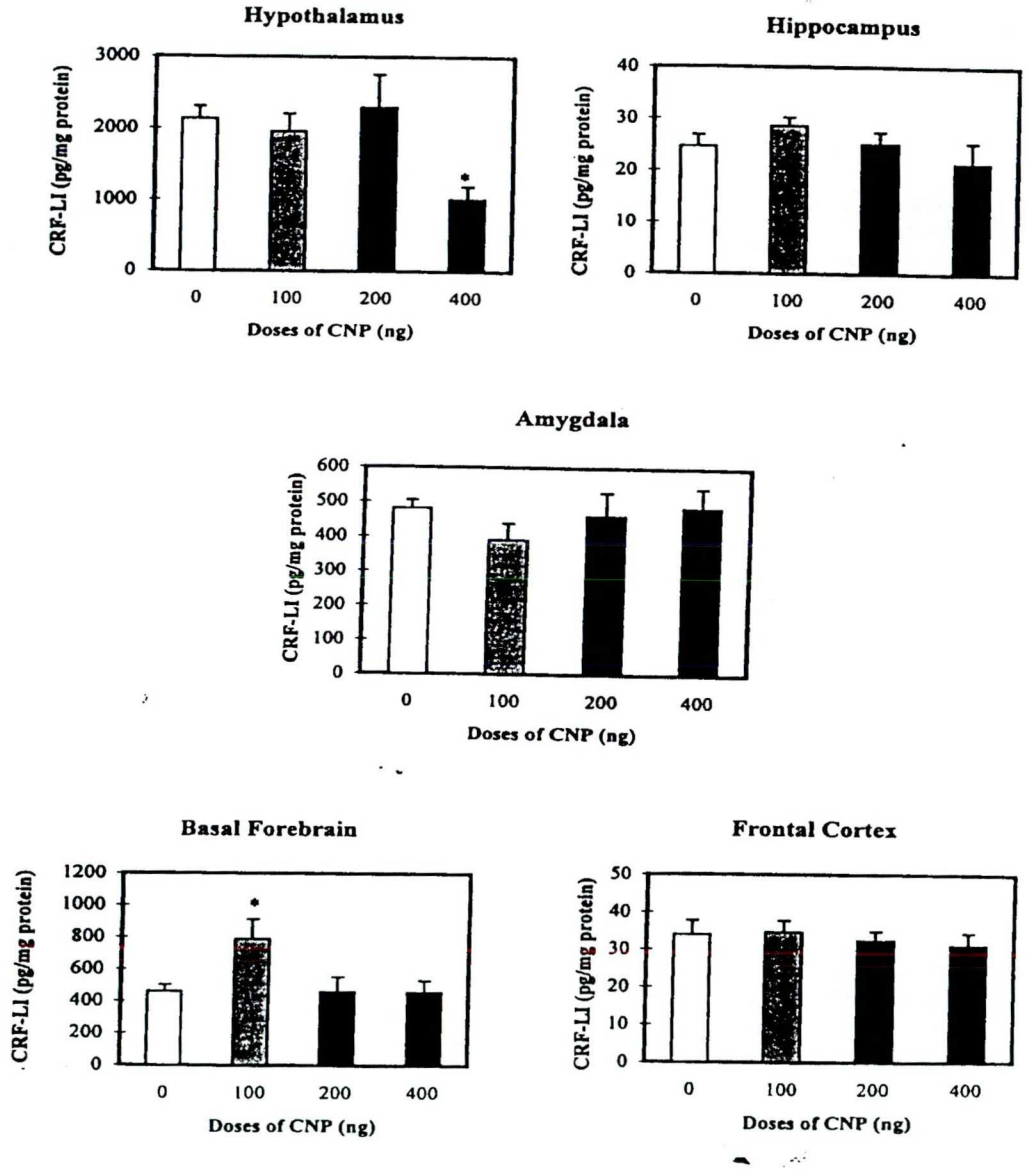

Figure 8. Effects of i.c.v. administered CNP on CRF-LI (pg/mg protein ± S.E.M.) in various brain areas. Each group contains 5-8 animals.

*$p<0.05$ vs. control group, **$p<0.001$ vs. control group

4. Discussion

4.1. New findings

- In addition to previous findings of others we described an evidence that behavioural (anxiogenic) and neuroendocrine (enhanced corticosterone secretion) responses to CCK 8 might be mediated at least in part by CRF system in the CNS. This in vivo evidence of the selective role of endogenous CRF mediation was concluded on our findings that pretreatment with different dilutions of (1:10 and 1:20 but not 1:100) CRF antiserum and i.c.v. administered different doses of a CRF receptor antagonist, alpha-helical CRF (in doses of 1,0 and 0,1 and 0,01 but not 0,001 μg) prevented the anxiogenic response to CCK 8 in a dose dependent manner in an elevated plus-maze test and inhibited the plasma corticosterone elevation. In our experimental setting we demonstrated that anxiety produced by centrally administered CCK 8 was antagonized by a blockade of the endogenous CRF system.

- We demonstrated that dopaminergic, cholinergic and opiate receptors are involved in the anxiogenic action of CCK, as blockers of the previously mentioned receptors can prevent the anxiogenic behavioural action of centrally administered CCK 8, indicating involvement of multiple neurotransmitter systems.

- 100, 150 and 200 ng doses of ANP_{1-28} abolished the normal preference of rats for the closed arms and increased the percentage of time spent in the open arms of the maze, which is consistent with 'anxiolytic-like' effect. Our studies demonstrated that this effect can be inhibited by dopaminergic, α- and β-adrenoreceptor antagonists, which suggest a multiple neurotransmitter system activation in mediation of peptide's 'anxiolytic-like' activity.

- After various doses of central administration of ANP_{1-28} the CRF-LI significantly increased in the hypothalamus, the hippocampus and the frontal cortex. In the amygdala ANP_{1-28} caused a marked but nonsignificant CRF-LI enhancement. In the basal forebrain CRF-LI decreased. This alteration of the brain CRF-ergic system activity support a hypothesis on involment of this system in neuroendocrine and behavioural actions of ANP_{1-28}.

- BNP and CNP in doses of 100, 200 ng and 400 ng increased the percentage of time spent in the open arms of the elevated plus-maze paradigm. Pretreatment with an β-adrenoreceptor antagonist and a muscarinegic cholinergic blocker antagonized the effect of 200 ng BNP in the elevated plus-maze test. The 'anxiolytic-like' effect of CNP was prevented by a dopamine receptor antagonist and an α- or β-adrenoreceptor blocker but not by a muscarinergic cholinergic blocker, a GABA-receptor blocker, a 5-HT receptor antagonist or an opiate receptor antagonist. Our results appear to indicate the involvement of different neurotransmitter systems in the mediation of BNP and CNP induced behavioural action.

- Centrally administered BNP in high dose (400 ng) significantly increased the CRF-LI in the hypothalamus and amygdala, while only a tendency towards an increase was found in the hippocampus. Centrally administered high dose (400 ng) of CNP caused a decrease in the CRF-LI in the hypothalamus while CRF-LI increased in the basal forebrain after a low dose (100 ng) of C type natriuretic peptide. Our data suggest that CRF neurotransmission may be involved in the mediation of some neuroendocrine and behavioural responses to other members of natriuretic peptide family.

4.2. Mediation of anxiogenic action of CCK

4.2.1. CCK and HPA axis

Results with special agonists to CCK receptor subtypes provide good evidence of the involvement of endogenous CCK in the control of anxiety. Activation of the CCK-B receptors induces anxiety (Harro et al., 1991; Rataud et al., 1991; Singh et al., 1991; Woodruff et al., 1991), while selective CCK receptor antagonists produce anxiolytic like effects (Dauge et al., 1989; Horwell et al., 1991). Based on this it was postulated that CCK might have anxiogenic properties and play a role in pathological anxiety. Stressful situations have been reported to increase CCK levels in some brain areas but in contrary to this release of CCK as measured by the behavioural effect of a CCK-receptor antagonist can be triggered by environmental safety cues (Wiertalk et al., 1992). Exposure of rats to the odour of a cat significantly increased the levels of CCK 4 in various brain structures and this effect was blocked by pretreatment with CCK-B antagonist L-365, 260 (Pavlasevic et al., 1993). Administration of the CCK-B agonist cholecystokinin tetrapeptide (CCK 4) induced panic attacks in man, being active in healthy volunteers (De Montigny et al., 1989) but especially potent in panic disorder patients in a dose dependent manner (Bradwejn et al., 1990) - implying the role of CCK-B receptor system in this illness. Decreased concentrations of CCK 8 in CSF have been observed in panic disorder patients compared with controls - suggesting anomalies or physiological alterations of the CCK system in this disorder. The behavioural and cardiovascular symptoms of panic disorder were not blocked by flumazenil, a benzodiazepine receptor antagonist, indicating that the panicogenic actions of CCK 4 are not mediated by benzodiazepine receptors. The 5-HT system might be the mediator of the above mentioned effect, since chronic treatment of patients with tricyclic antidepressants antagonizes the panicogenic effect of CCK 4.

Our findings were in accordance with previous works of several authors and have provided indirect in vivo evidence of the selective role of the endogenous CRF system in mediating the effects of CCK 8. Since central effects of CCK 8 and CRF are so alike, it might be postulated that these two systems might be in close connection. The CCK 8-induced stimulation of the adrenal gland as described previously by

Fekete and co-workers in 1981 and Porter and his collegues in the same year was significantly and dose dependently prevented by pretreatment with CRF antagonists. Our results may be interpreted in the light of the findings of others who described that CRF antiserum administered i.c.v. attenuates the elevation of plasma ACTH (Ono et al., 1985) and that ahCRF in vivo and in vitro inhibited the release of ACTH (Rivier et al., 1986). It was postulated that conditioned emotional stress stimulates colonic motility in rats through a mechanism involving the central release of CRF. Gue and co-workers have shown that i.c.v. injection of CCK 8 blocks the emotional stress and CRF-induced colonic motor alterations but this effect could be induced by many other substances such as neuropeptide Y and by 5-$HT1_A$ agonist which act through a mechanism involving the central release of CCK and/or modulation of CCK pathways, since their anti-stress effect is reversed by central CCK antagonists. Our data were in harmony with an observation that centrally administered CRF caused a marked reduction in CCK-like immunoreactivity in the medial frontal cortex, anterior cingulate cortex and nucleus accumbens (Takamasu et al., 1991).

Effects of CRF seen in increased emotional states as measured via different tests of anxiety are in accordance with our findings indicated before. CRF injected centrally has an anxiogenic effect in an open filed test, an operant conflict test, a social interaction test, an acoustic startle test, a defensive withdrawal test and an elevated plus maze paradigm (cf. review article of Dunn et al., 1990) and can be blocked by centrally administered ahCRF (Cole et al., 1987). Our experiments demonstrated that anxiety produced by centrally administered CCK 8 can be antagonised by a blockade of the endogenous CRF system.

The doses of CRF antagonists used throughout our study were chosen according to our previous experience (Sarnyai et al., 1992) and are lower then those usually given in conflict tests and other tests of anxiety. The difference in effective doses found might be explained by the different strains of rats used, as well as by different animal models used, although the exact explanation of these effective smaller doses is still unknown.

These results suggest that the anxiogenic and pituitary-adrenal-axis activating effects of CCK 8 are mediated at least in part by CRF. The connection between CCK 8 and the endogenous CRF system possibly might be of relevance as concerns the

possible use of CRF antagonists in a number of human psychopathological states in which endogenous CCK may play a role.

4.2.2. CCK and classic neurotransmitter systems

As a next step in our further work we investigated the possible involvement of different neurotransmitter systems in the previously described anxiogenic action of CCK 8 in rats. Based on the data of several biochemical and histochemical studies it is known that CCK may act as neuromodulator on mono-aminergic, endogenous opioid, cholinergic and GABAergic systems. The possibility that CCK might act on neurotransmitters was shown in earlier experiments done in our institution, which demonstrated that CCK altered the turnover of dopamine, norepinephrine and serotonin in different brain areas (Fekete et al., 1981 and 1982). Peripheral injection of CCK has increased the release of noradrenaline, dopamine and serotonin from hypothalamic nuclei (Kendrik et al., 1991). Crawley and her coworkers in 1988 have shown that CCK potentiates dopamine-mediated behaviours. Later on in 1991 based on the published data the same group has concluded that the neurophysiological and behavioural effects of CCK are considered to be mediated at least through dopaminergic neurotransmission (Crawly et al., 1991). This hypothesis is underlined by the fact that intracerebral perfusion with CCK 8 enhances dopamine release in the neostriatum and nucleus accumbens (Ruggeri et al., 1987), possibly by reducing D-2 receptor affinity. CCK is colocalized with dopamine in mesolymbic nerve terminals via direct presynaptic action on CCK receptors (Vickroy et al., 1989). Moreover, hyperactivity of the brain's dopaminergic system is widely citated etiological hypothesis of schizophrenia and therefore CCK is considered as one of several substances probably implicated in the pathophysiology of schizophrenia. Expression of CCK mRNA in the midbrain dopamine cells of schizophrenic patients is increased compared to that of normal controls (Schalling et al., 1990), therefore CCK antagonists could have antipsychotic actions.

In addition to dopaminergic involvement in effects of CCK evidence has been accumulated on the close interaction of CCK and GABA in the forebrain (Harro et al., 1991), since CCK is localized in GABA-synthesising neurons in the cerebral cortex,

hippocampus, and basolateral amygdala. Furthermore, peptide's release is under tonic control by GABA-mediated mechanisms.

Besides the above mentioned close correlations with different neurotransmitter system several findings suggest that CCK receptor regulation may also depend upon noradrenaline. Expression of proCCK mRNA in cell cultures is enhanced by noradrenaline (Monstein et al., 1990). Also, treatment with DSP-4, the neurotoxin that selectively destroys noradrenergic nerve terminals of projections from the locus ceruleus, can cause upregulation of CCK receptor binding in brain regions such as the frontal cortex and hippocampus, which receive noradrenergic input predominantly from the locus coeruleus. This finding is in line with similar changes in cortical CCK receptors after reserpine treatment and with changes related to anxiety, adaptational deficits and suicide.

Moreover, Weller and his collegues in 1988 have shown that CCK 8 antagonizes the effect of opiate antagonists on a variety of behaviours, including isolation-induced distress vocalisation in rat pups.

Investigating the role of different neurotransmitters, including the dopaminergic, noradrenergic, adrenergic, cholinergic, GABAergic, serotoninergic and opiate systems in CCK 8 induced anxiety in rats it is easy to predict that if transmitters are involved then receptor blockers to a given transmitter can prevent the behavioural action the peptide. With reference to receptor blockers used in our experiments, the doses selected were screened for dose-response in a previous series of experiments, in which we had to choose the minimal dose of receptor blocker that did not alter the behavioural paradigm per se (Telegdy 1987). The time of 30 minutes for treatment with CCK 8 or receptor blockers were also based on previous experiments performed in our institution. One might consider that CCK 8 at the dose used might have sedative action. When the same dose was used in open filed activity, no alteration was observed, as compared with the control (Fekete et al., 1984). The same conclusion was reached when action of CCK 8 was studied on active and passive avoidance paradigms, acquisition of active avoidance learning, extinction of bench-jumping active and food-motivated conditioned feeding behaviours and the retention of one-trial passive avoidance behaviour (Fekete et al., 1982 and Kádár et al., 1981). Moreover, dose of CCK 8 in micrograms was selected according to

observations in which the effective dose in behavioural and pituitary-adrenal axis activation was in microgram range (Crawley et al., 1981 and Itoh et al., 1982). Dopaminergic, cholinergic and endogenous opiod systems have been found to be involved in the CCK 8 caused 'anxiogenic-like' behaviour in our experiments. The fact that α and β adrenoreceptors or 5-HT receptors, as well as GABA receptor blockers were found ineffective is not attributed to the possibility that the dose was insufficient, because using the same paradigm we could demonstrate that the same dose of phenoxybenzamine or propranolol could block the action of atrial natriuretic and C type natriuretic peptides, while only phenoxybenzamine could block the anxiolytic action of brain natriuretic peptide (Biró et al., 1995 and 1996). The negative data with methysergide a HT_1 / HT_2 blocker do not exclude the possibility that other subtypes of 5-HT receptor might be involved as it was demonstrated in experiments done by several investigators. It was shown that anti-exploratory effects of ceruletide can be blocked by 5-HT_3 receptor antagonist ondansetron (Vasaar et al., 1993). Moreover, 5-HT can increase CCK release in cortex via 5-HT_3 receptor activation and conversely 5-HT release in guinea pig frontal cortex induced by exposure to the elevated plus maze test (Rex et al., 1993) can be potentiated by CCK B agonist CCK 4.

Although it is not known what is the correct explanation of neurotransmission seemed to be involved in anxiogenic action of CCK 8 in our experiments – it might be hypothesised that different timing and ways of administration of different doses of CCK peptides can affect the classic neurotransmitter systems in such a different way as was described above.

4.3. Mediation of anxiolytic action of natriuretic peptides

At least three subtypes of ANP receptors exists in CNS of mammals and participate not only in the regulation of the homeostasis of fluid, electrolytic balance and blood pressure but are suggested to be involved in the regulation of the reproductive, adrenocortical and neurohypophyseal axes as well.

4.3.1. ANP and classic neurotransmitter systems

Immunoreactive ANP is released from the rat hypothalamus (Yeung et al., 1991) and ANP mRNA transcripts have been identified in the rat hypopthalamus and porcine brain stem (Gardner et al., 1987). It has been shown that nuclei involved in the regulation of anterior pituitary hormone secretion contain cells immunoreactive for ANP, suggesting that ANP may moderate pituitary hormone release. Consistent with this hypothesis ANP has been shown to be released from rat hypothalamic fragments in vitro, to bind to anterior pituitary cells (Quiron et al., 1984) and to increase the cellular content of cyclic guanosine monophosphate (cGMP) not only in pituitary gland but in circumventricular organs as well. Consonant evidence from in vivo immunoneutralization studies suggest that ANP exerts an inhibitory effect on ACTH release and plasma corticosterone concentrations. This conclusion is supported by experiments where was found that secretagogue-evoked ACTH secretion in vitro and CRF-stimulated secretion of ACTH and cortisol in humans are suppressed by atriopeptines (Kellner et al, 1992). Previously described inhibition is in harmony with an experimental finding of Hattori and his coworkers published in 1986 that intravenous injection of ANP was able to inhibit haemorrhage-induced ACTH release.

'Anxiolytic-like' effects of centrally administered ANP_{1-28} in 100, 150 and 200 ng doses demonstrated in our experiments are in agreement with previous studies demonstrating similar increases in general activity of animals scored in case of other anxiolytic compaunds, such as chlordiazepoxide, diazepam and phenobarbitone.

Data on possible neurotransmission of ANP caused behavioural responses have been published previously and are in accordance with our experimental findings, involving cathecolamines and dopamine. In our institution dopaminergic and cholinergic neurotransmission have been detected in the delayed extinction in the active avoidance paradigm and the delayed response in the passive aviodance paradigm (Bidzseranova et al., 1991 and 1992). Besides dopaminergic and cholinergic, an α-adrenergic neurotransmission have been found by the same authors in the antiamnestic action of ANP. Furthermore, the hypothalamic CRF release induced by acethylcholine in rats was blocked by continous administration of ANP (Takao et al., 1988). It has been reported that intravenously administered dopamine

receptor blockers such as haloperidol or chlorpromazine in a dose of 50 μg inhibit natriuretic response to ANP (Martin-Grez et al., 1985). Moreover, it has been found that centrally administered ANP has an inhibitory effect on dopaminergic neurotransmission in the CNS of rats (Nakao et al., 1986).

Other catecholaminergic systems are also involved in the action of atrial natriuretic polypeptide. It has been shown that the basal release of immunoreactive atrial natriuretic peptide (IR-ANP) is a product of the activation of α- and β-adrenoreceptors by their endogenous ligands (Bush et al., 1990).

Although having known all these experimental findings on different neurotransmission of central actions of ANP there is still an open question on their activation and widespread interactions.

4.3.2. ANP and central CRF systems

Our experimental data that the ANP_{1-28} caused 'anxiolytic-like' behavioural state demostrated by increased plus-maze scores favour the idea that the inhibition of the CRF neurotransmission might be involved in mediation of this action. During testing of this hypothesis we have found that CRF-LI increased in the hypothalamus, the hippocampus and the frontal cortex, while decreased in the basal forebrain and a nonsignificant enhancement was found in the amygdala.

Although we are aware of a fact that measurements of the IR-CRF concentration alone does not distinguish between changes in synthesis, release, storage or degradation of the peptide, the increase of the IR-CRF level in certain brain areas might indicate the decreased release. To support this interpretation we might bound together the previously mentioned findings, namely that ANP is colocalized with CRF in the paraventricular nucleus and inhibits the release of the CRF from the hypothalamus as well as suppress the stress-induced HPA-axis activation. It may be hypothesized that the decrease of CRF level in the basal forebrain structures reflects the increased release and subsequent degradation of the CRF. Our results definitely have shown an alteration of endogenous CRF system by central administration of ANP, suggesting involvement of this system in neurotransission of Atrial Natriueretic Peptide induced "anxiolytic-like" behavioural and neuroendocrine actions.

4.3.3. BNP, CNP and classic neurotransmitter systems

While much is known of the CNS actions of ANP the other members of natriuretic peptide family are not so widely investigated. These peptides are of potential importance in neuroendocrine regulation, acting as neurotransmitters or neuromodulators in the brain, regulating hormonal and cardiovascular functions. BNP and CNP share similar cystein-ring structures with ANP (Sudoh et al., 1990 and Kojima et al., 1990), therefore interpretation of previous physiological studies using ANP antibodies has been rendered more difficult by the discovery of these peptides.

Itoh and his collegues have found in 1988 that intravenous administration of synthetic BNP into anaesthetised rats elicits natriuretic, diuretic and hypotensive activities comparable to those induced by an α helical ANP (α-hANP), raising the possibility that due to structural similarity BNP was acting on the ANP receptors.

Although some of the reported actions of CNP are the similar to those of ANP, effects that oppose those are also reported, for example while ANP inhibits prolactin secretion in the hypothalamus as was mentioned before, CNP acts within the same region as stimulating secretion of the same hormone (Huang et al., 1992). Anatomical localization studies indicated that CNP is contained in neuronal circuits having a direct relevance to several neuroendocrine systems. The heaviest concentration of CNP mRNA was observed in the anteroventral periventricular nucleus, in the region known to regulate the hypothalamo-pituitary-gondal axis and vasopressin release from the hypothalamo - neurohypophyseal system. In addition, the localization of CNP to the hypothalamic arcuate nucleus, the medial, median and periventricular preoptic area, the supraoptic, dorsomedial, ventral premamillary and lateral mamillary nuclei, and the posterior hypothalamic area has implications as concerns the hypothalamo-pituitary-gonadal function and the regulation of pituitary-adrenocortical secretion.

As it was described before in our experiments BNP in doses of 100, 200 and 400 ng as well as CNP in doses of 100 and 200 ng increased the percentage time spent in the open arms of the elevated plus-maze paradigm, abolishing normal preference of animals for the closed arms, therefore indicating an 'anxiolytic-like' effect.

Pretreatment with an α-adrenoreceptor antagonist or a muscarinegic cholinergic blocker antagonized the effect of 200 ng BNP scored in the elevated plus-maze test. On the other hand 'anxiolytic-like' effect of CNP was prevented by a dopamine receptor antagonist and an α- or β-adrenoreceptor blocker but not by a muscariengic cholinergic blocker, a GABA-receptor blocker, a 5-HT receptor antagonist or an opiate receptor antagonist. Having said all this our results appear to indicate the involvement of multiple neurotransmitter systems in the mediation of BNP and CNP induced behavioural action in rats, although their activation and interactions still remain to be answered.

4.3.4. BNP, CNP and central CRF systems

Behavioural data on 'anxiolytic-like' action of BNP and CNP discussed above implicated the possible involvement of central CRFergic neurotransmission in effects of brain and C-type natriuretic peptides. In our further experiments, designed to partially answer this hypothesis, we demonstrated that centrally administerd BNP in high dose (400 ng) significantly increased the CRF-LI in the hypothalamus and amygdala, while only a tendency towards an increase was found in the hippocampus. On the other hand centrally administered high dose (400 ng) of CNP decreased the CRF-LI in the hypothalamus, probably due to increased release while CRF-LI increased in the basal forebrain after administration of a low dose (100 ng) of CNP, most probably indicating the decreased release of the peptide. Alteration of CRF-LI, in the areas of the brain which are involved in the control of emotion and anxiety underline results of our behavioural experiments discussed previously. These experimental data support our idea that CRF neurotransmission may be partially involved in the mediation of some neuroendocrine and behavioural responses to natriuretic peptides.

As a final word to these studies our attemption was to provide experimental findings which might contribute to nowdays experienced rapid expansion of novel macromolecular target sites for CNS drug innovation, which in turn may lead to exciting innovative principles for treating affective disorders in the future.

5. References

Axelrod J., Reisine T.: Stress hormones their interaction and regulation. Science 224;452, 1984

Baldwin H.A., Raasanick S., Rivier J., Koob G.F., Britton K.T.: CRF antagonist reverses the 'anxiogenic-response' to ethanol withdrawal in the rat. Psychopharmacology (Berl) 103:227-232, 1991

Barden N., Menard Y., Rouleau D., Moore S., Dockray G.J., Dupont A.: Regional distribution of somatostatin and cholecystokinin-like immunoreactivity in rat and bovine brain. Peptides 2:299-302, 1981

Bidzseranova A., Varga J., Telegdy G.: The effect of receptor blockers on brain natriuretic-32-induced action on passive avoidance bahaviour in rats. Neuropeptides 22: 107-110, 1992

Bidzseranova A., Telegdy G., Tóth G.: The effects of receptor blockers on atrial natriuretic peptide induced action on passive avoidance behaviour in rats. Pharmacol Biochem Behav 40: 237-239, 1991

Bradwejn J., Koszycky D., Meteissian G.: Cholecystokinin-tetrapeptide induces panic attack in patients with panic disorder. Can J Psychiatry 35:83-85, 1990

Britton D.R., Britton K.T.: A sensitive open filed measure of anxiolytic drug activity. Physiol Behav 23:648-652, 1979

Bush A.J., Huang W., Copolov D.L., Lim A.T.W.: Hypothalamic atrial natriuretic peptide secretion plasticity: differential modulation of alpha and beta adrenoreceptors. Neuroendocrinology 52:65-69, 1990

Cannon W: Changes in pain, hunger, fear and rage. Appleton, New York, p.29, 1929

Chapell P.B., Smith M.A., Kilts C.D., Bissete G., Ritchie G., Anderson C., Nemeroff C.D.: Alterations in corticotropin releasing factor-like immunoreactivity in discrete brain regions after acute and chronic stress. J Neurosci 6:2908-2914, 1986

Crawley J.N., Hays S.E., O'Danouche T.L., Paul S.M., Goodwin F.K.: Neuropeptide modulation of social and exploratory behaviours in laboratory rodents. Pepides 2:123-129, 1981

Crawley J.N., Stivers J.A., Blumenstein K.L., Paul S.M.: Cholecystokinin potentiates dopamine-mediated behaviours: Evidence for modulation specific to a site of coexistence. J Neurosci 5:1972-1988, 1985

Crawley J.N.: Neuronal cholecystokinin. ISI Atlas Pharmacol 96:312, 1989

Crawley J.N.: Cholecystokinin-dopamine interaction. Trends Pharamcol Sci 12:232-236, 1991

Cole B.J., Britton K.T., Koob G.F.: Central administration of α-helical corticotropin-releasing factor attenuates the aquizition of a conditioned emotional response. Soc Neurosci Abstr 13:429, 1987

Csonka E., Fekete M., Nagy G. et al : Anxiogenic effect of cholecystokinin in rats. InPeptides. New York:Walter de Gruyter and Company 249-251, 1988

Dauge V., Steimes P., Derrien M., Beau N., Roques B.P., Feger J: CCK-8 effects on motivational and emotional states of rats involve CCK-A receptors of the posteromedian part of the nucleus accumbens. Pharmacol Biochem Behav 34:157-163, 1989

De Monitigny C.: Cholecystokinin tetrapeptide induced panic-like attacks in healthy volunteers. Arch Gen Psychiatry 46:511-517, 1989

Dunn A.J., Berridge C.W.: Physiological and behavioural response to corticotropin-releasing factor administration: Is CRF a mediator of anxiety or stress response? Brain Res (Brain Res Rev) 15:71-100, 1990

Fekete M., Szabó A., Balázs M., Penke B., Telegdy G.: Effects of intraventricular administration of cholecystokinin octapeptide sulphate ester and unsulfated cholecystoknin octapeptide on active and conditioned feeding behaviour of rats. Acta Physiol Hung 58:39-45, 1981

Fekete M., Bokor M., Penke B., Kovács K., Telegdy G.: Effects of cholecystokinin octapeptide and its fragments on brain monoamines and plasma corticosterone. Neurochem Int 3:165-169, 1981

Fekete M., Lengyel A., Hegedűs B., Penke B., Zarándy M., Tóth G., Telegdy G.: Further analysis of the effects of cholecystokinin octapeptide on avoidance behaviour in rats. Eur J Pharmacol 98:79-91, 1984

Franci C.R., Anselmo-Franci J., McCan S.M.: The role of endogenous atrial natriuretic peptide in resting and stress induced release of corticotropin, prolactin, growth hormone and thyroid stimulating hormone. Proc Natl Acad Sci USA, 89:11391-11395, 1992

Fink F., Dow R.C., Casley D.: Atrial natriuretic peptide is a physiological inhibitor of ACTH release: evidence from immunoneutralization in vivo. J Endocrinol 131: R9-12, 1991

Glowinski J., Iversen L.: Regional studies of catecholamines in the rat brain. The disposition of 3H dopa in variuos regions of the brain. J of Neurochem 13:655-669, 1966

Gue M., Tekamp A., Tabis N., Junien J.L. Bueno L.: Cholecystokinin blockade of emotional-stress and CRF-induced colonic motor alterations in rats - role of amygdala. Brain Res 658:232-238, 1994

Harro J., Vasar E., Bradwejn J.: CCK in animal and human research on anxiety. Tips Rev, Vol 14, p244-249, 1993

Harro J., Vasar E.: Evidence that CCK_B receptors mediate the regulation of exploratory behaviour in the rat. Eur J Pharmacol 193:379-381, 1991

Hattori T., Hashimoto K., Inogue H.: Effect of synthetic natriuretic peptide on haemorrhage-induced adrenocorticotropin secretion of rats. Endocrinol Japn 33(5):533-539, 1986

Hermann J.P., Langub M.C., Watson R.E.: Localization of C-type natriuretic peptide mRNA in the rat hypothalamus. Endocrinol 133(4):1903-1906, 1993

Horwell D.C.: Development of CCK_B antagonists. Neuropeptides 19:57-62, 1991

Huang F.L.S., Skala K.D., Samson W.K.: C-type natriuretic peptide mediates the hypothalamic actions of the natriuretic peptides to inhibit lutheinizing hormone secretion. Endocrinol 132:504-509, 1993

Ibanez-Santos J., Tsagarakis S., Rees L.H., Besser G.M., Grossman A.: Atrial natriuretic peptides inhibit the release of corticortopin-releasing-factor-41 from the rat hypothalamus in vitro. J Endocrinol 126:223-228, 1990

Itoh S., Ryoji H., Goro K., Kunihiro O.: Adrenocortical stimulation by a cholecystokinin preparation in the rat. Life Sci 25:1725-1730, 1979

Itoh S., Hiruta R., Katsuura G.: Effects of cholecystokinin octapeptide and vasoactive intestinal polypeptide on adrenocortical secretion in the rats. Jpn J Physiol 32:553-560, 1982

Itoh H., Nakao K., Yamade T., Shirakami G., Kangawa K., Minamino N., Matsuo H., Imura H.: Antidipsiogenic action of novel peptide 'brain natriuretic peptide' in rats. Eur J Pharmacol 150:193-196, 1988

Kádár T., Fekete M., Telegdy G.: Modulation of passive avoidance behaviour of rats by intracerebroventricular administration of cholecystokinin octapeptide sulphate ester and nonsulphated cholecystokinin octapeptide. Acta Phys Hung 58:269-274, 1981

Keller-Wood M., Dallman M.: Corticosteroid inhibition of ACTH secretion. Endocrinol Rev 5:1, 1984

Kellner M., Wiedmann K., Holsboer F.: Atrial natriuretic factor inhibits the CRH-stimulated secretion of ACTH and cortisol in man. Life Sci 50:1835-1842, 1992

Kendrick K., Leng G., Higichi T.: Noradrenalin, dopamine and serotonin release in the paraventricular and supraoptic nuclei of the rat in response to intravenous cholecystokinin injections. J Neuroendocrinol 3:139-144, 1991

Kojima M., Minamino N., Kangawa K., Matsuo H.: Cloning and sequence analysis of cDNA encoding a precursor for rat brain natriuretic peptide. Biochem Biophys Res Comm 159:1420-1426, 1989

Kojima M., Minamino N., Kangawa K., Matsuo H.: Cloning and sequence analysis of cDNA encoding a precursor for rat C-type natriuretic peptide (CNP). FEBS Lett 276:209-213, 1990

Makino S., Hashimoto K., Hirasawa R., Hattori T., Kageyama J., Ota Z.: Central interaction between endothelin and brain natriuretic peptide on pressor and hormonal responses. Brain Res 534:117-121, 1990

Martin-Grez M., Brigges J.P., Schubert G., Schnerrmann J.: Dopamine receptor antagonist inhibit the natriuretic response to atrial natriuretic factor. Life Sci 36:2171-2176, 1985

Monstein H.J., Folkesson R, Geijer T.: Procholecystokinin and proencephalin mRNA expression is modulated by cyclic AMP and noradrenaline. J Mol Endocrinol 4, 37-41, 1990

Nakao K., Katsumura G., Mari N.: Inhibitory effect of centrally administered atrial natriuretic polypeptide on the brain dopaminegic system in rats. Eur J Pharmacol 131:171-177, 1986

Nemeroff C.B.: The role of corticotropin-releasing factor in the pathogenesis of major depression. Pharmacopsychiatry 21:76-82, 1988

Nemeroff C.B., Owens M.J., Bisette G., Andorn A.C., Stanley M.: Reduced corticotropin releasing factor binding sites in the frontal cortex of suicide victims. Arch Gen Psych 45:577-579, 1988

Pavlasevic S., Bednar I, Quereshi G.A., Sodersten P.A.: Brain cholecytokinin octapeptide levels are incerased in a rat model of anxiety. Neuroreport 5:225-228, 1993

Pellegrino L.J., Pellegrino A.S., Cushman A.J.: Stereotactic atlas of the rat brain. New York. Plenum Press 8-57, 1979

Pellow S., Chopin P., File S., Briley M.: Validation of open-closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods 14:149-167, 1985

Poter L.M., Sander L.D.: The effects of cholecystokinin octapeptide on pituitary-adrenal hormone secretion. Regul Pept 2:245-252, 1981

Plotsky P.M., Cunningham E.T., Wiedmayer E.P.: Catecholaminergic modulation of corticotropin-releasing factor and adrenocorticotropin secretion. Endocr Rev 40:437-458, 1989

Quiron R., Dalphe M., De Lean A., Gutkowska S., Cantin M., Genert J.: Atrial natriuretic factor (ANF) binding sites in brain and related structures. Peptides 5:1167-1170, 1984

Rataud J., Darche F., Piot O., Stutzman J.C. Böhme G.A., Blanchard J.C.: Anxiolytic effect of CCK antagonists on plus-maze behaviour in mice. Brain Res 548:315-317, 1991

Ravard S., Dourish C.T.: Cholecystokinin and anxiety. Trends Pharmacol Sci 11:271, 1990

Rehfeld J.F., Nielsen F.C.: Molecular forms and regional distribution of cholecystokinin in the central nervous system. In Austin RG Landres Company 33-56, 1995

Rex A., Fink H., Marsden C.A.: The effect of CCK-4 and L 365,260 on cortical extracellular 5-HT release on exposure on the elevated plus-maze, in anxiety. Neurobiology, Clinic and Therapeutic Perspectives, Eds E Hamon H.Oilat and MH.Thelbot 232,207, 1993

Rivier C., Rivier J., Vale W.: Inhibition of adrenocorticotropic hormone secretion in the rat by immunoneutralization of corticotropin-releasing factor. Science 218:377-379, 1982

Rivier J., Rivier C., Vale W.: Synthetic competitive antagonist of corticotropin releasing factor. Effects of ACTH secretion in the rat. Science 224:889-81, 1984

Rivier C., Rivier J., Vale W.: Stress-induced inhibition of reproductive functions: Roles of endogenous corticotropin-releasing factor. Science 231:607-609, 1986

Ruggeri M., Ungerstedt A., Agnati L.F., Mutt V., Harfstrand A., Fuxe K.: Effects of cholecystokinin peptides and neurotensin on dopamine release and metabolism in the rat rostral and caudal part of the nucleus accumbens using intracerebral dialysis in the anesthetized rat. Neurochem Int 10:509-520, 1987

Ono N., Samson W.K., McDonald J.K., Lumpkin M.D., Bedran de Castro J.C., McCan S.M.: Effects of intravenous and intraventricular injection of antisera directed against corticotropin-releasing factor on the secretion of anterior pituitary hormones. Proc Natl Acad Sci USA 82:7787-7790, 1985

Owens M.J., Nemeroff C.B.: Physiology and pharmacology of corticotropin-releasing factor. Pharmacol Rev, Vol 43, No 4, p425-473, 1991

Sarnyai Z., Biró É., Penke B., Rivier J., Telegdy G.: The cocaine-induced elevation of plasma corticosterone is mediated by endogenous corticotropin-releasing factor (CRF) in rats. Brain Res 589:154-156, 1992

Schalling M.K., Friberg K., Sergogy K., Riederer P., Bird E, Schiffman S.N., Mailleux P., Vanderhaagen J., Kuga S., Goldstein M., Kitahama K., Luppi P.H., Jouvet M., Hoekfelt T: Analysis of expression of cholecystokinin in dopamine cells in the ventral mesencephalon of several species and in humans with schizophrenia. Proc Natl Acd Sci USA 87:8427-8431, 1990

Selye J: The general adaptation syndrome and the diseases of adaptation. J Clin Endocrinol Metab 6:117, 1946

Shibasaki T., Naruse M., Naruse K.: Atrial natriuretic factor is released from rat hypothalamus in vitro. Biochem Biophys Res Comm 136:590-596, 1986

Sheeehan M.J., Bellerache J.S.: Facilitation of GABA release by cholecystokinin and cerulein in rat cerebral cortex. Neuropeptides 3:429-434, 1984

Siegel R.A., Duker E.M., Pahnke U., Wuttke W.: Stress-induced changes in cholecystokinin and substance P concentrations in discrete regions of the rat hypothalamus. Neuroendocrinology 46:75-81, 1987

Singh L., Lewis A.S., Field M.J., Hughes J., Woodruff G.N.: Evidence for an involvement of the brain cholecystokinin B receptor in anxiety. Proc Natl Acad Sci USA 88:1130-1133, 1991

Sudoh T., Minamino M., Kangawa K., Matuso H.: C-type natriuretic peptide (CNP). A new member of natriuretic peptid family identified in porcine brain. Biochem Biophysic Res Comm 168:863-870, 1990

Takamasu Y., Yamamato H., Ogunremi O., Matsuzaki I., Moroji T.: The effects of corticotropin-releasing hormone on peptidergic neurons in the rat forebrain. Neuropeptides 20:255-265, 1991

Takao T., Hashimotot K. Ota Z.: Effect on natriuretic peptide on acethylcholine-induced release of corticotropin-releasing factor from rat hyopthalamus in vitro. Life Sci 42:1199-1203, 1988

Telegdy G.: Neuropeptides and brain function. Basle:Karger 1-332, 1987

Tuomisto J., Mannisto P.: Neurotransmitter regulation of anterior pituitary hormones. Pharmacol Rev 37:240, 1985

Vasar E., Peuranen E., Opik T., Harro J., Mannisto P.T.: Ondansetron, an antagonist of 5-HT3 receptors, antagonizes the anti-exploratory effect of cerulein, an agonist of CCK receptors in the elevated plus maze test. Psychopharmacol (Berl) 110:213-218, 1993

Weller A., Blass E.M.: Behavioural evidence for cholecystokinin-opiate interactions in neonatal rats. A J Physiol 255:R901-R907, 1988

Vickroy T., Bianchi B.R.: Pharmacological and mechanistic studies of cholecystokinin-facilitated (3H)dopamine efflux from rat nucleus accumbens. Neuropeptides 13:43-50, 1989

Wiertalk E.P., Maier S.F., Watkins L.R.: Cholecystokinin antianalgesia: safety cues abolish morphine analgesia. Science 256:830-833, 1992

Wittert G.A., Espiner A., Ricgards A.M., Donald R.A., Livesy J.H., Yandle T.G.: Atrial natriuretic factor reduces vasopressin and angiotensin II but not the ACTH response to acute hypoglycaemic stress in normal men. Clin.Endocrinol 38:183- 189, 1993

Woodruff G.N., Hill D.R., Boden P., Pinnock R., Singh L., Hughes J.: Functional role of the brain CCK receptors. Neuropeptides 19:45-56, 1991

Yeung V.T.F., Lai C.K., Cockram C.S.: Atrial natriuretic peptide in the central nervous system. Neuroendocrinology 53:18-24, 1991

Zenker N., Bernstein D.E.: The estimation of small amounts of corticosterone in the rat plasma. J Biol Chem 231:695-701, 1958

6. Acknowledgements

I would like to thank Professor Gyula Telegdy, Chairman of the Department of Pathophysiology, Albert Szent-Györgyi Medical University for providing me with the facilities to carry out my experiments and also for his continuos support in development of my thesis.

I also wish to acknowledge the work and kind help of my previous colleagues and friends from Endocrine Unit of First Department of Medicine of Albert Szent-Györgyi Medical University, namely dr.János Gardi, and Prof.János Julesz. I am escepially thankful for precious consultations and discussions with dr.Miklós Vecsernyés.

I am deeply indebted to my previous teachers and friends dr.Zoltán Sarnyai and dr.Márta Gálfi, not only for their excellent scientific and professional advice as well as encouragement but also for ensuring that these years have been enjoyable and productive.

It is also a pleasure to acknowledge the significant help of Ms.Edit Prágai in the technical parts of my experiments.

A special thanks-you goes to my family, without whom I would not have had the opportunity to complete my work, namely to my grandmother Margit Bánszky and my brother dr.Oszkár Biró.

7. Annex

Publications in international journals from 1993 until 1997 referenced by Science Citation index

Bíró É., Sarnyai Z., Szabó G.: The role of endogenous CRH in the mediation of the behavioural and nueroendocrine response to cholecystokinin octapeptide sulphate esther (CCK) in rats. Neuroendocrinology 57:340-345, 1993

Handley, S.	*Neuroendocrinol. CNS Drugs*	*Vol.2., 397-414 (1994)*
Heinrich, SC.	*Ann NY Acad Sci*	*771: 92-104 (1995)*
Bourim, M.	*Fund Clin Pharmacol*	*10:116-126 (1996)*
Kellner, M.	*Psychophar.*	*133: 55-61 (1997)*
Adamec, RE.	*Behav. Neurosci*	*111: 435-449 (1997)*
Roosendall, B.	*Psychoneuroendocrinol*	*21: 681-693 (1996)*
Shlik, I.	*CNS drugs*	*8:134-152 (1997)*

Laczi F., Iványi T., Sarnyai, Z., Vecsernyés, M., Lengyel, G., Szabó, G., Bíró, É., Gardi, J., Julesz, J., Telegdy, G.: The role of central corticoliberin in the ether stress-induced secretion of neurohypophyseal hormones and corticosterone in the rat. Neuropeptides 26: 33-37 (1994)

Peters, J.	*Clin. Exp. Hypertens*	*18, 933 (1996)*

Laczi F., Szabó, G., Vecsernyés M., Bíró, É., Gardi, J., Julesz, J., Telegdy, G.: The role of central corticoliberin in the hyperosmosis-induced secretion of neurohypophyseal hormones and corticosterone in the rat. Neuropeptides 27: 15-18 (1994)

Raff, H. *Am. J. Physiol* *39, R94 (1996)*

Sarnyai, Z., Bíró É., Babarczy, E., Vecsernyés, M., Laczi, F., Szabó, G., Kriván, M., Kovács, G.L., Telegdy, G.: Oxytocin modulates behavioural adaptation to repeated treatment with cocaine in rats. Neuropharmacol. 31: 593-598 (1992)

Levy, A.D. *Front. Neuroendocrinol* *15, 85 (1994)*
Johns, J.M. *Behav. Neurosci* *108, 107 (1994)*
Belej, T. *Brain Res* *736, 287 (1996)*
Thomas, A. *Life Sci* *58, 1615 (1996)*
Johns, J.M. *Neuropeptides* *31, 439 (1997)*

Sarnyai, Z., Vecsernyés, M., Laczi, F., Bíró, É., Szabó, G., Kovács, G.L.: Effects of cocaine on the content of neurohypophyseal hormones in the plasma and in different brain structures in rats. Neuropeptides 23: 27-31 (1992)

Levy, A.D. *Front. Neuroendocrinol* *15, 85 (1994)*
Fodor, G. *Nat. Prod. Rep* *11, 443 (1994)*
Johns, J.M. *Behav. Neurosci* *108, 107 (1994)*
Blake, M.J. *J. Pharm. Exp. Ther* *268, 522 (1994)*
Rivier, C. *Mol. Cell. Neurosci* *5, 189 (1994)*
Belej, T. *Brain Res* *736, 287 (1996)*
Gardi, J. *Life Sci* *60, 2111 (1997)*

Sarnyai, Z., Bíró, É., Gardi, J., Vecsernyés, M., Julesz, J., Telegdy, G.: Alterations of corticotropin-releasing factor-like immunoreactivity in different brain regions after acute cocaine administration in rats. Brain Res 616: 315-319 (1993)

Rivier, C.	*Mol. Cell. Neurosc.*	*5, 189 (1994)*
Teoh, SK.	*J. Pharm. Exp. The.*	*270, 1134, (1994)*
Cratty, M.S.	*Brain Res*	*675, 297 (1995)*
Madras, B.K.	*Synapse*	*18, 261 (1995)*
Richter, R.M.	*Neurosci. Let.*	*187, 169 (1995)*
Bíró, É.	*Life Sci*	*59, 1351 (1996)*
Zhou,Y.	*J. Pharmacol. Exp. The.*	*279, 351 (1996)*
Simar, M.R.	*Neuroendocrinol*	*63, 93 (1996)*
Gardi, J.	*Neuropeptides*	*31, 15 (1997)*
Gardi, J.	*Life Sci*	*60, 2111 (1997)*
Simar, M.R..	*Psychoneuroendocrino.*	*22, 141 (1997)*
Mello, N.K.	*Pharm. Biochem. Behav*	*57, 571 (1997)*
Goeders, N.E.	*Psychoneuroendocrinol.*	*22, 237 (1997)*

Sarnyai Z., Bíró, É., Gardi, J., Vecsernyés, M., Julesz, J., Telegdy, G.: Brain corticotropin-releasing factor mediates anxiety-like behavior induced by cocaine withdrawal in rats. Brain Res 675: 89-97 (1995)

Bíró, É.	*Life Sci*	*59, 1351 (1996)*
Gardi, J.	*Neuropeptides*	*31, 15 (1997)*
Shaham, Y.	*J. Neurosci*	*17, 2605 (1997)*
Moreau, J.L.	*Neuroreport*	*8, 1697 (1997)*
Ambrosio, E.	*Synapse*	*25, 272 (1997)*
David, M.J.	*Neuropsy. Clin. Neurosc.*	*9, 382 (1997)*
Moy, S.S.	*Psychopharmacol*	*131, 354 (1997)*
Wu, J.C.	*Neuropsychopharmaco.*	*17, 402 (1997)*
Johns, J.M.	*Dev. Neurosci*	*19, 368 (1997)*
Koob G.F.	*Pharm. Biochem. Behav*	*57, 513 (1997)*

Sarnyai Z., Bíró É., Penke B., Rivier J., Telegdy G.: The cocaine-induced elevation of plasma corticosterone is mediated by endogenous corticotropin-releasing factor (CRF) in rats. Brain Research, Vol. 589, No.1., 154-156. 1992.

Zhou, Y.	*J. Pharmacol Exper Ther*	*279: 351-358 (1996)*
Marita, K..	*J. Pharmacol Exper Ther.*	*279: 379-385 (1996)*
Sinar, M.R.	*Neuroendocrinol*	*63: 93-100 (1997)*
Farre, M.	*J. Pharm Exper Ther*	*283: 164-176 (1997)*
Carey, R.	*J. Neurosci. Met*	*73: 1-8 (1997)*
Stanulis, E.D.	*J. Pharm Exper. Ther.*	*280: 284-291 (1997)*
Torres, G.	*Mol Brain Res*	*43: 225-232 (1996)*
Gardi, J.	*Neuropeptides*	*31,15 (1997)*
Goeders, N.E.	*Phar Biochem Behav*	*57:571-599 (1997)*
Ambrosio, E.	*Synapse*	*25:272-276 (1997)*
Goeders, N.E.	*Psychoneuroendocrinol*	*22:237-259 (1997)*