C-type natriuretic peptide exerts stimulatory effects on the corticotropin-releasing hormone-induced secretion of hormones in normal man

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Abstract

C-type natriuretic peptide and atrial natriuretic peptide have been reported to bind to distinct receptors and to exert opposing effects on different systems. Although it is known that atrial natriuretic peptide inhibits the corticotropin-releasing hormone-stimulated hormone release in man, the corresponding action of C-type natriuretic peptide has so far not been characterized. We investigated the effects of 30-min infusions of 150 and 300 µg C-type natriuretic peptide on adrenocorticotropin, cortisol, and prolactin release stimulated by 100 µg corticotropin-releasing hormone and on cardiovascular parameters in 8 healthy male volunteers. Compared with placebo, 300 µg C-type natriuretic peptide significantly ($P<0.05$) enhanced the stimulation of cortisol (area under curve (arbitrary units): 520 ± 35 vs 651 ± 55) and prolactin (area under curve: 29 ± 3 vs 37 ± 5). Adrenocorticotropin levels were increased, but the differences did not reach statistical significance (maximum increment: 27 ± 4 vs 36 ± 2 pg/ml). C-type natriuretic peptide at a dose of 150 µg had no clear effect on these hormones and C-type natriuretic peptide also produced no cardiovascular or subjective effects. Our data suggest stimulatory effects of C-type natriuretic peptide on corticotropin-releasing hormone-induced hormone release and offer further evidence for a complex role of different natriuretic peptides in endocrine regulation.

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Introduction

C-type natriuretic peptide (CNP), the most recently identified member of the natriuretic peptide family, was first isolated in 1990 (1). Although the other natriuretic peptides, atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP), are mainly of cardiac origin in man, CNP is found most abundantly in the human central nervous system. The CNP concentration has been reported to be 10-fold higher than the ANP concentration in the brain and the cerebrospinal fluid (2), and especially high concentrations (25 times higher than ANP) have been found in the hypothalamus (3). ANP and BNP act primarily via the type A natriuretic peptide receptor (NPR-A) and CNP binds preferentially to the type B natriuretic peptide receptor (NPR-B), both of which are coupled to a guanylyl cyclase system (4). Therefore, these peptides have different biological actions. Preclinical studies in rats and sheep have shown opposing effects of CNP and ANP on water drinking (5) and on adrenocortical and prolactin secretion (6, 7).

In contrast to ANP and BNP, no natriuretic effect of CNP or inhibition of the endocrine and cardiovascular response to angiotensin II has been shown in man (8, 9). Although suppressive effects of ANP on the corticotropin-releasing hormone (CRH)-stimulated release of adrenocorticotropin (ACTH), cortisol, and prolactin have been characterized (10–12), so far no data are available on the corresponding action of CNP on the stimulated secretion of these hormones in man. Therefore, we investigated the effects of 2 dosages of CNP on the hormonal response to CRH in 8 healthy male volunteers to test the hypothesis that CNP exerts effects different to those of ANP.

Subjects and methods

Eight healthy male volunteers (21–33 years old, mean 24±6 years) of normal weight (body mass index 21.7–24.8 kg/m², mean 23.8 kg/m²²) were studied. Each subject was given a thorough medical and psychiatric examination. Individuals with a personal or family history of psychiatric disorders or a recent stressful life event were excluded, as were shift workers and individuals who had recently made a transmeridian flight. Persons who had smoked, drunk any alcohol, or
had any medical treatment during the previous 3 months were also excluded. None of the subjects was sleep-deprived or had irregular sleeping habits. Written informed consent was obtained from each subject prior to the investigation. The protocol had been approved by the Ethics Committee for Human Experiments of the Max Planck Institute of Psychiatry, Department of Psychiatry, Munich.

Each subject participated in 3 different experimental sessions, separated by at least 4 days. All subjects were studied in a single-bedded soundproof room under video observation. The subjects arrived at 1700 h after having had a standardized meal at 1200 h. An intravenous catheter was inserted into a forearm vein of each arm at 1705 h and connected to the adjacent laboratory. From 1730 to 1830 h blood samples were drawn every 30 min, from 1845 to 1915 h every 5 min, from 1930 to 2000 h every 15 min, and from 2000 to 2200 h every 30 min. Blood pressure and heart rate were registered concomitantly with an automatic device. At 1900 h 100 µg human (h) CRH (Clnalfa, Läufelfingen, Switzerland) were given intravenously within 30 s as a bolus injection. In addition, an infusion of 30 ml cooled 0-9% saline (placebo), 30 ml saline containing 150 µg CNP (Clnalfa), or 30 ml saline containing 300 µg CNP was given from 1845 to 1915 h at a rate of 60 ml/h in a randomized order in a prospective single-blind study design.

For cortisol, ACTH, and prolactin immunoreactivity determinations, ethylene-diamine-tetraacetic acid (Merck, Darmstadt, Germany; 1 ml/ml blood) and kallikrein inhibitor (Bayer, Leverkusen, Germany; 350 IU/ml blood) were added to the native blood samples. All blood samples were immediately centrifuged at 4000 g for 10 min, and then the plasma was frozen and stored at −80°C until analysis. For cortisol analysis, a commercial radioimmunoassay kit was used (ICN Biomedicals Inc., Carson, CA, USA). The sensitivity was 7 ng/ml plasma, and the intra- and interassay coefficients of variation at 10, 40, and 100 ng/ml were below 5%. Plasma ACTH immunoreactivity was measured using a commercially available immunoradiometric assay (Nichols, San Juan Capistrano, CA, USA). The sensitivity was 4 pg/ml plasma, and the intra- and interassay coefficients of variation were below 10% at 20 and 50 pg/ml. For prolactin determinations a commercially available immunoradiometric assay was used (Nichols). Minimum detectable amounts were 0.2 ng/ml plasma and intra- and interassay coefficients of variation were below 8% at levels of 2 and 8 ng/ml plasma.

Statistics

The parameters’ area under the curve (AUC) between 1930 and 2030 h (calculated with the trapezoid rule), the maximum stimulated concentration, and the maximum increment (difference between maximum plasma concentrations from 1900 to 2000 h and baseline values from 1830 to 1900 h) were tested for significant treatment effects in the 3 conditions using a one-factorial analysis of variance (ANOVA) with repeated measures design for the endocrine (cortisol, ACTH, prolactin) and cardiovascular (heart rate, systolic and diastolic blood pressure) data. Treatment was the within-subjects factor with 3 levels corresponding to ‘placebo’, ‘150 µg CNP’ and ‘300 µg CNP’. If significant main effects were identified, tests with contrasts followed to locate the differences. To keep the type I error ≤ alpha = 0.05 (nominal level of significance), a Bonferroni correction was used. All values are expressed as means ± s.e.m.

Results

Cortisol and ACTH

After infusion of CNP and injection of CRH an enhancement of cortisol secretion was observed, although infusion of CNP alone did not show any effects until 1900 h. Significant treatment effects were identified for the AUC values for cortisol [F(2,14) = 4.8, P < 0.05], the maximum increments [F(2,14) = 5.8, P < 0.05], and the maximum concentrations [F(2,14) = 4.4, P < 0.05]. Further analysis with contrasts in ANOVA localized significant differences between the placebo and the 300 µg CNP condition for the variables AUC (tests with contrasts, [F(1,7) = 7.2, P < 0.05], maximum increment [F(1,7) = 7.4, P < 0.05], and maximum concentration [F(1,7) = 10.1, P < 0.02]). Significant effects were also found between placebo and 150 µg CNP for the maximum increment (tests with contrasts, [F(1,7) = 5.9, P < 0.05]) and between 150 and 300 µg CNP for the variable AUC [F(1,7) = 11.2, P < 0.02]. The respective values are given in Fig. 1 and Table 1. As indicated in Table 1 and Fig. 2, plasma ACTH levels after CRH were slightly enhanced by 300 µg CNP. However, there were no significant

![Figure 1: Effects of CNP infusions (hatched box) on the CRH-stimulated (arrow) release of cortisol in healthy human volunteers (means ± s.e.m.; n = 8). (●), Placebo; (○), 150 µg CNP; (■), 300 µg CNP.](image-url)
treatment effects in ANOVA for AUC values, maximum increments or maximal concentrations. In addition, no effects emerged during the CNP infusions alone until 1900 h.

**Prolactin**

A CNP stimulatory effect was also evident for prolactin after CRH, although no effects of CNP only were detected. Significant treatment effects were seen in the maximum increments (F[2,14] = 3·9, P < 0·05) and the maximum concentrations after stimulation (F[2,14] = 4·4, P < 0·05). For AUC values only, a tendential significance was revealed (F[2,14] = 3·7, P = 0·05). Significant differences were localized between the 300 µg CNP and placebo condition for AUC (F[1,7] = 9·0, P < 0·02). Significant effects were also seen between the 150 and 300 µg CNP conditions for both AUC (F[1,7] = 6·5, P < 0·05) and the maximum stimulated concentration (F[1,7] = 8·3, P < 0·05), but only marginal effects were seen for the maximum increment (test with contrasts, F[1,7] = 5·5, P = 0·05). The respective values are given in Table 1 and Fig. 3.

**Cardiovascular parameters**

Heart rate and systolic and diastolic blood pressure before and after CRH remained unaffected by the two CNP dosages. No significant treatment effects for AUC, maximum increments or maximum levels were detected (see Table 2).

**Side effects**

All CNP infusions were well tolerated and none of the volunteers reported any subjective symptoms during CNP administration, in particular no vegetative signs such as palpitations, vertigo or flushes were reported.

**Discussion**

The major result of our study is that an infusion of 300 µg CNP over a 30-min period significantly enhanced the CRH-induced release of cortisol and prolactin in normal man. No heart rate or blood pressure alterations were observed that could have interfered with the hormonal release. Thus, the effects of 300 µg CNP on cortisol and prolactin secretion are different from those of 150 µg CNP and 150 µg ANP in the same paradigm (10–12).

Cargill et al. (13) determined endogenous basal plasma CNP levels under resting conditions in the very low picogram range (0·9–1·0 pg/ml), which might be explained by the short half-life time of 2·6 min (8).

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**Table 1** The effect of infusions of placebo and two dosages of CNP on the release of cortisol, ACTH and prolactin following stimulation by 100 µg CRH. Values are means ± s.e.m.

<table>
<thead>
<tr>
<th></th>
<th>Placebo (1)</th>
<th>150 µg CNP (2)</th>
<th>300 µg CNP (3)</th>
<th>Tests with contrasts within ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AUC (arbitrary units)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortisol</td>
<td>520 ± 35</td>
<td>533 ± 35</td>
<td>651 ± 55</td>
<td>ns</td>
</tr>
<tr>
<td>ACTH</td>
<td>158 ± 16</td>
<td>151 ± 13</td>
<td>178 ± 8</td>
<td>ns</td>
</tr>
<tr>
<td>Prolactin</td>
<td>29 ± 3</td>
<td>26 ± 4</td>
<td>37 ± 5</td>
<td>ns</td>
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<tr>
<td><strong>Maximum increment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortisol (ng/ml)</td>
<td>101 ± 10</td>
<td>118 ± 11</td>
<td>142 ± 19</td>
<td>*</td>
</tr>
<tr>
<td>ACTH (pg/ml)</td>
<td>27 ± 4</td>
<td>26 ± 2</td>
<td>36 ± 2</td>
<td>ns</td>
</tr>
<tr>
<td>Prolactin (ng/ml)</td>
<td>2·8 ± 1·2</td>
<td>2·7 ± 1·3</td>
<td>4·6 ± 1·7</td>
<td>ns</td>
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<tr>
<td><strong>Maximum concentration</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Cortisol (ng/ml)</td>
<td>154 ± 9</td>
<td>160 ± 10</td>
<td>191 ± 19</td>
<td>ns</td>
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<tr>
<td>ACTH (pg/ml)</td>
<td>47 ± 6</td>
<td>47 ± 3</td>
<td>56 ± 2</td>
<td>ns</td>
</tr>
<tr>
<td>Prolactin (ng/ml)</td>
<td>9·3 ± 1·3</td>
<td>8·6 ± 1·6</td>
<td>12·4 ± 2·2</td>
<td>ns</td>
</tr>
</tbody>
</table>

*P < 0·05; ns, not significant.

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* AUC, 1930–2030 h.
CNP exerts stimulatory effects upon secretion of pituitary hormones

Concentrations of CNP in the portal venous blood of the pituitary have not yet been determined in man or in animals. Hence, it remains to be investigated whether CNP is secreted from the hypothalamus in similar amounts to ANP. Studies with intravenous administration of lower dosages in man have failed to show endocrine effects of CNP. In one study, CNP was given in the morning during high basal hypothalamic–pituitary–adrenocortical (HPA) system activity, which possibly masked any stimulatory effects. Hunt et al. (8) infused 11 ng/kg/min for 120 min and did not detect any effect on basal plasma ACTH, cortisol, vasopressin, or catecholamines. Barr et al. (14) also reported no endocrine effects of 30-min infusions of 10 and 50 ng CNP/kg/min, the latter dose being similar to the dose in our study. In these studies, CNP also failed to show significantly different responses to angiotensin II infusion.

In line with our investigation, a CNP stimulatory effect on the activity of the HPA system was reported in sheep: intraventricular infusion increased plasma ACTH and cortisol levels significantly at baseline and augmented the cortisol and vasopressin responses to acute moderate hemorrhage (7). However, in an earlier study with intracerebroventricular (i.c.v.) infusions of CNP, this group found suppressed cortisol levels after an abrupt initial surge that was possibly caused by blood pressure alterations (15). The intravenous administration of similar dosages of ANP and CNP in vivo in dogs revealed a greater arterial blood pressure decrease with CNP (16), even though only a modest specific vasodilatory action was shown in vitro (17). This finding was also confirmed by i.c.v. administration of CNP in sheep (15). Barr et al. (14) reported a significant reduction of systolic and diastolic blood pressure and a diminished cardiac output without increases in heart rate in man during a 50 ng/kg/min infusion of CNP for 30 min; 10 ng/kg/min CNP did not produce the same effect. Intra-arterial CNP infusion in man is less vasodilative than ANP infusion (18). In contrast, our study shows that neither dosage of CNP had any significant influence on blood pressure or heart rate, as had been observed previously with lower i.v. dosages of CNP in man (8, 9). Therefore, an indirect activation of the hormonal secretion via hemodynamic effects can be ruled out as an explanation for the increase in cortisol and prolactin.

As the secretion of cortisol was significantly stimulated in the absence of pronounced CNP effects on pituitary ACTH secretion in our study, either a direct CNP adrenocortical action, or an action via the splanchnic nerves and medullary–adrenocortical interactions (19) must be taken into account. An expression of NPR-B receptors has been reported not only in the hypothalamus and the pituitary, but also in the adrenals (20). Although in vitro information about the modulation of cortisol or corticosterone release by CNP in the adrenals is so far lacking, an inhibition of the ACTH-induced

Table 2 The effect of infusions of placebo and two dosages of CNP on cardiovascular parameters following 100 µg CRH. Values are means ± s.e.m.

<table>
<thead>
<tr>
<th></th>
<th>Placebo (1)</th>
<th>150 µg CNP (2)</th>
<th>300 µg CNP (3)</th>
<th>Tests with contrasts within ANOVA</th>
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<tr>
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<td></td>
<td></td>
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<td>1 vs 2</td>
</tr>
<tr>
<td><strong>AUC (arbitrary units)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate</td>
<td>265 ± 13</td>
<td>255 ± 11</td>
<td>265 ± 9</td>
<td>ns</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>449 ± 15</td>
<td>455 ± 9</td>
<td>448 ± 4</td>
<td>ns</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>269 ± 9</td>
<td>271 ± 10</td>
<td>259 ± 8</td>
<td>ns</td>
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<tr>
<td><strong>Maximum increment</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate (per min)</td>
<td>12 ± 3</td>
<td>9 ± 3</td>
<td>14 ± 4</td>
<td>ns</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>9 ± 3</td>
<td>4 ± 2</td>
<td>6 ± 2</td>
<td>ns</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>4 ± 1</td>
<td>4 ± 1</td>
<td>5 ± 2</td>
<td>ns</td>
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<td><strong>Maximum level</strong></td>
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<tr>
<td>Heart rate (per min)</td>
<td>78 ± 4</td>
<td>73 ± 4</td>
<td>80 ± 5</td>
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<td>Systolic BP (mm Hg)</td>
<td>122 ± 6</td>
<td>119 ± 3</td>
<td>120 ± 3</td>
<td>ns</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>72 ± 3</td>
<td>71 ± 2</td>
<td>69 ± 2</td>
<td>ns</td>
</tr>
</tbody>
</table>

AUC, 1930–2030 h.
BP, blood pressure; ns, not significant.

Figure 3 Effects of CNP infusions (hatched box) on the CRH-stimulated (arrow) release of prolactin in healthy human volunteers (means ± s.e.m.; n = 8). (△), Placebo; (●), 150 µg CNP; (■), 300 µg CNP.
release of aldosterone has been observed in bovine tissue (21). On the other hand, CNP has been reported to inhibit the release of vasopressin, a synergist of CRH at the corticotrope (22), pointing to a complex puzzle of different actions of CNP at different sites.

A stimulatory effect on prolactin secretion from CNP administered i.c.v. has also been reported in rats (6, 23). Application of cytotoxin cell targeting methodology for NPR-B and NPR-A receptors does not show a physiological role of CNP in the regulation of basal prolactin secretion. There is no evidence for a direct action of CNP on the tuberoinfundibular dopamine system, and distinct hypothalamic cell populations are necessary to transduce the stimulatory effects of CNP and the inhibitory effects of ANP (6). A direct action of CNP on the lactotrope was not seen in dispersed pituitary cells incubated under static or dynamic conditions (23). However, data about the penetration of CNP through the blood–brain barrier are still lacking. Prolactin stimulation is mainly under the control of the prolactin-inhibiting factor dopamine. Moreover, dopamine release from the tuberoinfundibular system (24) is regulated in a complex manner and involves a hypothalamic cascade of cholinergic, opioidergic and serotonergic neurons. Further investigations applying different antagonists are needed to clarify the mechanism of prolactin stimulation by CNP and to determine whether a prolactin-releasing factor may also be involved.

From our data, further experiments with higher dosages of CNP are desirable to clarify the dose-response of the endocrine actions of CNP. Due to the considerable expense of CNP for human experimentation, investigations with higher dosages are more feasible in animals. Support for these investigations comes indirectly from a preclinical study with AT20 pituitary cells, in which cGMP response curves to CNP failed to show any signs of saturation even at con-centrations up to 30 μmol/L, indicating a relatively low affinity of CNP for the NPR-B receptor (25). However, despite elevating cGMP levels, CNP had no effect on basal or CRH-stimulated ACTH release from the AT20 cells due to a suggested lesion at some point distal to the production of cGMP, possibly resulting from the absence of cGMP-dependent protein kinase (26). Preliminary investigations in AT20 cells in our laboratory point in the same direction (authors’ unpublished results). If regular pituitary cells show a similar affinity, then relatively high concentrations of CNP (at least 10−9 mol/L) must be released into the hypothalamic–pituitary portal circulation to elicit potential endocrine actions. However, CNP effects in the absence of detectable changes in cGMP concentrations have also been described (27). Although information about the physiological concentrations of CNP in the hypothalamic–pituitary portal blood is so far not available, the localization and concentration of hypothalamic CNP favors a possible modulatory role in neuroendocrine regulation (3). In addition, the role of vascular endothelial cells in peripheral CNP secretion remains to be discussed (28). In summary, the opposing effects of natriuretic peptides need further characterization in human and preclinical studies. Further clarification is particularly necessary for antagonists specific for the individual receptors.

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CNP exerts stimulatory effects upon secretion of pituitary hormones


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