Corticotropin-releasing hormone microinfusion in the central amygdala diminishes a cardiac parasympathetic outflow under stress-free conditions

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INTRODUCTION

Corticotropin-releasing hormone (CRH), a 41-amino-acid peptide, is known to exert activating effects on the hypothalamus-pituitary-adrenal axis, by releasing adrenocorticotropic 24,51,67. In addition, CRH is involved in the central regulation of autonomic and behavioural activity. Numerous studies on the effects of intracerebroventricular (i.c.v.) CRH injections revealed overall increases in sympathetic-mediated responses, like rises in heart rate and blood pressure 13,29,42,44, rises in catecholamine and glucose levels 2,13,14,42,44 and increases in behavioural activity 7,10,12,22,32,42,47,50,60,61,63,69,70. However, CRH in the CNS also inhibits the cardiac parasympathetic nervous tone 26-28. The exact anatomical sites responsible for the behavioural and cardiovascular actions of centrally administered CRH are unknown yet.

CRH immunoreactive neurons and terminals are widely distributed throughout the central nervous system 24,51,64. Among the forebrain regions that contain CRH-immunoreactive neurons are the PVN, the central nucleus of the amygdala (CeA) and the bed nucleus of the stria terminalis (BNST). The CeA contains the majority of the forebrain CRH-immunoreactive neurons that innervate the brainstem nuclei such as the parabrachial nucleus, midbrain central grey and dorsal vagal complex 18,33,34,26. From the CeA, CRH has only efferent pathways to the brainstem areas, no ascending CRH neurons from the brainstem nuclei to the CeA are found to date 33.

The CeA is an important part of the neural circuitry mediating behavioural, autonomic and neuroendocrine adjustments to environmental stimuli 35. Numerous studies revealed that it is involved in the parasympathetic regulation of both the acute and the conditioned
stress response. For example, bilateral CeA lesions attenuate the conditioned stress-induced, vagus-mediated gastric ulceration in rats. In addition, lesioning of the CeA causes a disruption of the characteristic parasympathetic and passive coping responses; such as bradycardia together with immobile behaviour, evoked by both conditioned and unconditioned aversive footshocks.

The present study was designed to investigate the effects of CRH, infused into the CeA, on behaviour, cardiac and endocrine responses in basal, non-stressed conditions. In view of the involvement of the CeA in the parasympathetic output and the CRH connections to the brainstem vagal complex, it is likely that CRH peptidergic regulation in the CeA is involved in the regulation of the parasympathetic cardiovascular output and the behavioural components associated with passive coping response. The CRH-receptor antagonist, α-helical CRH (α-hCRH) 9–41, was used to test the specificity of the observed exogenous CRH effects.

MATERIALS AND METHODS

Animals

Nineteen male Wistar rats, weighing 304–340 g at the beginning of the experiment, were used. The animals were housed individually in perspex cages (25 × 25 × 30 cm) and kept in a temperature-controlled room (20 ± 2°C) with a 12-h light–dark cycle (lights on from 08.30 to 20.30 h). The experiments were performed during the light period of the cycle (between 10.00 and 15.30 h). Food and water were available ad libitum.

Surgery

Each experimental animal was first provided with a permanent silicon catheter (0.95 mm o.d., 0.50 mm i.d.) in the right atrium, inserted via the right jugular vein. This method allows frequent blood sampling in undisturbed, freely moving rats. Bilateral permanent stainless-steel brain cannulae (o.d. 0.3 mm, i.d. 0.15 mm) for drug infusion were aimed just above the central amygdala (coordinates: 6.7 mm rostral to interaural, lateral 4.0 mm to the midline and ventral 6.2 mm below the dura) according to Paxinos and Watson. Together with the heart catheter, the brain cannulae were permanently fixed to the skull of the rat by means of stainless-steel screws and dental cement. In order to record the electrocardiogram (ECG), transcutaneous steel electrodes made of standard paperclips were implanted, one between the scapulae and the other in the middle of the back. The animals were kept under ether anaesthesia during the entire surgical procedure.

Drug treatment

Synthetic rat/human CRH (CRF; Sigma Chemical Co., St. Louis, MO) and the CRH receptor antagonist, α-hCRH 9–41 (α-hCRF; Sigma Chemical Co., St. Louis, MO) were dissolved in artificial cerebrospinal fluid (aCSF) with ascorbic acid (100 μg/ml aCSF). CRH was administered in a dose of 30 ng/rat per cannula, as in a pilot-experiment, in which two lower doses of CRH, 3 ng and 300 ng, were tested, under the same conditions as described in this paper, no change in heart rate and behavioural response was found. α-hCRH was administered in doses of 0.1 μg (low dose) and 1 μg/rat (high dose) per cannula. The vehicle was sterile aCSF containing (in mM): NaCl 127.64, KCl 2.55, CaCl₂ 1.26 and MgCl₂ 6H₂O 0.93.

All compounds were infused in a total volume of 1 μl in each brain cannula during a 7-min period.

ECG recording and analysis

The ECG of freely moving rats was monitored telemetrically by means of a miniature FM transmitter (EDB-ROY, Haren, The Netherlands) as described earlier. The transmitter was connected to the transcutaneous electrodes and secured around the chest of the rat by means of a strap. The transmitted signals were received on a commercial FM radio and stored on tape. For off-line analysis, the recorded ECG samples were processed through a cardiotachometer pulse generator which generated a square wave pulse at each R wave (Olivetti, M24). The time between the onset of two consecutive pulses, the interbeat interval (IBI), was measured within the range of 100 to 220 ms. IBIs were recalculated in beats/min.

Blood sampling

Before the start of the experiment, the animals were habituated to the full experimental procedure for at least 3 days. Fifty minutes before the start of an experiment, the animals were connected to a polyethylene blood-sampling tube. Blood samples of 0.3 ml were withdrawn for determination of plasma catecholamines and corticosterone concentrations. Samples were immediately transferred to chilled (0°C) centrifuge tubes containing 0.01% EDTA as antioxidant, and 10 μl heparin solution (500 IU/ml) as anticoagulant. Blood was centrifuged at 4°C for 10 min at 5000 rpm. The supernatant was stored at −70°C. Withdrawn blood was replaced by transfusion of heparinized donor blood, after each blood sample taken, in order to minimize the changes in blood volume. Donor blood was obtained from unstressed rats of the same strain also provided with permanent heart catheters. Plasma catecholamine determination was performed by high-performance liquid chromatography (HPLC) with electrochemical determination (ECD) as described earlier. Plasma corticosterone was measured by means of reversed-phase HPLC according to the method of Dawson with minor modifications. Samples were redissolved in ultra-pure water and spin-filtered through a 0.2-μm membrane filter (Millipore) before detection.

Behavioural measurements

Behaviour was recorded on the basis of the following criteria: resting/sleeping = inactive with eyes open or closed; immobility = completely motionless, absence of skeletal and vibrissae movements except those associated with respiration; exploring = investigation of any part of the home cage; grooming = wiping the fur with forepaws and tongue (washing); burying = pushing the bedding material with rapid movements of the snout or forepaws; rearing = sniffing in the air with both forepaws from the floor; eating = chewing food pellets or faeces; sniffing = sniffing in the air with paws on the floor.

All behavioural elements were recorded by means of a keyboard-operated microprocessor (EDB, Haren, The Netherlands). The duration and the frequency of these elements were recorded and expressed as the percentage of the total observation period.

Experimental procedures

The experiments were performed in the animals’ home cages under stress-free conditions. The rats were habituated to the strap holding of the transmitter, and to the infusion and blood-sampling procedure for a few hours during 3 days before the start of the experiments. After 10 or more days recovery from surgery, the rats were tested for the first time. A cross-over design was used in which each rat served as its own control. Each animal was pretreated either with α-hCRH or vehicle, 50 min before CRH or aCSF infusion. The experimental protocol is given in Table I.

Each animal received vehicle and CRH infusion after vehicle pretreatment and either one of the pretreatment dose of α-hCRH (Table I). Each treatment was separated by a wash-out period of at least 1 week. The pretreatment was given at t = −50 min at the lap of the experimentor. Immediately after the pretreatment, the rat was placed into its home-cage, connected with ECG transmitter, blood sampling and infusion tubes, filled with the second drug infusion
solution. Immediately after the second control sample, at \( t = 0 \) min, either the CRH or aCSF was microinfused in the undisturbed, freely moving rat. Each 1-min sample of heart rate and behaviour was directly followed by a blood sample, before \( (t = -10 \text{ and } t = -1 \text{ min}) \), during \( (t = 3 \text{ and } t = 6 \text{ min}) \) and after the second microinfusion \( (t = 8, 10, 12, 17, 27, 47, 67 \text{ min}) \). Corticosterone was determined in all blood samples. Noradrenaline and adrenaline were determined in the blood samples taken at \( t = -1, 6, 12, \text{ and } 47 \text{ min} \).

### Histology

At the end of the experiments, the rats were deeply anaesthetized with sodium pentobarbital (90 mg/kg i.p.) and perfused intracardially with saline followed by a 4% formaldehyde solution. The brains were postfixed in the same fixative for at least 1 week. Frozen sections of 40 \( \mu \text{m} \) were cut, and the tip of the cannula placement was determined on unstained sections.

### Statistics

Cardiac, hormonal and behavioural data were evaluated using an analysis of variance with repeated measures (ANOVA) and followed by the correlated Student t-test. A probability level of \( P < 0.05 \) was taken as significant for all tests.

### RESULTS

#### Histology

Histological examination revealed that four of the animals had to be excluded from further analysis because of improper bilateral cannulae placement. The cannulae tips had to be localized just above or entering the dorsal edge of the CeA. Fig. 1 shows examples of cannulae placements that were considered as proper (⋆) and improper (○) bilateral CeA placements.

#### Cardiac responses

Fig. 2 shows that local infusion of 30 ng of CRH in the CeA produced an increase in heart rate response compared to the vehicle-treated group. The tachycardia started during the infusion and lasted for the entire registration period. The ANOVA showed a significant main effect of treatment \( (F_{1,17} = 11.823, \ P < 0.005) \) and an interaction between treatment and time \( (F_{4.68} = 4.309, \ P < 0.005) \). The CRH-induced tachycardia was reduced by the CRH-receptor antagonist in high dose \( (1 \mu \text{g}) \), and partially reduced by the low dose of the \( \alpha\)-hCRH \( (0.1 \mu \text{g}) \) (Fig. 2). Comparing the high dose \( \alpha\)-hCRH + CRH-treated group with the vehicle-treated group, the ANOVA revealed a significant interaction effect \( (F_{4.56} = 3.285, \ P < 0.05) \). Analysis of the separate sample times revealed one significant difference in heart rate response between the high dose \( \alpha\)-hCRH + CRH infusion and the vehicle-group (at \( t = 6 \text{ min} \)). No significant differences were found between the vehicle and the low dose \( \alpha\)-hCRH + CRH group, and between the CRH and the low dose \( \alpha\)-hCRH + CRH group.

#### Catecholamine response

Fig. 3 shows the plasma adrenaline (Fig. 3a) and noradrenaline (Fig. 3b) levels in the four groups before, during and after the second infusion. No response was seen in both catecholamines on the infusion of CRH or the vehicle in all the four groups (Fig. 3). Analysis of variance did reveal neither a significant treatment effect nor treatment × time interaction between the four groups.

#### Corticosterone response

No significant differences in plasma corticosterone between the vehicle and CRH-group were found (Fig. 4). The corticosterone levels of both groups stayed on the same level after either drug or vehicle treatment. However in the \( \alpha\)-hCRH + CRH infusion groups, significant differences were found in relation to the vehicle group and the CRH group. Both doses of the antagonist + CRH treatment revealed slowly rises in corticosterone levels, which reach their maximum at the last sample. The \( \alpha\)-hCRH in high dose compared to the CRH group showed a significant effect \( (F_{1.17} = 6.444, \ P < 0.05) \) and a significant time effect \( (F_{4.68} = 8.5222, \ P < 0.0001) \). The Student t-test indicated that only at \( t = 17 \) and \( t = 27 \text{ min} \) significant differences

### TABLE 1

<table>
<thead>
<tr>
<th>Test</th>
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<tbody>
<tr>
<td>1</td>
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<td>aCSF</td>
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<tr>
<td>2</td>
<td>aCSF</td>
<td>CRH (30 ng)</td>
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<tr>
<td>3a</td>
<td>( \alpha)-hCRH 1.0 ( \mu \text{g} )</td>
<td>CRH (30 ng): high dose</td>
</tr>
<tr>
<td>3b</td>
<td>( \alpha)-hCRH 0.1 ( \mu \text{g} )</td>
<td>CRH (30 ng): low dose</td>
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were found between the high dose α-hCRH + CRH and the CRH-treatment. The low dose α-hCRH +
CRH group showed significant increases in corticosterone level compared to both the vehicle and the
CRH groups (vehicle–α-hCRH + CRH (0.1 μg), treatment: $F_{1,19} = 6.664, P < 0.05$; time: $F_{4,52} = 12.353, P <
0.0001$; treatment × time: $F_{4,52} = 3.703, P < 0.01$; CRH–α-hCRH + CRH (0, 1 μg), treatment: $F_{1,17} =
6.444, P < 0.05$; time: $F_{4,52} = 8.522, P < 0.001$; treatment × time $F_{4,52} = 24.498, P < 0.08$). The data at $t =
17$ and $t = 27$ min demonstrated significant increases between the low dose α-hCRH + CRH and the vehicle and the CRH-group.

**Behavioural responses**

**Resting / sleeping**

Resting or sleeping is the major element the animals displayed during the experiments, the other behaviours
appeared more rarely.

Resting behaviour in the vehicle group remained at a constant level, during the whole experiment (Fig. 5a).
Infusion of CRH in both the CRH and the high dose α-hCRH + CRH group resulted in a reduction in resting
behaviour (Fig. 5a). Comparison between the vehicle the CRH group, and the high dose α-hCRH + CRH
revealed significant treatment effects (aCSF-CRH, $F_{1,27} = 5.234, P < 0.05$; aCSF-α-hCRH + CRH
(1 μg), $F_{1,21} = 4.137, P < 0.05$), no time or treatment ×
time interaction effects were found. At $t = 6$ min the
duration of resting was significantly reduced in rats receiving the α-hCRH + CRH and CRH treatments,
compared to the vehicle group. The reduction in resting behaviour lasted until the infusion period was
stopped, after which it slowly returned to basal level (at $t = 27$). The results of resting behaviour in the low dose α-hCRH + CRH group are not shown, as no differences between this group and the vehicle-group were found.

**Immobility**

No effects of the infusion of either aCSF or CRH were found on the duration of immobile behaviour in
Fig. 4. Plasma corticosterone levels before, during and after microinfusion into the CeA of CRH pretreated with artificial-CSF (CRH), CRH pretreated with α-hCRH, high dose (hCRH + CRH 1 μg), or CRH pretreated with α-hCRH, low dose (hCRH + CRH 0.1 μg) in stress-free conditions. In the control experiments, artificial CSF pretreated with αCSF was infused (CSF). Data are expressed as averages ± S.E.M. The infusion period is indicated by the horizontal bar.

the vehicle group the CRH group and the high dose α-hCRH + CRH group (Fig. 5b). Only in the CRH group and the low dose α-hCRH + CRH group, the level of immobile behaviour was overall higher compared to the vehicle group. Analysis of variance revealed significant treatment effects between the vehicle and the CRH-group (F1.27 = 9.029, P < 0.01) and the vehicle and the low dose α-hCRH + CRH group (F1.20 = 9.211, P < 0.01). Significant time and treatment × time interactions were found between the vehicle and the low dose α-hCRH + CRH group (time: F4.80 = 9.733, P < 0.0001; interaction: F4.80 = 6.971, P < 0.001), CRH and low dose α-hCRH + CRH group (time: F4.76 = 4.858, P < 0.01; interaction: F4.76 = 2.502, P < 0.05) and between the two α-hCRH + CRH groups (time: F4.52 = 3.937, P < 0.05; interaction: F4.52 = 3.051, P < 0.05). The rats treated with the low dose α-hCRH + CRH infusion showed a rapid increase in immobile behaviour at t = 10 and t = 12, and a rapid recovery from immobility after t = 12 (Fig. 5b). Significant differences were found between the vehicle group and the low dose α-hCRH + CRH group at t = 10 and t = 12, and the vehicle-group with the CRH-group at t = 3. The results of immobility in the high dose α-hCRH + CRH group are not shown, as no differences between this group and the vehicle group were found.

Exploring

Analysis of variance revealed significant treatment × time interactions between the vehicle-CRH group (F4.108 = 2.334, P < 0.05) and the vehicle-high dose antagonist + CRH group (F4.48 = 2.911, P < 0.05) were found. The occurrence of exploration was significantly increased in the CRH and the high dose α-hCRH + CRH group at t = 6 min compared to the vehicle group. No effects were found between the CRH and the two α-hCRH + CRH groups.

The occurrence of the other behavioural responses, i.e. grooming, burying, rearing, sniffing and eating, did not differ significantly between the different treatments.

Fig. 5. Time spent in various behaviours before, during and after microinfusion into the CeA of CRH pretreated with CSF (CRH-group), CRH pretreated with α-hCRH, high dose (hCRH + CRH 1 μg), or CRH pretreated with α-hCRH, low dose (hCRH + CRH 0.1 μg) in stress-free conditions. In the control experiments, artificial CSF pretreated with αCSF was infused (CSF). Data are expressed as averages ± S.E.M. The infusion period is indicated by the horizontal bar. Resting (5A) and immobile (5B) behaviour are shown.
DISCUSSION

The major finding of the present study is that local CRH microinfusions, into the CeA, increased heart rate without affecting plasma adrenaline, noradrenaline and corticosterone concentrations in rats under stress-free conditions. Simultaneously, the behaviours, immobility and exploring were increased. Pretreatment of the CRH-receptor antagonist resulted in a dose-dependent blockade of the CRH-induced tachycardia. However, pretreatment of the CRH-receptor antagonist did not cause a blockade in the CRH-induced behavioural activation. In contrast with the CRH group, both CRH-receptor antagonist treatments showed an increase in corticosterone response, while the catecholaminergic levels remained at a constant level.

The results indicate that the CRH system in the CeA is involved in cardiovascular and behavioural control, whereas adrenal neuroendocrine mechanisms do not seem to be affected by CRH in the CeA in stress-free conditions.

The obtained tachycardia by CRH infusion in the CeA can be explained by three possible mechanisms. A direct stimulation of the sympathetic output or a direct inhibition of the parasympathetic vagal output or indirectly by an increase in behavioural activity may be responsible for the rise in heart rate after CRH administration. Indeed an increase in exploring is observed, which is considered to be accompanied by a rise in sympathetic outflow. Rises in plasma adrenaline and noradrenaline are generally taken as a measurement for the activation of the sympathetic nervous system. No elevations in peripheral adrenaline and noradrenaline levels were found as a consequence of the CRH-infusion. Compared to other experiments, the present plasma catecholamine levels can be considered as basal values. Two conclusions can be drawn, first it can be concluded that the tachycardia is not the result of a general activation of the sympathetic nervous system. Rather, a reduction in the parasympathetic activity seems to be responsible for the CRH-induced increase in heart rate in stress-free conditions. Secondly, these low catecholamine levels indicate that the experiment is carried out under stress-free conditions indeed. In conclusion, the balance in activity of the sympathetic and parasympathetic components of the autonomic nervous system seems to be altered by the CRH infusion, with a shift towards the sympathetic side.

The corticosterone results revealed no leakage of infused CRH to the periphery, with subsequent activation of the pituitary-adrenal axis, in the CRH group, as no increases in peripheral plasma corticosterone levels during the infusion were found. These results are in agreement with other groups who asserted that the CeA does not seem to be involved in the regulation of plasma corticosterone in stress-free conditions. Although, the CeA seems to be involved in the corticosterone response to different stress-situations. One group asserted that CRH mechanisms in the CeA seem to play an important role in the corticosterone response after stress.

The basal plasma levels of corticosterone were slightly elevated in comparison to the usual mean 24 h average of 5 μg/dl. These slightly elevated basal levels of plasma corticosterone in all the groups cannot be a result of the animals being stressed at the beginning and during the experiments, as the behavioural data showed that during the experiment the rats were resting/sleeping during most of the time.

The behavioural responses to CRH given in the CeA revealed some discrepancy with the behavioural responses observed after i.c.v. CRH injections. The decrease in resting behaviour, after CRH infusion in the CeA, was accompanied by an increase in exploratory and immobility behaviour, but not by an increase in grooming. The increase in locomotor behaviour and immobile behaviour is in accordance with i.c.v. CRH reports. However, in this experiment no effect of locally applied CRH in the CeA on grooming behaviour was found, which disagree with the increase in grooming found after i.c.v. CRH infusion. Accordingly, regionally specific neuromodulatory properties of CRH seem to play an important part in the different regulation of autonomic and behavioural responses in the CNS. This regional specificity of CRH was suggested earlier by Diamant and colleagues and Koob and co-workers.

The present results differ in some respect from reports by other investigators injecting CRH locally in the amygdala area. One group reported an elevation of noradrenaline levels, while another group asserted that the enhanced effect they found on memory of aversive learning by infusion of CRH in the amygdala, was a result of activating the release of adrenaline by CRH. The discrepancy between these results and the results of the present study may be due, at least in part, to differences in volume injected, the concentration used, and the exact area of injection/infusion of the peptide. Although the possibility of diffusion of CRH to adjacent responsive structures, as to the basolateral nucleus of the amygdala, has to be considered, the various CRH-induced responses at the
CeA, such as the diminishing parasympathetic response, may indicate a site specificity for the CRH-evoked effects.

The effects of CRH can be blocked by a local application of the α-hCRH, the CRH receptor antagonist. This indicates that CRH receptors are involved. At the level of the CeA these CRH-binding sites may represent autoreceptors. As CRH is given at the level of the cell body of the CRH neurons in the CeA, the present data of an inhibition of the parasympathetic response may be explained by an autoreceptor-mediated inhibition of CRH connections with the parasympathetic-regulating brainstem nuclei. This hypothesis is supported by recent electrophysiological research by Rainnie and colleagues in which differential effects of CRH on the membrane properties of basolateral (BLA) and central amygdala (CeA) neurons in vitro was found. This group suggested that the differential action of CRH on the BLA and CeA neurons was caused by activation of a heterogenous population of CRH receptors in the amygdala. They proposed that the CRH-mediated inhibition in CeA neurons was due to an autoinhibition caused by somatodendritic CRH autoreceptors.

Another possible explanation of the CRH-induced cardiovascular effects can be that CRH can act also on receptors located on neurons which do not contain CRH and project to structures which are involved in cardiovascular control, i.e. the lateral hypothalamus. Uryu and colleagues have recently described, that part of the CRH axon terminals in the CeA arises from neurons in the lateral hypothalamic area and the dorsal raphe nucleus, the amygdalophetal pathway, and the others from intra-amygdaloid CRH neurons. Accordingly, the amygdalophetal and intra-amygdaloid pathways might participate in the regulation of autonomic responses and behavioural activation, since both the lateral hypothalamic area and the amygdala are considered to contribute to the organization of autonomic, somatosensoric and somatomotoric responses.

The CRH receptor antagonist, α-hCRH, has been used in the present experiment only as a tool to demonstrate the specificity of the exogenous CRH administration. Some groups have described a partial agonistic effect of the CRH-receptor antagonist. The transient agonistic activity of the α-hCRH will not be detected when the α-hCRH is injected 30 to 60 min before exposing the animal to a stressful stimulus, whereas binding of CRH, secreted in reaction to the stress or administered exogenously, to its receptor may still be adequately blocked. The apparent necessity to use the antagonist at large amounts points towards a low affinity of the antagonist for the CRH receptor.

The differences found between administration of the α-hCRH alone and as a pretreatment before a CRH infusion can have the following explanation. The CRH-receptor antagonist can probably block both the tonic (endogenous release of CRH), and the phasic (stress-related release, or exogenous administered) release of CRH. In the present experiment, the phasic release of CRH, and the blockade by the antagonist, was examined, because infusion of CRH probably resulted in a raise in concentration of CRH in the synaptic clefts. While in the experiments of the other groups using α-hCRH alone mostly the blockade by α-hCRH of the tonic release of CRH, endogenous amount of CRH, was examined. Besides, it is suggested that the CRH-receptor antagonist can produce effects which are unrelated to its action at the CRH-receptor which can also be an explanation for the behavioural response seen after pretreatment by the α-hCRH infusion.

However, the possibility cannot be ruled out that the reduction in resting behaviour, the increase in exploration and the increase in plasma corticosterone by the combined α-hCRH and CRH infusion is due to possible partial agonistic properties of α-hCRH. Another explanation for the additive effects of α-hCRH + CRH compared to CRH may be the spread of α-hCRH to other adjacent responsive structures.

Although some groups reported that the CRH and the α-hCRH interact with the same receptor population in a competitive manner, different receptor affinities to CRH and its antagonist may be another explanation for the results mentioned above, and have different affinities to CRH and its antagonist.

Our results and earlier work done in this laboratory are consistent with the aforementioned statement that the CeA selectively regulates the parasympathetic output of acute and conditioned stress responses. The present results leads to the hypothesis that CRH at the level of the CeA inhibits parasympathetic output in non-stressed situations. This results in a shift in the balance in activity of the sympathetic and parasympathetic components of the autonomic nervous system towards the sympathetic side. Furthermore, it is hypothesized that mediation of CRH-autoreceptors on the CRH neurons in the CeA, with connections to parasympathetic brainstem nuclei, can be responsible for the diminishing parasympathetic response found under stress-free conditions. To verify this hypothesis further research is necessary.

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