Full title:

The interaction of Urocortin II and Urocortin III with amygdalar and hypothalamic cotricotropin-releasing factor (CRF) - reflections on the regulation of the hypothalamic-pituitary-adrenal (HPA) axis

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Short title:

The interaction of Urocortin II and Urocortin III with CRF

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Abstract

Urocortin II (Ucn III) and Urocortin III (Ucn III) are selective agonists of the CRF receptor type 2 (CRFR2). The aim of the present experiments was to investigate the effects of Ucn II and Ucn III on the central CRF and peripheral glucocorticoids in rats. Increasing doses (0.5-1-2-5 μg/2 μl) of Ucn II or Ucn III were administered intracerebroventricularly, then CRF concentration was determined by immunoassays in two different brain regions, the amygdala and the hypothalamus, and in two different time paradigms, 5 minutes and 30 minutes after the administration of peptides. In parallel with the second determination, plasma corticosterone concentration was measured by chemofluorescent assay. The amygdalar CRF amount was increased significantly by 0.5 and 5 μg of UCN II and 2 and 5 μg of UCN III in the 5 minutes experiments and by 5 μg of UCN II and 0.5 and 5 μg of UCN III in the 30 minutes experiments. The hypothalamic CRF content was not affected considerably in the 5 minutes paradigm, but it was influenced significantly in the 30 minutes paradigm, with 0.5 and 1 μg of UCN II and 0.5 to 2 μg of UCN III decreasing, and 2 and 5 μg of UCN II and 5 μg of UCN III increasing the hormone concentration, respectively. The plasma corticosterone concentration was decreased by 1 and 2 μg of UCN II and UCN III and increased by 0.5 and 5 μg of UCN III. The present results demonstrate that central administration of Ucn II and Ucn III modulate time-dependently and dose-dependently the amygdalar and the hypothalamic CRF concentration, and, directly or indirectly, the plasma corticosterone concentration. The present experiments suggest that the role of CRFR2 in the regulation of the HPA axis can be inhibitory or stimulatory, depending on the actual concentration of their agonists.

Introduction

The corticotropin-releasing factor (CRF) is a hypothalamic neurohormone and an extrahypothalamic neurotransmitter, which regulates the neuroendocrine, autonomic and behavioral responses to stressors through two distinct types of CRF receptors: CRFR1 and CRFR2 (Chang et al., 1993; Vale et al., 1981). Activation of CRFR1 was shown to induce stress reactions, while, until recently, activation of CRFR2 was thought to reduce stress responsiveness (Bale and Vale, 2004; Reul and Holsboer, 2002). The urocortins are members of the same CRF family of peptides, with similar chemical structure, but different pharmacological profile (Fekete and Zorrilla, 2007; Suda et al., 2004). Urocortin I (Ucn I) has equal affinity for both CRFRs (Vaughan et al., 1995); Urocortin II (Ucn II) and Urocortin III

(Ucn III) have much higher affinity for CRFR2, than CRFR1 (Lewis et al., 2001; Reyes et al., 2001). Therefore, these two peptides are considered selective agonists for CRFR2.

CRF is the principal activator of the hypothalamic-pituitary adrenal (HPA) axis (Carrasco and Van de Kar, 2003; Tsigos and Chrousos, 2002). Various stressors activate different sources of CRF, such as the central nucleus of the amygdala (CeA) and the paraventricular nucleus of the hypothalamus (PvN). CRF, in synergy with arginine-vasopressin (AVP), stimulates the secretion of the adrenocorticotrophic hormone (ACTH) from the anterior pituitary, which, in turn, stimulates the production of the glucocorticoids in the adrenal cortex. Therefore, the increase glucocorticoid concentration in the plasma reflects the activation of the HPA axis, but it also exerts a negative feedback effect on the hypothalamic CRF and a positive feedback effect on the amygdalar CRF production. Nevertheless, amygdalar CRF may stimulate the hypothalamic CRF release through a feed-forward effect; this represents the limbic activation of the HPA axis (Herman et al., 2005; Jankord and Herman, 2008).

Previous studies demonstrated that CRF and UCN I stimulate the HPA axis in rodents by activating CRFR1, which are expressed abundantly in the central nervous system (CNS), especially in the cerebral cortex and the anterior pituitary (Skelton et al., 2000; Turnbull and Rivier, 1997; Vale et al., 1981; Vaughan et al., 1995). Other studies suggested that activation of CRFR2 inhibits the HPA axis, CRFR2 being distributed predominantly in the periphery and limited centrally in the amygdalar and the hypothalamic nuclei (Valdez et al., 2002; Valdez et al., 2003). However, recently it was shown that administration of UCN II and UCN III, that is the activation of CRFR2 stimulates the HPA axis in rats (Jamieson et al., 2006; Maruyama et al., 2007). Moreover, it seems that the participation of CRFR2 in the regulation of the HPA axis is stressor-specific (physical vs. psychological) and species-specific (mice vs. rats).

The aim of the present experiments was to investigate the effects of Ucn II and Ucn III on the central CRF and peripheral glucocorticoids in rats. UCN II or UCN III was administered into the lateral cerebral ventricle of the rat and CRF concentration was measured in two different brain regions: the amygdala and the hypothalamus. The hormone concentrations were determined by immunoassays in two different time paradigms: 5 minutes and 30 minutes after the intracerebroventricular (icv) administration of the peptides. In parallel with the second determination, the plasma corticosterone concentration was determined by a chemofluorescent assay.

Materials and methods

Animals

Male Wistar rats (N = 106) weighing 150-200 g were used. During the experiments they were kept and handled in accordance with the instructions of the University of Szeged Ethical Comittee for the Protection of Animals in Research. The animals were housed in their home cages at constant room temperature (23 °C) on a standard illumination schedule, with 12-h light and 12-h dark periods (lights on from 6:00 a.m.). Commercial food and tap water were available ad libitum. The animals were allowed to acclimatize for at least 7 days before surgery, and they were handled daily to minimize the effects of nonspecific stress.

In vivo cannulation

Under intraperitoneal anesthesia with Phenobarbital 35 mg/kg (Nembutal, CEVA-Phylaxia, Hungary), the rats were implanted with a stainless steel Luer cannula (10 mm long) aimed at the right lateral cerebral ventricle. The cannula was secured to the skull with dental cement and acrylate. The lateral cerebral ventricle was targeted according to the Stereotaxic Atlas of the Rat Brain (Pellegrino et al., 1979): 0.2 mm posterior to the bregma, 1.7 mm lateral to the bregma and 3.7 mm deep from the dural surface. The animals were allowed at least 5 days to recover after the surgery and the permeability of the cannula was tested with methylene blue after the experiments.

Treatments

The animals (N=60) were treated through the cannula implanted into the right lateral cerebral ventricle. Saline solution (2 μl NaCl 0.9 %, Biogal, Hungary) was administered for the control groups and increasing doses (0.5, 1, 2 and 5 μg/2 μl) of UCN II (Bachem Ltd, Switzerland) or UCN III (Bachem Ltd, Switzerland) were injected for the peptide-treated groups. The saline solution and the UCNs were administered between 8:00-10:00 a.m. to avoid circadian changes of the central and peripheral hormones of the HPA axis. Two sets of experiments were performed. In the first one the animals were decapitated 5 minutes after treatment, their brains were removed and their amygdalae and their hypothalami were isolated and homogenised for determination of CRF concentration. In the second set, the animals were sacrificed 30 minutes after treatment, their brains were processed likewise and their trunk blood was collected for plasma corticosterone determination.

In vitro homogenisation

Brain tissues were dissected in a Petri dish filled with ice-cold Krebs solution (composition: 113 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, 11.5 mM glucose, 1.2 mM KH_2PO_4, 2.5 mM $CaCl_2$, pH 7.4, Reanal, Hungary). Both the amygdalae and the hypothalami were isolated according to the Stereotaxic Atlas of the Rat Brain (Pellegrino et al., 1979), with the bregma as point of reference. The coordinates were as follows: rostrocaudal 0 - -2 mm, mediolateral +3 - +6 mm, and dorsoventral +7 - +10 mm for the amygdala; rostrocaudal +2.6 - -2.6 mm, mediolateral +1.5 - -1.5 mm, and dorsoventral +7 - +10 mm for the hypothalamus. The brain tissues were homogenised following the instructions of Suda and Joanny (Joanny et al., 1989; Suda et al., 1985).

CRF determination

For determination of the amygdalar and hypothalamic CRF contents, we used the mouse/rat CRF high-sensitivity Enzyme-Linked Immuno-Sorbent Assay (ELISA) kit provided by CosmoBio Company, Ltd., Japan. This kit is based on a sandwich ELISA and shows no cross-reactivity to UCNs (mouse/rat or human).

Corticosterone determination

The plasma corticosterone was extracted and detected by the chemofluorescent assay described by Zenker and Bernstein, as modified by Purves and Sirett (Purves and Sirett, 1965; Zenker and Bernstein, 1958).

Statistics

Statistical analysis of the results and data reduction were performed by analysis of variance (ANOVA, SigmaPlot Software). The differences between groups were tested by one-way ANOVA followed by Holm-Sidak post hoc comparison test. A probability level of 0.05 or less was accepted as indicating a statistically significant difference.

Results

UCN II produced significant augmentations of the amygdalar CRF amount in the 5 minutes and in the 30 minutes experiments (Figure 1). 0.5 and 5 μg of UCN II were the most effective doses in the first set of experiments [$F(4,30) = 14.118$, $p<0.001$], but only the effect of the highest dose was effective in the second set of experiments $F(4,30) = 16,112$, $p<0.001$].

UCN II did not influence the hypothalamic CRF content in the 5 minutes experiments. In contrast, it induced dose-dependent changes of the hypothalamic CRF content in the 30 minutes experiments. 0.5-2 μg of UCN II decreased, while 5 μg of UCN II increased the hormone concentration, respectively [$F(4,30) = 40.200$, $p<0.001$] (Figure 2).

The plasma corticosterone concentration was also changed dose-dependently, with the 1 and 2 μg of UCN II decreasing it significantly [$F(4,30) = 14.118$, $p<0.001$], at 30 minutes following the administration of the peptide (Figure 3).

UCN III induced significant augmentation of the amygdalar CRF amount in both the 5-minutes and the 30 minutes paradigm [$F(4,30) = 20.459$, $p<0.001$]. In the first set of experiments 2 and 5 μg, in the second set of experiments 0.5 and 5 μg of the peptide produced significant effects [$F(4,30) = 25,825$, $p<0.001$] (Figure 4).

UCN III did not induce any changes in the hypothalamic CRF content in the 5 minutes paradigm. Although UCN III influenced dose-dependently the hypothalamic CRF content in the 30 minutes paradigm: low peptide doses (0.5 and 1 μg) decreased, high peptide doses increased the hormone concentration significantly [$F(4,30) = 158.254$, $p<0.001$] (Figure 5).

The plasma corticosterone concentration was influenced in a dose-dependent manner also. It was increased significantly by 0.5 and 5 μg of UCN III and decreased significantly by 1 and 2 μg of UCN III [$F(4,30) = 21,788$, $p<0.001$], respectively, both determined 30 minutes following the administration of the peptide (Figure 6).

Discussion

The present study demonstrates that central Ucn II and Ucn III interacts with the amygdalar and the hypothalamic CRF production in a time-dependent manner. Our results coincides with previous reports, according to which UCN II and UCN III induces changes in CRF and/or AVP hnRNA and/or mRNA expression (Jamieson et al., 2006; Maruyama et al., 2007). One study showed that icv administration of similar doses of UCN II and UCN III increased the expression of the hypothalamic CRF hnRNA and AVP hnRNA after 30 minutes in the PvN of Wistar rats (Maruyama et al., 2007). Another study proved that icv administration of both low and high doses of UCN III induced hypothalamic expression of AVP mRNA, but not CRF mRNA in Sprague-Dawley rats after 4 hours, though it did not exclude the possibility of interaction of UCN III with either CRF or AVP earlier than 4 hours (Jamieson et al., 2006). Based on the present results, we presume that central activation of CRFR2 in the amygdala inhibits the CRF release (from the 5 minutes experiments), then

further inhibits the CRF release or stimulates the amygdalar CRF synthesis (from the 30 minutes experiments), at least, in the presence of the high concentrations of their agonists. Central administration of CRFR2 agonists first does not influence the hypothalamic CRF release (in the 5 minutes experiments), then it stimulates the hypothalamic CRF synthesis or inhibits the hypothalamic CRF release (in the 30 minutes experiments), in most of the doses of UCNs used, except the highest dose of UCN II. We suppose that the increase of the hypothalamic CRF concentration in this case may be due to the feed-forward effect achieved by the increased amygdalar CRF secretion on the paraventricular CRF production.

The present study also demonstrates that the CRFR2 agonists acts on the adrenal glucocorticoid secretion in a dose-dependent manner. The results of the present study may seem confusing, as UCN II and UCN III have different, even opposite impacts on the plasma corticosterone concentration. Actually, previous studies also reflect this dichotomy. On one hand, experiments with mice suggested that activation of CRFR2 stimulates the HPA axis and mediates anxiolitic and antidepressive effects (Bale et al., 2000; Kishimoto et al., 2000). On the other hand, experiments with rats demonstrated that administration of CRFR2 agonists activates the HPA axis and promotes anxiogenic and depressive behavior (Jamieson et al., 2006; Maruyama et al., 2007). Moreover, a third group of experiments resulted in lack of neuroendocrine (Pelleymounter et al., 2004) or behavioral responses (Zhao et al., 2007) following central administration of UCN II and UCN III in rodents. However, the contradictions raised can be explained by the dose-dependent actions of Ucn II and Ucn III. We observed that intermediate doses of CRFR2 agonists decrease and lower or higher doses of CRFR2 agonists increase or do not change the plasma corticosterone concentrations. We propose that these dose-dependent changes are modulated by the time-dependent changes of the amygdalar and hypothalamic CRF synthesis/release. The only exception to this premise is represented by the lowest dose of UCN III, the effect of which can be explained by the participation of another ACTH secretagogue, such as paraventricular AVP (Jamieson et al., 2006; Maruyama et al., 2007). The increased corticosterone concentration in this case is associated with an increased amygdalar CRF and a decreased hypothalamic CRF concentration, which may be caused by the positive and negative feedback effects exerted by plasma glucocorticoids on the amygdalar and paraventricular CRF production, respectively. (Fekete and Zorrilla, 2007; Suda et al., 2004).

In our previous experiments, increased brain CRF (2-4 ng/ml) and plasma corticosterone concentration (25-45 μg/100 ml) have been measured in rats exposed to non-specific stress. Icv administration of UCN II and UCN III induced a similarly significant

response. However, different stressors may activate different brain regions (the basolateral nucleus of the amygdala or the PvN of the hypothalamus) (Sajdyk et al., 2004; Skorzewska et al., 2011), resulting in the elevation of plasma corticosterone concentration, which, in turn, can influence bidirectionally the amygdalar CRF and hypothalamic CRF concentration (Herman et al., 2005; Jankord and Herman, 2008). Moreover, these brain regions may present distinct CRFR distributions and reciprocal afferentations. Therefore, the participation of CRFR1 or CRFR2 in the regulation of the HPA axis is not a matter of simple dualism, but varies according to the specific brain regions and/or neuron subpopulations involved in the stress response (Janssen and Kozicz, 2013).

Taking the previous and the present results into consideration, we conclude that central administration of Ucn II and Ucn III modulate time-dependently and dose-dependently the amygdalar and the hypothalamic CRF concentration, and, directly or indirectly, the plasma corticosterone concentration. Consequently, the role of CRFR2 in the regulation of the HPA axis can be inhibitory or stimulatory, depending on the actual concentration of their agonists.

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Figure(s)

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Figure 1

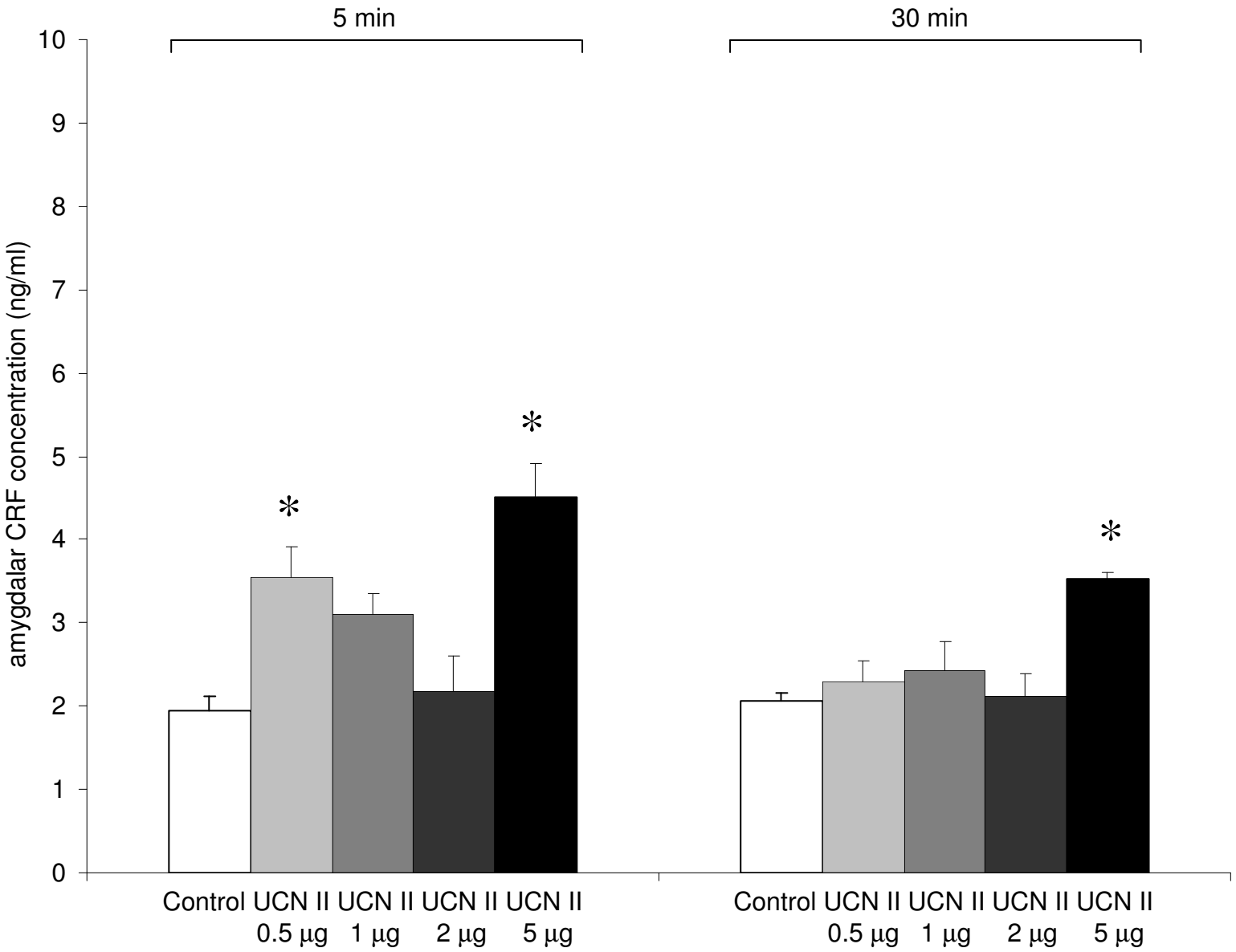

Figure 2

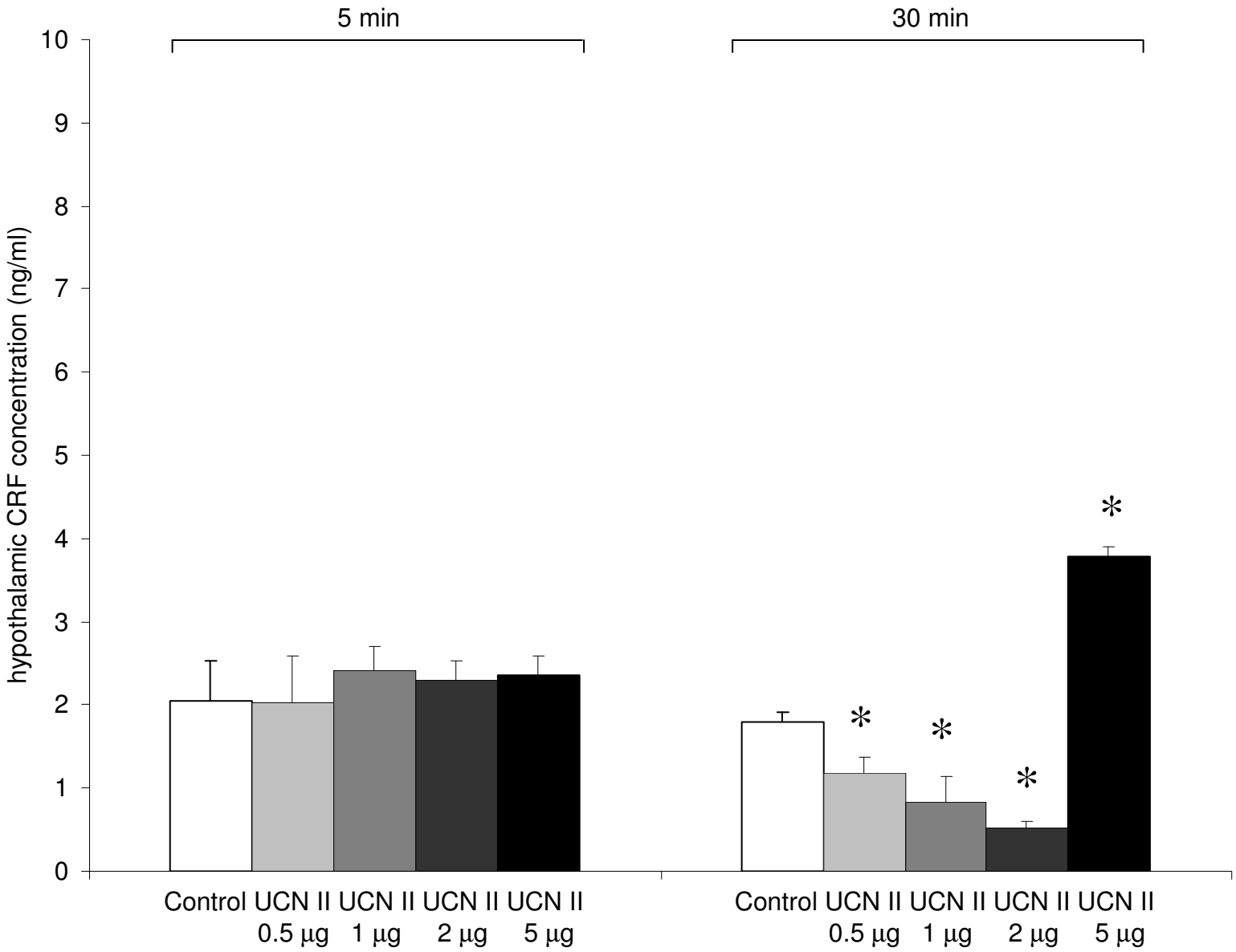
5 min
30 min
hypothalamic CRF concentration (ng/ml)
0
1
2
3
4
5
6
7
8
9
10
Control
UCN II 0.5 µg
UCN II 1 µg
UCN II 2 µg
UCN II 5 µg
Control
UCN II 0.5 µg
UCN II 1 µg
UCN II 2 µg
UCN II 5 µg
*
*
*
*

Figure 3

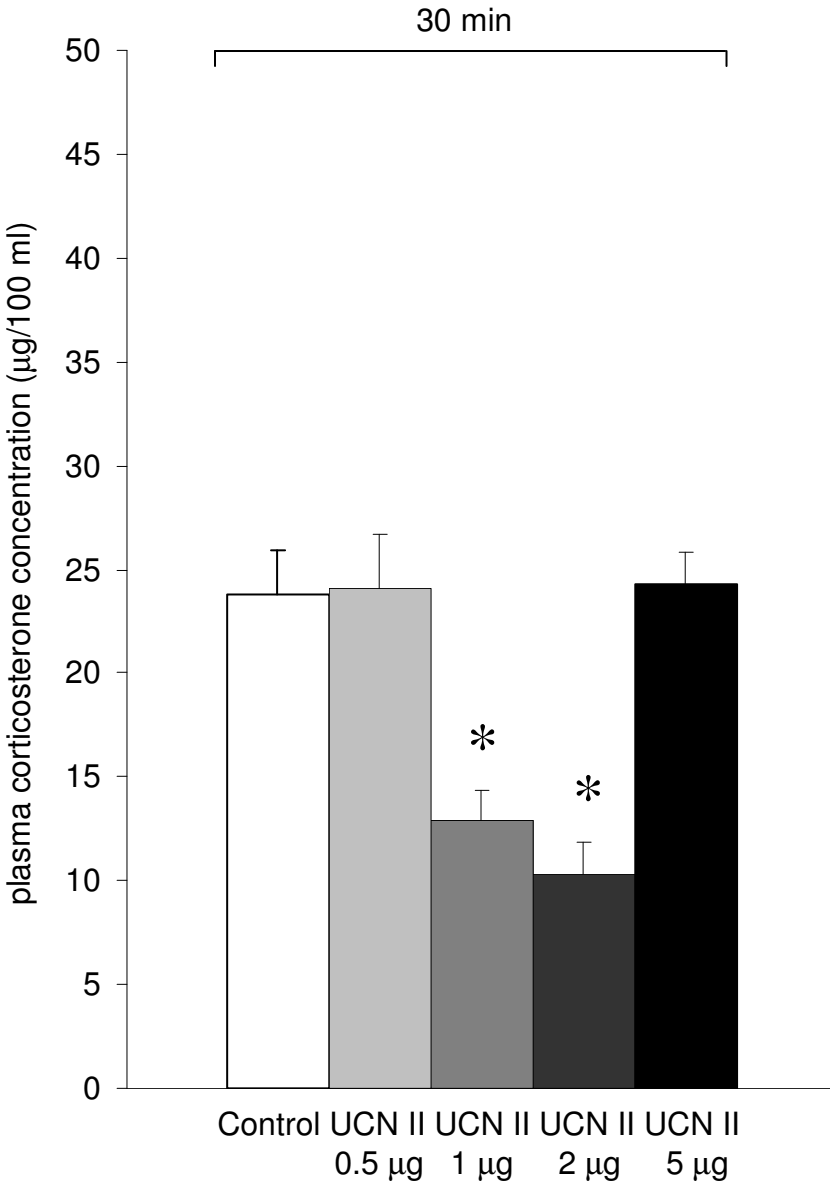
30 min
plasma corticosterone concentration (µg/100 ml)
50
45
40
35
30
25
20
15
10
5
0
*
*
Control
UCN II 0.5 µg
UCN II 1 µg
UCN II 2 µg
UCN II 5 µg

Figure 4

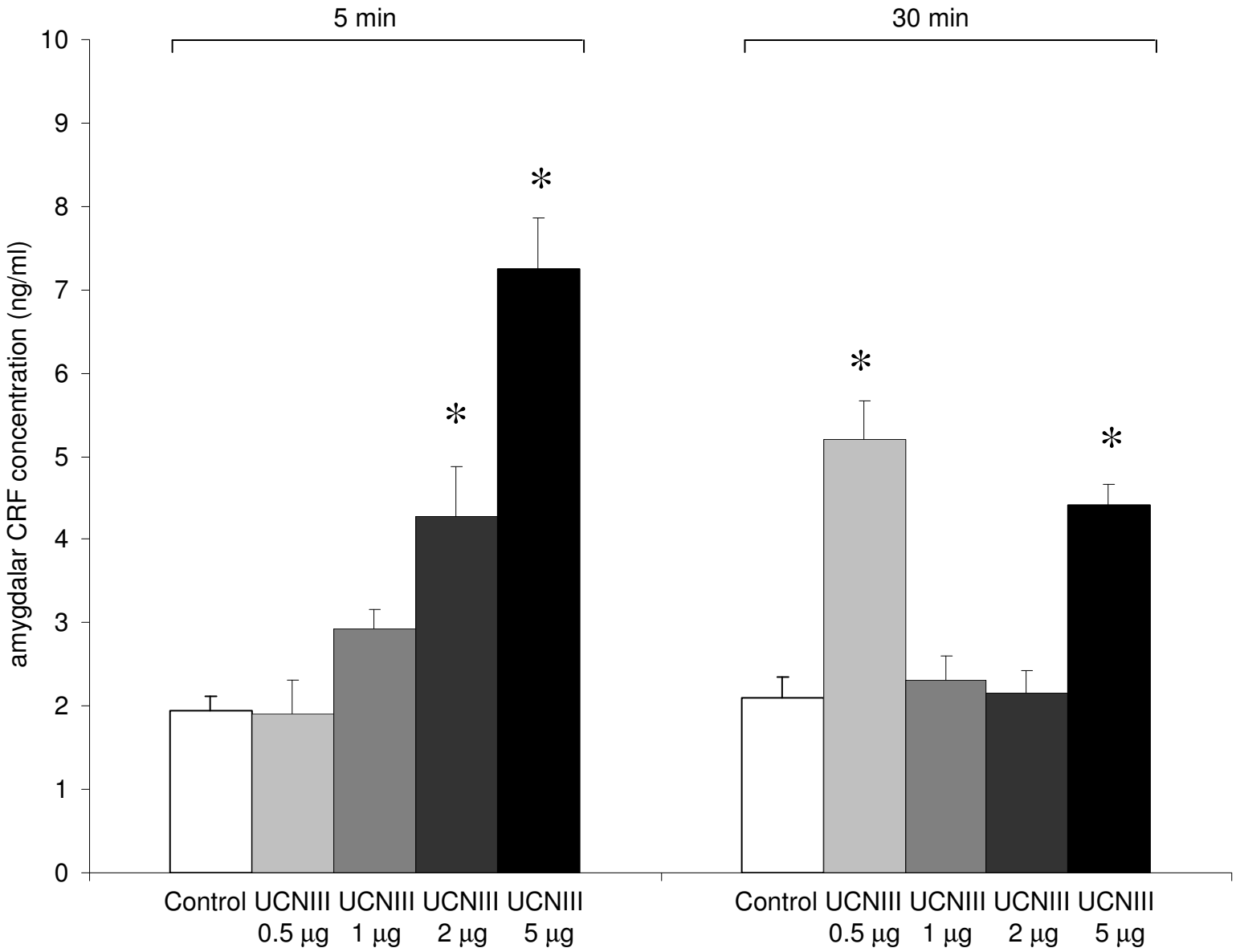
5 min
30 min
amygdalar CRF concentration (ng/ml)
0
1
2
3
4
5
6
7
8
9
10
*
*
*
*
Control
UCNIII 0.5 µg
UCNIII 1 µg
UCNIII 2 µg
UCNIII 5 µg
Control
UCNIII 0.5 µg
UCNIII 1 µg
UCNIII 2 µg
UCNIII 5 µg

Figure 5

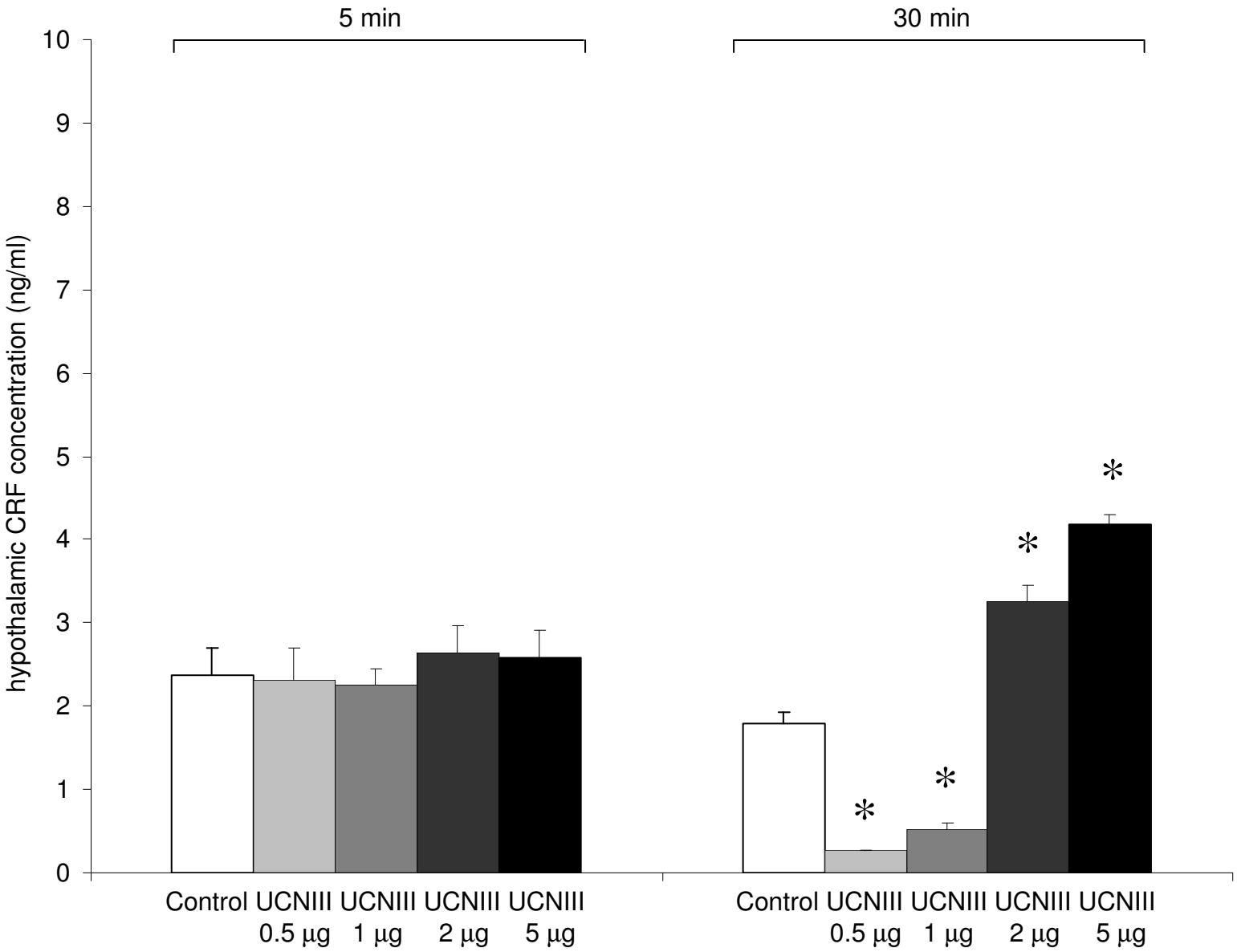
5 min
30 min
hypothalamic CRF concentration (ng/ml)
0
1
2
3
4
5
6
7
8
9
10
Control
UCNIII 0.5 µg
UCNIII 1 µg
UCNIII 2 µg
UCNIII 5 µg
Control
UCNIII 0.5 µg
UCNIII 1 µg
UCNIII 2 µg
UCNIII 5 µg
*
*
*
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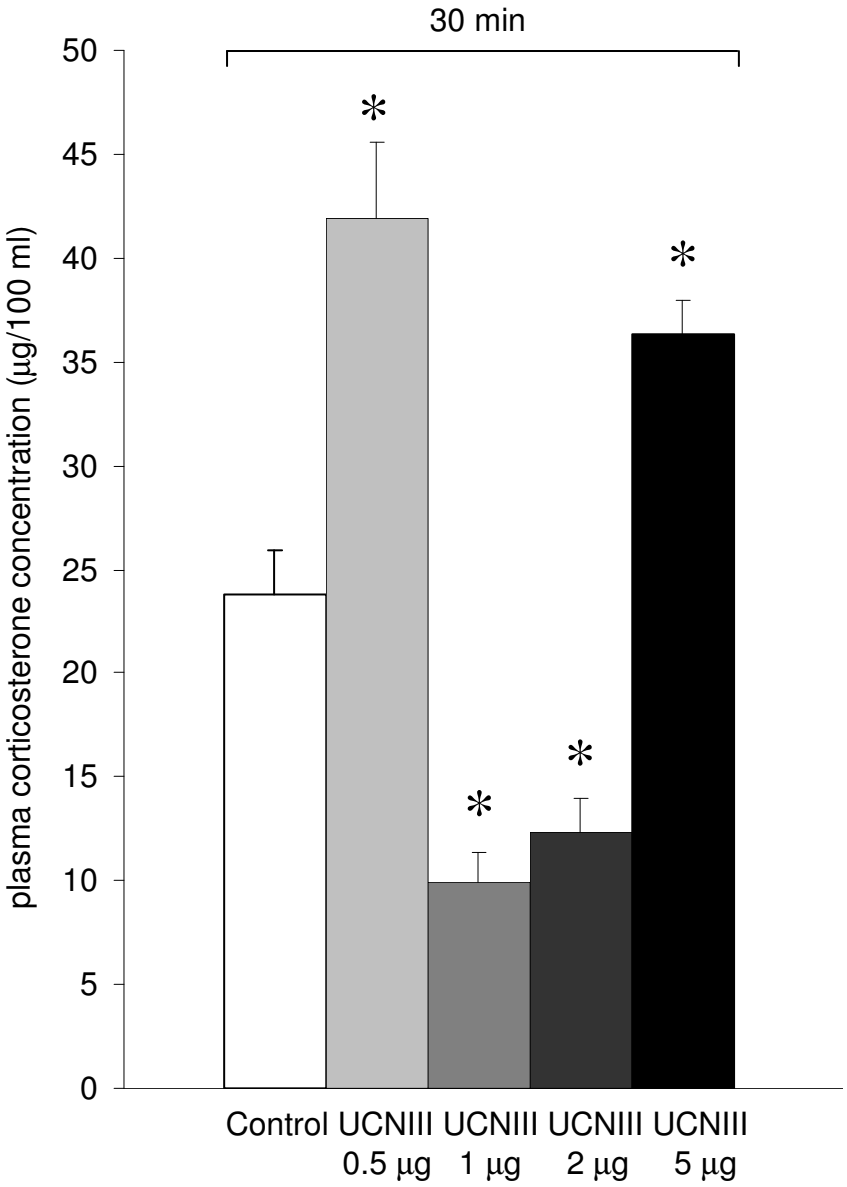
Figure 6


30 min
plasma corticosterone concentration (µg/100 ml)
50
45
40
35
30
25
20
15
10
5
0
*
*
*
*
Control
UCNIII 0.5 µg
UCNIII 1 µg
UCNIII 2 µg
UCNIII 5 µg

Legends for figures

Figure 1. The effects of UCN II on the amygdalar CRF concentration determined 5 minutes and 30 minutes following the icv administration of the peptide. Values are presented as means ± SEM; * indicates a statistically significant difference ($p<0.05$).

Figure 2. The effects of UCN II on the hypothalamic CRF concentration determined 5 minutes and 30 minutes following the icv administration of the peptide. Values are presented as means ± SEM; * indicates a statistically significant difference ($p<0.05$).

Figure 3. The effects of UCN II on the plasma corticosterone concentration determined 30 minutes following the icv administration of the peptide. Values are presented as means ± SEM; * indicates a statistically significant difference ($p<0.05$).

Figure 4. The effects of UCN III on the amygdalar CRF concentration determined 5 minutes and 30 minutes following the icv administration of the peptide. Values are presented as means ± SEM; * indicates a statistically significant difference ($p<0.05$).

Figure 5. The effects of UCN III on the hypothalamic CRF concentration determined 5 minutes and 30 minutes following the icv administration of the peptide. Values are presented as means ± SEM; * indicates a statistically significant difference ($p<0.05$).

Figure 6. The effects of UCN III on the plasma corticosterone concentration determined 30 minutes following the icv administration of the peptide. Values are presented as means ± SEM; * indicates a statistically significant difference ($p<0.05$).