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The effects of CRF and urocortins on the hippocampal glutamate release

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ABSTRACT

Corticotropin-releasing factor (CRF) is a hypothalamic neurohormone and an extrahypothalamic neurotransmitter that regulates the hypothalamic-pituitary-adrenal (HPA) axis. The urocortins (UCN I, UCN II and UCN III) are CRF-related peptides, which may also regulate the HPA axis directly or indirectly, by modulation of extrahypothalamic neurotransmitters, such as amygdalar GABA and hippocampal glutamate.

Our previous *in vitro* superfusion studies have already demonstrated that CRF and UCN I stimulate the amygdalar GABA release in rats. The aim of the present study was to investigate the effects of CRF, UCN I, UCN II and UCN III on the glutamate release elicited electrically from rat hippocampal slices in similar *in vitro* conditions. In order to investigate the participation of CRF receptors (CRFR1 and CRFR2) in this process, hippocampal slices were pretreated with antalarmin, a selective antagonist of CRFR1 or astressin 2B, a selective antagonist of CRFR2.

CRF and UCN I at 100 nM decreased significantly the hippocampal glutamate release evoked by electrical stimulation. In contrast, 100 nM of UCN II and UCN III did not affect significantly the hippocampal glutamate release enhanced by electrical stimulation. The decreasing effects of CRF and UCN I were reversed by antalarmin, but not by astressin 2B, both being administered in equimolar doses.

Our results demonstrate that CRF and UCN I inhibit the glutamate release in the hippocampus *via* CRFR1 and that CRFR2 does not participate to this process. Based on the previous and the present results we conclude that CRFR1 agonists can activate the HPA axis not only directly, but also indirectly by increasing the amygdalar GABA release and decreasing the hippocampal glutamate release.

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1. Introduction

Corticotropin-releasing factor (CRF) is a hypothalamic neurohormone and an extrahypothalamic neurotransmitter that mediates the endocrine, autonomic and behavioral responses to stress (Vale et al., 1981). As a hypothalamic neurohormone, CRF activates the hypothalamic-pituitary-adrenal (HPA) axis (Carrasco and Van de Kar, 2003). CRF is secreted from the paraventricular nucleus (PVN) of the hypothalamus and released into circulation at the level of median eminence; reaching the anterior pituitary it stimulates the secretion of adrenocorticotrop hormone (ACTH), which on its turn stimulates the production of glucocorticoids in the adrenal cortex (Tsigos and Chrousos, 2002). The increase of plasma glucocorticoid concentration not only reflects the activation of the HPA axis, but it exerts negative feedback effects on the hypothalamus, the anterior pituitary and the hippocampus and positive feedback effect on the amygdala (Herman and Cullinan, 1997).

As an extrahypothalamic neurotransmitter, CRF may also modulate the HPA axis (Herman and Cullinan, 1997). CRF is also synthesized in the central nucleus of the amygdala (CeA) found in the vicinity of the medial nucleus of the amygdala (MeA) and the ventral subiculum (vSub) region of the hippocampus (Reul and Holsboer, 2002). Neurons from these regions (especially Mea and vSub) send GABAergic or glutamatergic projections to the GABAergic neurons of the bed nucleus of the stria terminalis (BNST) and the peri-paraventricular nuclei (peri-PVN), which exert a tonic GABAergic inhibition upon the paraventricular CRF synthesis/release (Bale and Vale, 2004; Cullinan et al., 2008). Thus, the

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amygdala through GABAergic–GABAergic disinhibition increases, whereas the hippocampus through glutamatergic-GABAergic inhibition decreases the activity of the HPA axis, respectively (Herman et al., 2003, 2004).

CRF acts through two distinct receptors, CRFR1 and CRFR2, which seem to have antagonistic effects in the brain (Chang et al., 1993; Van Pett et al., 2000). Activation of CRFR1 induces HPA axis activation, anxiety and depression, while activation of CRFR2 produces anxiolytic and antidepressive effects (Bale and Vale, 2004; Reul and Holsboer, 2002). However, our recent studies 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, the urocortins (Bagosi et al., 2013, 2014). The urocortins (UCN I, UCN II and UCN III) are CRF-related peptides, with similar chemical structure, but different pharmacological profile, than that of CRF (Fekete and Zorrilla, 2007; Suda et al., 2004). In contrast with CRF, which binds preferentially to CRFR1, UCN I has equal affinity for both CRF receptors (Vaughan et al., 1995). UCN II and UCN III bind selectively to CRFR2 (Lewis et al., 2001; Reyes et al., 2001).

Our previous *in vitro* superfusion experiments have demonstrated that CRF and UCN I stimulate the amygdalar GABA release in rats and that this stimulatory effect is mediated *via* CRFR1, and not CRFR2 (Bagosi et al., 2008). The aim of the present experiments was to investigate the effects of CRF and urocortins on the hippocampal glutamate release in rats in similar *in vitro* conditions.

2. Materials and methods

Male Wistar rats (Animal Husbandry Services, Domaszék, Hungary) weighing 150–250 g were used. During the experiments they were kept and handled in accordance with the instructions of the University of Szeged Ethical Committee for the Protection of Animals in Research which are concordant with the European Communities Council Directive of 24 November 1986 (86/609/EEC). The rats were decapitated and their brains were rapidly removed. All efforts were made to minimize animal suffering and to reduce the number of animals used (N = 2 for each experiment). The hippocampus was isolated and 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) according to the Stereotaxic Atlas Of The Rat Brain (Pellegrino et al., 1979). The extracted hippocampus was cut with a McIlwain tissue chopper and slices of 300 μM were produced.

The hippocampal slices were investigated with *in vitro* superfusion method described originally by Gaddum (Gaddum, 1953). The slices were incubated for 30 min in 8 ml of Krebs solution, submerged in a water bath at 37 °C and gassed through a single-use needle with a mixture of 5% CO_2 and 95% O_2. During the incubation [^{3}H]glutamate (Biotrend Ltd., Germany) at 20 μM with a specific activity of 40 Ci/mmol was injected into the incubation medium. Two tritiated slices were transferred to each of the four cylindrical perspex chambers of the superfusion system (Experimetria Ltd, Hungary). A multichannel peristaltic pump (Gilson Minipuls 2, USA) was used to maintain a constant superfusion rate of 200 μl/min. The slices were superfused for 30 min to allow tissue equilibrium. In order to determine the effects of different CRFR agonists on the hippocampal glutamate release equimolar doses (100 nM) of CRF, UCN I, UCN II or UCN III (Bachem Ltd., Germany) were added to the perfusion medium, 20 min after the superfusion had started. In order to determine the participation of CRF receptors in this process 100 nM of selective CRFR1 antagonist antalarmin or 100 nM of selective CRFR2 antagonist astressin-2B (Sigma–Aldrich Inc., USA) were preadded to the perfusion medium, 10 min after the superfusion had started. The concentrations of the non-selective agonists were selected based on preliminary experiments in which 100 nM of CRF and 100 nM UCN I proved the most effective in stimulating the hippocampal glutamate release. Using equimolar concentrations of the selective agonists/antagonists was inspired by our previous studies investigating the amygdalar and the hypothalamic GABA release (Bagosi et al., 2008, 2012).

The superfusates were collected in Eppendorf tubes by a multichannel fraction collector (Gilson FC 203B, UK) every 2 min. After the first 2 min electrical stimulation consisting of square-wave impulses (total duration: 2 min, voltage: 100 V, pulse length: 5 ms, frequency: 10 Hz) was delivered to each of the four chambers, as gold electrodes were previously attached to both halves of the superfusion chambers and connected to an ST-02 electrical stimulator (Experimetria Ltd., Hungary). The fraction collecting lasted 32 min, thus 16 fractions were obtained.

After the fraction collecting had finished, 3 ml of scintillation fluid (Ultima Gold, Perkin–Elmer Inc., USA) were added to each fraction and the remnants of the superfused brain slices, which were previously solubilized in 200 ml of Krebs solution using an ultrasonic homogenizer (Branson Sonifier 250, USA). The radioactivity in the fractions and the homogenized tissue samples was measured with a liquid scintillation spectrometer (Tri-carb 2100 TR, Packard Inc., USA). The fractional release was calculated as a percentage of the radioactivity present in the collected sample compared to the total radioactivity of the corresponding tissue.

The fractional release of glutamate was calculated by the area-under-the-curve (AUC) method and statistical analysis of the results was performed by analysis of variance (SigmaPlot v11.0, Systat Software Inc., USA). Differences between two areas were determined by Student's t test or two-way ANOVA followed by Tukey post-hoc test and a probability level of 0.05 or less was accepted as indicating a statistically significant difference.

3. Results

CRF and UCN I (both of 100 nM concentration) decreased significantly the hippocampal [^{3}H]glutamate release elicited by electrical stimulation [$t(6, 11) = 2.816$; $p < 0.05$ for CRF *vs.* the control, and $t(6, 11) = 3.352$; $p < 0.05$ or UCN I *vs.* the control] (Figs. 1 and 2). In contrast, UCN II and UCN III (both of 100 nM concentration) did not affect significantly the hippocampal [^{3}H]

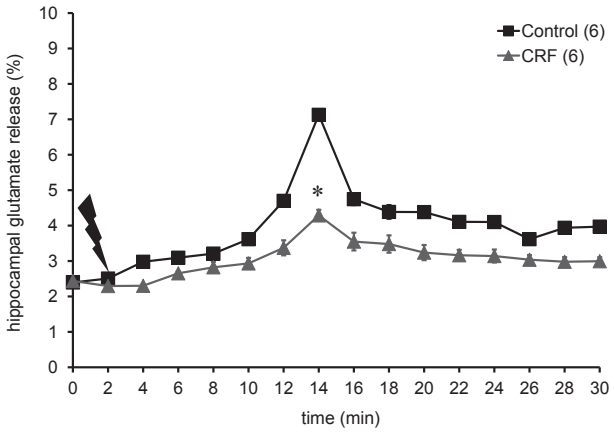

Fig. 1. The effects of CRF on the hippocampal glutamate release in rats. CRF (100 nM) decreased significantly the fractional [^{3}H]glutamate release from rat hippocampal slices following electrical stimulation. Values are presented as fractional release (%) ± SEM; the number of animals used was 2 for each experiment and the numbers of slices is indicated in brackets. A probability level of 0.05 or less was accepted as a statistically significant difference and indicated with * $p < 0.05$ for CRF *vs.* control.

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