CENTRAL CORTICOTROPIN–RELEASING FACTOR (CRF) MAY ATTENUATE SOMATIC PAIN SENSITIVITY THROUGH INVOLVEMENT OF GLUCOCORTICOIDS

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Corticotropin-releasing factor (CRF) is an important regulator of physiological functions and behavior in stress. Analgesia is one of the characteristics of stress reaction and CRF is involved in providing stress-induced analgesia, however, the underlying mechanisms remain to be determined. Exogenous CRF mimics stress effects on pain sensitivity and causes analgesic effect. The present study was performed to investigate the participation of endogenous glucocorticoids in analgesic effects induced by central administration of CRF in anesthetized rats. The participation of glucocorticoids was studied by pharmacological suppression of the hypothalamic-pituitary-adrenocortical (HPA) axis as well as an occupation of glucocorticoid receptors by its antagonist RU 38486. Since CRF administration causes the release of β-endorphin from the pituitary, the opioid antagonist naltrexone was used to determine the contribution of opioid-dependent mechanism to CRF-induced analgesia. An electrical current threshold test was applied for measurement of somatic pain sensitivity in anesthetized rats. Intracerebroventricular administration of CRF (2 µg/rat) caused analgesic effects (an increase of pain thresholds) and an increase in plasma corticosterone levels. Pretreatment with naltrexone did not change analgesic effects of central CRF as well as corticosterone levels in blood plasma. However, pharmacological suppression of the HPA axis leading to an inability of corticosterone release in response to CRF resulted in an elimination of CRF-induced analgesic effects. Pretreatment with RU 38486 also resulted in an elimination of CRF–induced effects. The data suggest that CRF-induced analgesic effects may be mediated by nonopioid mechanism associated with endogenous glucocorticoids released in response to central CRF administration.

Key words: analgesia, corticotropin-releasing factor, glucocorticoid receptors, glucocorticoids, opioid receptors, somatic pain sensitivity
previously studied the role of glucocorticoids in systemic CRF-induced analgesic effects on somatic pain sensitivity. It has been shown that systemic CRF-induced analgesia may be mediated by glucocorticoids (27). However it remains unclear whether central CRF-induced effects are also mediated via glucocorticoid-dependent mechanisms because different mechanisms may be involved in systemic and central CRF-induced effects on pain sensitivity. For example, it has been shown in hot plate testing that systemic CRF-induced analgesic effects are mediated by opioids in rats (14), while central CRF-induced effect are provided by nonopioid noradrenergic mechanisms (17, 19). Moreover, central CRF administration by itself has produced conflicting results about somatic pain sensitivity. Earlier studies failed to demonstrate that central CRF changes pain sensitivity (24, 28, 29). At the same time later findings demonstrated that central CRF may change somatic pain sensitivity (15, 17, 25, 30, 31).

The goal of the present study is to investigate the contribution of endogenous glucocorticoids to analgesic effects induced by central administration of CRF in anesthetized rats. The participation of endogenous glucocorticoids was studied by pharmacological suppression of the HPA axis as well as an occupation of glucocorticoid receptors by its antagonist RU 38486. Since CRF causes the release β-endorphin from the pituitary, the opioid antagonist naltrexone was used to separate the contribution of β-endorphin released in response to central CRF administration from the contribution of glucocorticoids.

MATERIAL AND METHODS

Animals

Adult male Sprague-Dawley rats (Stolbovoe, Moscow, Russia) weighing 220–300 g were used. Four animals per cage were acclimatized to standard laboratory conditions (12:12 h light-dark cycle, temperature 20±1°C, free access to food and water) for 7 days before use. Care and treatment of animals were according to European Communities Council guidelines in animal research (86/609/EEC) and the local animal care committee at the Pavlov Institute of Physiology RAS.

Drugs

The following drugs were used: rat/human CRF (Sigma, Saint Louis, USA), naltrexone hydrochloride (Dupont de Nemours, Saint Louis, USA), CRF, naltrexone hydrochloride were dissolved in 0.9% sterile saline immediately before administration. RU 38468 was dissolved in 1,2-propylene glycol (Vector, Sankt-Petersburg, Russia). Pentobarbital sodium (Nembutal) was used as solution (Phylaxia-Sanofi, Budapest, Hungary).

Nociceptive testing

Electrical current threshold test was used for measurement of somatic pain sensitivity (32-34). Pain threshold was the minimum current necessary to provoke tail withdrawal reaction. A rat was placed on a platform and the tip of the tail was lowered into a glass of saline. Electrical stimulation was delivered through a pair of wire electrodes: one of which was applied to the tail skin, the other one was placed into the glass of saline. Sinusoidal current (500 Hz) was increased gradually from 0.07 to 1 or 2 mA. Digital amperes meter was included to the united circuit to measure current intensity. When the tail was pulled out, the electrical stimulation was ceased and current intensity induced the tail withdrawal reaction was monitored.

To exclude the activation of the HPA axis induced by uncontrolled stress and decrease individual variability of pain thresholds we performed nociceptive testing on anaesthetized rats similarly as other researches (28, 35). Animals were anaesthetized by Nembutal (40 mg/kg in 2 ml/kg, i.p.) 20 min before recording of pain thresholds as described previously (32, 33). To avoid the possibility of tissue damage a cut off current (maximal possible pain threshold) of 1 mA was used for testing of pain sensitivity.

Corticotropin-releasing factor administration

CRF was administered in lateral ventricle at a dose of 2 µg/rat in 7 µl/rat immediately after measurement of baseline pain thresholds. Control animals were given a vehicle of CRF (saline). Nociceptive testing was performed at following time points after CRF or CRF vehicle administration: 3, 8, 15, 20 and 30 min. This testing schedule was based on our previews data (32, 33). Analgesia was defined as an increase of pain thresholds after CRF injection compared to baseline pain thresholds before CRF injection and to pain thresholds after CRF vehicle injection at appropriate time points.

Pretreatments to corticotropin-releasing factor administration

1. Occupation of opioid receptors by naltrexone

The nonselective antagonist of opioid receptor naltrexone was used for the occupation of opioid receptors (36). The dose of naltrexone and the time of its administration were determined according to the data of literature (14). Naltrexone was injected at a dose of 1 mg/kg in a volume of 2 ml/kg, i.p., and 15 min before the measurement of baseline pain threshold. Control animals were injected with saline instead of the antagonist. Immediately after measurement of baseline thresholds CRF or its vehicle were administered to rats with pretreatment of opioid antagonist or its vehicle.

2. Pharmacological suppression of the hypothalamic-pituitary-adrenocortical axis activity

The pharmacological suppression of the HPA axis activity was created by cortisol administration at a pharmacological dose of 300 mg/kg (i.p., 2.5% suspension, 12 ml/kg) one week before experiment (37). Control animals received a saline (12 ml/kg, i.p.), instead of cortisol. One week after cortisol or vehicle pretreatments rats were injected with CRF or its vehicle. It is important to note that CRF was injected when the exogenous hormones had already been eliminated but corticosterone response to CRF was still inhibited.

3. Occupation of glucocorticoid receptors by RU 38468

Glucocorticoid receptors were occupied by RU 38486. The dose of the antagonist and time of its administration were selected on the base of our previous studies (38, 39). RU 38468 was injected at a dose of 20 mg/kg in 5 ml/kg, s.c., 140 min before the measurement of baseline pain threshold (39). Control animals were injected with the antagonist vehicle 1,2-propylene glycol. Immediately after measurement of baseline thresholds CRF or its vehicle were administered to rats with the antagonist pretreatment and control animals.

Experimental protocol

Experimental design is described in Table 1. All rats were first implanted i.c.v. with cannula (day 1). Experiments were started...
when animals were recovered (day 8). Pain sensitivity was tested on day 8 (experiment 1, 2 and 4) and on day 15 (experiment 3). Pain thresholds were measured 2 min before (baseline pain thresholds) and 3, 8, 15, 20, 30 min after CRF or CRF vehicle (saline) injection (Table 1). After recording the pain thresholds at 30 min the rats were decapitated and sample of blood were collected at 31 min for assay of corticosterone (Table 1).

**Experiment 1. Effect of central corticotropin-releasing factor on tail withdrawal reaction induced by electrical current and on corticosterone blood levels**

Anesthetized rats (n=21) without pretreatment were tested for baseline pain thresholds. Then, all animals were randomly assigned to one of two groups injected by CRF or CRF vehicle (saline) and CRF (or saline)-induced pain thresholds were determined.

**Experiment 2. Effect of opioid receptor antagonist naltrexone on corticotropin-releasing factor-induced analgesia**

Anesthetized rats (n=28) were given either naltrexone (n=12) or its vehicle (n=16) and, then, they were tested for baseline pain thresholds. All rats pretreated with naltrexone (or its vehicle) were injected with CRF or saline and CRF (or saline) -induced pain thresholds were measured.

**Experiment 3. Effect of cortisol pretreatment on corticotropin-releasing factor-induced analgesia**

All rats (n=40) were assigned to one of two groups treated by cortisol (n=19) or vehicle (n=21). One week after cortisol or vehicle pretreatment (on day 15) all rats were anesthetized for baseline pain thresholds testing. Then, they were given either CRF or saline and CRF (or saline)-induced pain thresholds were measured.

**Experiment 4. Effect of glucocorticoid receptor antagonist RU 38486 on corticotropin-releasing factor-induced analgesia**

All rats (n=34) were randomly assigned to one of two groups treated by RU 38486 (n=16) or RU 38486 vehicle (n=18). Then, animals were anesthetized for testing of baseline pain thresholds. After pain testing the rats pretreated with RU 38486 or RU 38486 vehicle were injected by CRF or saline and CRF (or saline)-induced pain thresholds were determined.

**Surgery and intracerebroventricular injection**

Rats were anesthetized with nembutal (5 mg/kg in 2 ml/kg) and placed in a stereotaxic instrument. A cannula was implanted unilaterally at the left lateral ventricle according to the coordinates of the stereotaxic atlas (40): 0.8 mm posterior to the bregma and 1.5 mm lateral to the suture in the third cerebral ventricle for subsequent intracerebroventricular (i.c.v.) infusion of either CRF or saline. The cannula lowered to 4.0 mm below the surface of the skull. A screw was placed into the skull and the entire assembly was held in place with dental cement. Animals were tested 1 week after implantation. Cannula was attached to a Hamilton syringe via PE20 tubing. The infusions were made using the manually held Hamilton syringe. After the experiments cannula placement was confirmed by i.c.v. administration of dye.

**Collection of blood samples and estimation of blood corticosterone levels**

When the procedure of nociceptive testing was completed rats were immediately decapitated (at 31 min after CRF injection) and their trunk blood for measurement of corticosterone levels was collected. The blood samples for measurement of corticosterone levels were centrifuged at 4°C, and plasma was frozen for hormonal analysis. Corticosterone level of plasma was measured by microfluorometry (37).

**Data and statistical analysis**

Data were presented as absolute measurements of electrical thresholds (mA). Data was expressed as the mean ±SEM. We used the nonparametric Mann-Whitney test for comparing pain thresholds and Student t-test to analyze corticosterone levels. In each case, the required level for significance was considered to be *P*<0.05.

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<th>Table 1. Summary of experiments.</th>
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RESULTS

Effect of central corticotropin-releasing factor on tail withdrawal reaction induced by electrical current and on corticosterone blood levels

Baseline pain thresholds before injection of CRF or its vehicle were 0.46±0.04 mA (n=10) and 0.45±0.03 mA (n=11), respectively.

Central administration of CRF to rats without any preliminary pretreatment resulted in a profound increase in pain thresholds (Fig. 1A). The CRF-induced analgesic effect was seen for up to 30 min after CRF injection. At that time interval CRF-induced pain thresholds were significant higher compared to baseline pain thresholds and saline. Arrow (0 min) is the moment of CRF or saline injection.

Central administration of CRF to these animals caused a dramatical increase in the blood plasma corticosterone levels compared with the levels in control rats received CRF vehicle (Fig. 1B).

Fig. 1. Effect of central administration of CRF on tail withdrawal reaction induced by electrical current (A) and corticosterone level in blood plasma (B) in rats without any pretreatment. Data are presented as the mean ±S.E.M. Significant difference at p<0.05; * - from baseline pain thresholds and saline. Arrow (0 min) is the moment of CRF or saline injection.

Effect of opioid receptor antagonist naltrexone on corticotropin-releasing factor-induced analgesia

Naltrexone did not change neither baseline pain sensitivity nor CRF-induced analgesic effect (Fig. 2A). There were no significant differences between the baseline as well as CRF-induced pain thresholds in rats pretreated with naltrexone and the control animals (Fig. 2A). Baseline pain thresholds in rats pretreated with naltrexone or vehicle were 0.44±0.06 mA (n=12) and 0.47±0.05 mA (n=16), respectively.

Pretreatment with the opiate antagonist naltrexone had no effect on the CRF-induced corticosterone levels (Fig. 2B).

Fig. 2. Effect of opioid antagonist naltrexone on CRF-induced analgesia (A) and corticosterone level in blood plasma (B). Data are presented as the mean ±S.E.M. Significant difference at p<0.05; * - from baseline pain thresholds and saline groups (naltrexone+saline and vehicle+saline). Arrow (0 min) is the moment of CRF or saline injection.

Effect of cortisol pretreatment on corticotropin-releasing factor-induced analgesia

Cortisol pretreatment did not change the baseline pain threshold. There were no significant differences between the baseline pain thresholds in rats with the cortisol pretreatment (0.40±0.02 mA, n=19) and control rats with saline pretreatment (0.46±0.02 mA, n=21).

Central CRF administration to the control rats resulted in an increase of pain thresholds compared to baseline threshold as well as to pain thresholds observed after CRF vehicle injection.
The present data indicate that analgesic effects of central CRF on somatic pain sensitivity are abolished by pharmacological suppression of the HPA axis or RU 38486, but not by naltrexone. The data suggest that CRF-induced analgesic effects may be mediated by nonopioid mechanism associated with endogenous glucocorticoids released in response to central CRF administration.

Central CRF administration has produced conflicting results on somatic pain sensitivity (13). The results of the present study are in a good agreement with data demonstrating analgesic effect of central CRF (17, 25). Accordingly our results the opioid peptides do not participate in analgesia induced by central CRF in anesthetized rats. The nonopioid mechanism of central CRF-induced analgesic effects on somatic pain sensitivity were also supported by other studies performed in both anesthetized (24) and unanesthetized (17) rats.

DISCUSSION

The present data indicate that analgesic effects of central CRF on somatic pain sensitivity are abolished by pharmacological suppression of the HPA axis or RU 38486, but not by naltrexone. The data suggest that CRF-induced analgesic effects may be mediated by nonopioid mechanism associated with endogenous glucocorticoids released in response to central CRF administration.

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The data obtained provide evidence that at least one of the nonopioid mechanisms of central CRF-induced analgesia is associated with the HPA axis and mediated through glucocorticoids. Two experimental approaches were used to investigate the participation of endogenous glucocorticoids in CRF-induced analgesia: pharmacological suppression of the HPA axis and occupation of glucocorticoid receptors by its antagonist RU 38486. The cortisol pretreatment was used for suppression of the HPA axis. It has been previously founded that cortisol injection at a pharmacological dose one week before experiment causes long-lasting deficiency of stress-induced production of the HPA axis hormones: CRF, ACTH and corticosterone (37). Corticosterone deficiency one week after cortisol pretreatment has been confirmed in the present study. The inhibition of CRF-induced corticosterone levels was accompanied by a disappearance of central CRF-induced analgesia. The findings indicate that the deficiency of corticosterone production might be responsible for the elimination of CRF-induced analgesia in cortisol pretreated rats in our experimental conditions. The results suggest that the HPA axis through glucocorticoids is involved in CRF-induced analgesic effects on somatic pain sensitivity.

Further evidence of the participation of glucocorticoids in central CRF-induced analgesic effects also comes from the experiments with glucocorticoid receptors antagonist RU 38486. Administration of RU 38486 resulted in an increase in plasma corticosterone level that complies with the data published earlier (41, 39). The data provide evidence of the occupation of glucocorticoid receptors. The disappearance of analgesic effect in rats pretreated with RU 38486 suggests that glucocorticoids are involved in central CRF-induced analgesia. The elimination of analgesic effects of CRF by pretreatment with the glucocorticoid receptor antagonist resulted from the elimination of corticosterone action, despite the elevated plasma corticosterone levels. It means that the occupation of glucocorticoid receptors by corticosterone is needed for CRF-induced analgesic effects in rats. We consider the results with RU 38486 pretreatment as a support for the involvement of glucocorticoids in providing analgesic effect of central CRF.

Our previous (27, 42) and present results allow us to compare the contribution of the HPA axis and glucocorticoids to systemic and central CRF-induced analgesic effects under similar experimental conditions in anesthetized rats. Both central and systemic CRF-induced effects were not reversed by naltrexone. These data suggest that both central and systemic CRF-induced effects may be mediated by nonopioid mechanisms. However, although central CRF-induced analgesic effects were completely eliminated by pharmacological blockade of the HPA axis or glucocorticoid receptor antagonist RU 38486, systemic CRF-induced analgesic effects were not completely suppressed by these pretreatments (27, 42). The findings suggest that in our experimental conditions glucocorticoids play an important role in providing of central CRF-induced analgesic effects. At the same time glucocorticoid-independent mechanisms also contribute to systemic CRF-induced analgesic effects additionally to glucocorticoid-mediated mechanisms (27).

Glucocorticoid-dependent mechanisms of CRF-induced analgesia are in a good agreement with the data of literature (including our results) on the role of CRF and glucocorticoids in the development of stress-induced analgesia (43, 44) and regulation of physiological functions (7, 38, 45).

At the same time according to some data of literature analgesic effects of central CRF may be provided by mechanisms not associated with endogenous glucocorticoids (18, 25). The differences in results may be explained by some reasons including the type of stimulus for testing of pain sensitivity. It has been shown that adrenalectomy attenuates central CRF-induced analgesic effects in mechanical but not thermal paw withdrawal reaction (25). The finding suggests that glucocorticoids may participate in the CRF-induced effects on pain sensitivity to mechanical but not thermal stimuli.

Testing of tail flick reflex under anesthesia is widely applied in nociceptive researches (46-48). In our study we tested tail flick withdrawal under pentobarbital anesthesia. At present we can conclude that central CRF-induced analgesia is actually occurring through involvement of glucocorticoids in anesthetized rats. At the same time it is known that glucocorticoids may contribute to stress-induced analgesia in both anesthetized (6) and unanesthetized (43, 44) animals. This fact allows us to consider glucocorticoid-dependent mechanism as one of common mechanisms of pain regulation under stress conditions. Additionally, some data of literature (49-51) suggest that analgesic mechanisms in anesthetized and unanesthetized animals are not essentially different because the same pathways may be involved in the pain regulation and analgesia in anesthetized and unanesthetized rats.

The participation of the HPA axis through endogenous glucocorticoids in central CRF-induced analgesia complies with the data demonstrating the role of the paraventricular nucleus (PVN) of hypothalamus in the HPA axis activation (52) as well as in the inhibition of pain sensitivity (53). Activation of CRF-producing neurons in the paravascular part of the hypothalamic PVN leads to analgesia that is mediated by mechanisms that do not involve vasopressin or opioids (53). Our previous results demonstrate that CRF-producing neurons of PVN can participate in the nonopioid stress-induced analgesia through glucocorticoid-dependent mechanism (6).

One of the brain structures providing the inhibition of pain sensitivity during stress is the periaqueductal gray matter of the midbrain (PAGM) (54). We demonstrated previously that analgesia induced by the PAGM stimulation may be mediated by glucocorticoids released in response to the stimulation (55). The PAGM stimulation activates the HPA axis and antinociceptive system resulting in an increase of CRF, ACTH and plasma corticosterone levels as well as pain thresholds, respectively (55). Implantation of corticosterone into the PAGM resulted in an increase of pain thresholds in rats (55). These findings suggest that in general the PAGM may be involved in glucocorticoid-dependent mechanisms of analgesia.

In summary the present results suggest that the analgesic effects of central CRF on somatic pain sensitivity in anesthetized rats may be mediated by nonopioid mechanisms. Central CRF-induced analgesia observed in anesthetized rats may be provided by mechanisms associated with endogenous glucocorticoids and mediated through glucocorticoid receptors.

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REFERENCES

4. Ohmura Y, Yoshioka MM. The roles of corticotropin releasing factor (CRF) in responses to emotional stress: is CRF release a cause or result of fear/anxiety? CNS Neurol Disord Drug Targets 2009; 8: 459-469.


47. Shin MS, Helmuttter FJ. Antinociception following application of DAMGO to the basolateral amygdala results from a direct interaction of DAMGO with mu opioid receptors in the amygdala. *Brain Res* 2005; 1064: 56-65.
48. Xiao DQ, Zhu JX, Tang JS, Jia H. 5-hydroxytryptamine 1A (5-HT1A) but not 5-HT1 receptor is involved in mediating the nucleus submedius 5-HT-evoked antinociception in the rat. *Brain Res* 2005; 1046: 38-44.

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