DOI 10.1007/s11055-016-0260-7
Neuroscience and Behavioral Physiology, Vol. 46, No. 4, May, 2016

Effects of Corticotropin-Releasing Factor (CRF) on Somatic Pain Sensitivity in Conscious Rats: Involvement of Types 1 and 2 CRF Receptors

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Translated from Rossiiskii Fiziologicheskii Zhurnal imeni I. M. Sechenova, Vol. 100, No. 11, pp. 1287–1296, November, 2014. Original article submitted August 18, 2014.

Corticotrophin-releasing factor (CRF) is involved in regulating pain sensitivity and can elicit analgesic effects in animals and humans. The aim of the present work was to investigate the involvement of types 1 and 2 CRF receptors (CRF-1 and CRF-2 receptors) in mediating the analgesic action of CRF on somatic pain sensitivity when given systemically to conscious rats. Somatic pain sensitivity was tested in terms of the latent period (LP) of the tailflick reaction in response to thermal stimulation (the tail flick test). The involvement of CRF-1 and CFR-2 receptors was studied by systemic administration of their specific antagonists NBI 27914 and astressin 2B, respectively. Systemic administration of CRF increased the latent period of the pain reaction (it had an analgesic effect). Prior administration of NBI 27914 or astressin 2B eliminated the analgesic effect of CRF. In addition, administration of NBI 27914 affected the basal latent period of the pain reaction, increasing it. These data provide evidence that the analgesic effect of CRF may be mediated by both CRF-1 and CRF-2 receptors. CRF-1 receptors, unlike CRF-2 receptors, may also be involved in regulating the basal level of pain sensitivity.

Keywords: corticotropin-releasing factor, somatic pain sensitivity, CRF-1 and CRF-2 receptors, rats.

Corticotropin-releasing factor (CRF) is involved in regulating somatic pain sensitivity in stress. Exogenous CRF can induce both weakening [15, 31] and enhancement [12, 16] of somatic pain sensitivity. Although exogenous CRF has been seen to have double effects on pain sensitivity, the results of most studies have provided evidence of suppression of somatic pain sensitivity (an analgesic effect) in both animals [6, 9, 21] and humans [18, 20].

The peripheral action of CRF on somatic pain sensitivity has received the most study. Peripheral administration of CRF, both systemically (intravenous [9], intraperitoneal [1], intracardiac [5]) and locally [23], induces analgesic effects both on the background of inflammation [23] and without inflammation [9]. The action of CRF is mediated via CRF receptors of types 1 and 2 (CRF-1 and CRF-2 receptors) [10]. There are few data on the involvement of CRF receptors in mediating the analgesic effect of CRF when given peripherally, and these have been obtained on the background of inflammation. Use of selective antagonists of CRF receptors have demonstrated the involvement of both CRF-1 and CRF-2 receptors, located on leukocytes, in mediating the analgesic actions of CRF when given locally at the inflammation site in somatic tissues [22]. Additionally, data have been obtained showing that CRF-1 receptors can contribute to the development of inflammation-induced hyperalgesia [8, 11].

Despite the fact that the action of CRF on somatic pain sensitivity is apparent not only in inflammation, but also in the absence of inflammation [9, 15], there are no data on the involvement of CRF receptors in mediating the peripheral action of CRF on somatic pain sensitivity in normal conditions (in the absence of inflammation). Our previous studies [2] demonstrated the involvement of CRF-2 receptors in mediating the analgesic effect evoked by systemic administration of CRF in anesthetized rats. Administration of CRF-1 and CRF-2 receptors in mediating this effect in conscious animals has not previously been studied.

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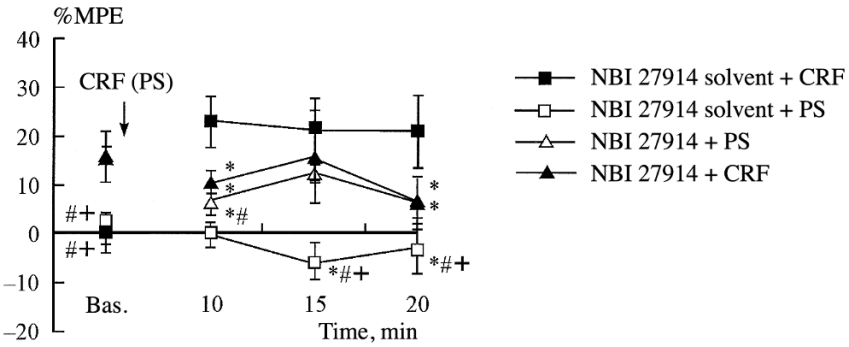

Fig. 1. Effects of NBI 27914 on the analgesic effect induced by systemic administration of CRF to conscious rats. Significant differences, $p < 0.05$: *from the [NBI 27914 solvent + CRF] group; #from the [NBI 27914 + CRF] group. Bas. – latent period of pain reaction 60 min after administration of NBI 27914 or its solvent. PS – physiological saline. Each group consisted of 7–8 animals.

The aim of the present work was to investigate the involvement of CRF-1 and CRF-2 receptors in mediating the analgesic action of CRF on somatic pain sensitivity when given systemically in conscious rats.

Methods

Experiments were performed on male Sprague–Dawley rats weighing 260–300 g previously acclimated to laboratory animal house conditions: temperature 20–22°C, 12:12 h light regime (light switched on at 08:00 and off at 20:00), and unrestricted access to water and food.

CRF (Sigma, St. Louis, USA) was given i.p. (40 μg in 2 ml/kg). The CRF dose was selected on the basis of published data [15] and results from our previous studies [1]. Controls received solvent (physiological saline) in place of CRF.

The involvement of CRF-1 and CRF-2 receptors in the development of CRF-induced analgesia was studied by blocking these receptors with their selective antagonists NBI 27914 and astressin 2B, respectively (Sigma, St. Louis, USA). NBI 27914 was given i.p. at a dose of 5 mg in 1 ml/kg and astressin 2B was given s.c. at a dose of 200 μg in 5 ml/kg. Antagonist doses were selected on the basis of results from our previous studies [2] and published data [4, 11, 19]. Astressin 2B was dissolved in distilled water and given 30 min before administration of CRF or physiological saline [2, 19]. The solvent for NBI 27914 was a solution containing 95% physiological saline and 5% ethanol, supplemented with Tween [11]. NBI 27914 was given 60 min before administration of CRF or physiological saline. Control animals received the corresponding solvents in place of NBI 27914 or astressin 2B.

Somatic pain sensitivity was tested in terms of the latent period of the tail flick reaction in rats in response to irradiation of the ventral surface of the tail with a focused light beam [32]. This model meets ethical requirements for experiments studying pain sensitivity in conscious animals and is widely used in experimental studies [17]. Irradiation intensity was selected such that the initial (basal) latent period of the tail-flick reaction was generally 4–6 sec. To avoid damaging the skin of the tail, the duration of exposure to the stimulus was restricted to a maximum of 15 sec. The effects of CRF-1 and CRF-2 antagonists on the analgesic action of CRF were compared by expressing the latent periods recorded after administration of CRF and its solvent as percentages of the maximum possible effect (%MPE). %MPE was calculated as [7]: $[(LP_t - LP_b)/(LP_m - LP_b)]\cdot 100$, where LP_t is the test latent period of the pain reaction and LP_b is the basal (initial) latent period, and LP_m is the maximum possible latent period of the pain reaction, which in our experiments was 15 sec.

Four series of experiments were performed. Experiments of series 1 and 3 investigated the effects of NBI 27914 and astressin 2B, respectively, on the analgesic effects of CRF; series 2 and 4 investigated the effects of NBI 27914 and astressin 2B, respectively, on basal pain sensitivity.

Four groups of animals were used in series 1 and 3, given agents in the following combinations: 1) solvent for antagonist + solvent for CRF (physiological saline); 2) solvent for antagonist + CRF; 3) antagonist + solvent for CRF (physiological saline); 4) antagonist + CRF. All animals initially underwent testing for the initial basal latent period. Rats were then divided into two groups: one group received NBI 27914 or astressin 2B and the other (the control group) received their corresponding solvents. At 30 min following administration of astressin 2B (or its solvent) or 60 min after injection of NBI 27914 (or its solvent), the basal latent period of the pain reaction was again tested. Rats were then given CRF or its solvent (physiological saline) and CRF- (or physiological saline)-induced pain reaction latent periods were measured 10, 15 and 20 min after injections.

Experiments of series 2 and 4 used two groups of rats: one group was given NBI 27914 or astressin 2B, respectively, and the other received their solvents. Initial basal latent periods were first measured, after which the experimental rats were given NBI 27914 or astressin 2B and control animals received the corresponding solvents. Basal pain reaction latent periods were again tested 30, 40, 60, 80, and 100 min after injections. After testing of pain sensitivity, rats were decapitated and blood samples were collected from the ab-

TABLE 1. Plasma Corticosterone Levels (μg/dl) 20 min after Administration of CRF or Physiological Saline (PS) after Administration of NBI 27914, Astressin 2B, or Their Solvents to Conscious Rats

No.	Group	PS	CRF	Significant differences between PS and CRF
1	NBI 27914 solvent	14.8 ± 1.8 (6)	23.6 ± 1.8 (8)	$p < 0.05$
2	NBI 27914	15.5 ± 1.9 (7)	21.97 ± 1.8 (8)	$p < 0.05$
3	Astressin 2B solvent	14.1 ± 0.6 (6)	25.2 ± 1.2 (11)	$p < 0.05$
4	Astressin 2B	15.5 ± 2.7 (3)	22.6 ± 1.9 (4)	$p < 0.05$

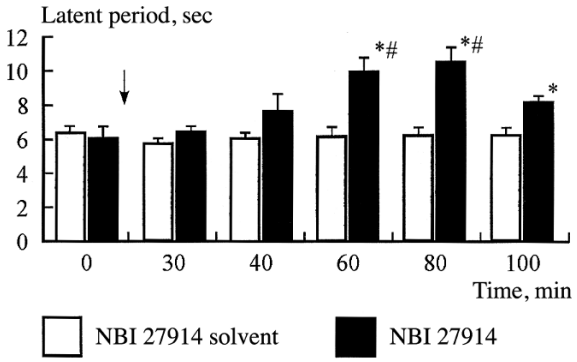

Fig. 2. Effects of NBI 27914 on the basal somatic pain sensitivity level in conscious rats. Significant differences, $p < 0.05$: *from NBI 27914 solvent; #from the basal latent period (before administration of antagonist or its solvent). Each group consisted of 4–7 rats.

dominal vessels for analysis of plasma corticosteroid contents using a spectrophotometric micromethod.

Data were analyzed statistically in MedCalc v. 12.7.0.0 (Statistics for Biomedical Research, MedCalc Software, Belgium). Statistical evaluation of differences between groups was based on the *t* test or its modifications for different variances (for comparisons of plasma corticosterone levels) or the Mann–Whitney or Wilcoxon tests (for comparison of the latent periods of pain reactions).

Results

Initial basal latent periods measured in control rats before administration of solvents for NBI 27914 or astressin 2B were 5.2 ± 0.1 sec (n = 15) and 6.1 ± 0.1 sec (n = 17), respectively.

Administration of CRF to control animals given NBI 27914 solvent or astressin 2B solvent (Fig. 3), as expected, induced an analgesic effect. The analgesic effect of CRF was apparent as an increase in the latent period of the pain reaction from the corresponding latent periods after administration of solvent (Fig. 1, 3). Development of the analgesic effect of CRF was accompanied by an increase in plasma corticosterone production (see Table 1).

The initial basal latent period measured before administration of NBI 27914 was 5.1 ± 0.2 sec (n = 15). Administration of NBI 27914 eliminated the analgesic effect of CRF at 10 and 20 min after injection of CRF (Fig. 1). At these time points, the latent periods of induced by CRF on the background of NBI 27914 was significantly shorter than the latent periods induced by CRF in control animals, but did not differ from the corresponding latent periods after administration of its solvent. At the same time, there were no significant differences between the latent periods at 15 min induced by CRF in control animals and rats given NBI 27914 (Fig. 1).

In addition, studies of the actions of NBI 27914 on the analgesic effect of CRF showed that NBI 27914 had an effect in its own right (without administration of CRF) on somatic pain sensitivity: administration of antagonist suppressed pain sensitivity. This follows from the fact that basal latent periods before administration of CRF (or its solvent) in both groups of rats given NBI 27914 were significantly greater than the corresponding latent periods in control animals given NBI 27914 solvent (Fig. 1).

The action of NBI 27914 on basal pain reaction latent periods was studied in more detail in additional investigations with the aim of understanding when its action starts to appear and how long it lasted (Fig. 2). The effect of NBI 27914 seen in the previous series of experiments (an increase in the basal latent period) started to become apparent 60 min after administration of antagonist and persisted through the following 20 min (Fig. 2), as evidenced by increases in the latent period of the pain reaction at 60 and 80 min after administration of NBI 27914 as compared with the initial basal latent period and the corresponding latent periods in control rats given antagonist solvent.

The increase in the basal latent period evoked by NBI 27914 was accompanied by a decrease in the plasma corticosterone content. The basal plasma corticosterone level 100 min after administration of NBI 27914 was 3.96 ± 0.79 μg/dl (n = 4) and was significantly ($p < 0.05$) lower than the corresponding level after administration of its solvent (8.72 ± 0.79 μg/dl, n = 4). At the same time, at 80 min after injection, we did not see any effect of NBI 27914 on corticosterone levels produced in response to CRF or physiological saline, as evidenced by the absence of any significant differences between plasma corticosterone contents in rats given NBI 27914 and controls given its solvent (see Table 1).

The initial basal latent period measured before administration of astressin 2B was 6.24 ± 0.1 sec (n = 9). Administration of astressin 2B eliminated the analgesic effect of CRF which was apparent as an increase in the latent period of

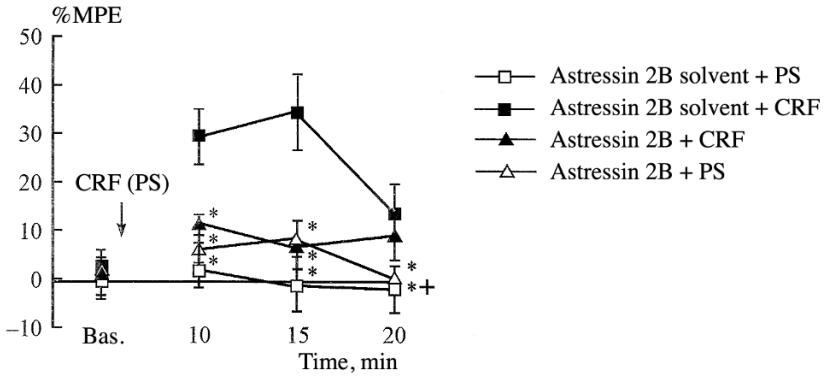

Fig. 3. Effects of astressin 2B on the analgesic effect of systemic administration of CRF to conscious rats. Significant differences, $p < 0.05$: *from the [astressin 2B solvent + CRF] group; +from the [astressin 2B + CRF] group. Bas. – latent period of pain reaction 30 min after administration of astressin 2B or its solvent. PS – physiological saline. Each group consisted of 5–11 animals.

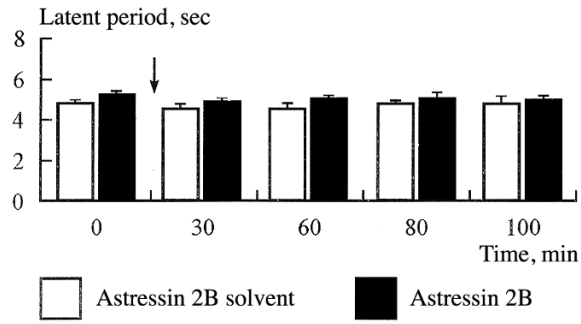

Fig. 4. Effects of astressin 2B on the basal somatic pain sensitivity level in conscious rats. Each group consisted of 4–5 rats.

the pain response at 10 and 15 min after injection (Fig. 3). At these time points, the latent periods induced by CRF on the background of astressin 2B were not different from the corresponding latent periods after administration of its solvent but were significantly shorter than the corresponding latent periods in control rats given astressin 2B solvent (Fig. 3).

Despite the fact that astressin 2B had clear effects on the analgesic action of CRF, it had no effect on basal pain sensitivity, as evidenced by the absence of any significant difference between latent periods in rats given astressin 2B and the corresponding latent periods in control animals (Fig. 4).

Administration of astressin 2B produced no changes in plasma corticosterone levels. Basal plasma corticosterone 100 min after administration of astressin 2B was 8.8 ± 0.34 μg/dl (n = 4) and was not significantly different from the corresponding level after administration of its solvent (7.2 ± 0.7 μg/dl, n = 5). There were also no differences between the corresponding corticosterone levels induced by administration of CRF or physiological saline in rats given astressin 2B and control animals (see Table 1).

Discussion

The data obtained here support previous results [3, 15] on the analgesic action of CRF on somatic pain sensitivity after systemic administration of this substance. A new fact obtained in the present study is that both types of CRF receptor, CRF-1 and CRF-2 receptors, are involved in mediating the analgesic effect of CRF. In fact, prior administration of both the CRF-1 receptor antagonist NBI 27914 and the CRF-2 receptor antagonist astressin 2B eliminated the analgesic action of CRF.

The involvement of CRF-1 receptors in mediating the analgesic action of CRF is evidenced by the elimination of this effect on exposure to NBI 27914. The data indicate that NBI 27914 affects not only the analgesic effect of CRF, but also basal pain sensitivity. Administration of NBI 27914 led to an increase in the basal latent period, which was comparable with the CRF-induced increase in the latent period. This is probably why there was no significant difference between the latent periods induced by CRF in rats given NBI 27914 and control animals at 15 min (Fig. 1).

The results providing evidence that NBI 27914 affects basal pain sensitivity are in good agreement with published data on increases in the threshold of pain reactions on systemic administration of NBI 27914 (i.p., 5 mg/kg) [11, 14]. We showed that the increase in the basal latent period evoked in inflammation by systemic administration of NBI 27914 may also be apparent in normal conditions (in the absence of inflammation), which is a new observation made in the present study.

It should be noted that our results obtained with systemic administration of NBI 27914 are in good agreement with recent data obtained with central administration of this agent [13]. As NBI 27914 crosses the blood-brain barrier [30], it can be suggested that mediation of the action of systemically administered NBI 27914 on somatic pain sensitivity involves central CRF-1 receptors.

The actions of NBI 27914 in our experiments was accompanied by suppression of plasma corticosterone production 100 min after administration. As activation of the hypothalamo-hypophyseal-adrenocortical system (HHACS) is

mediated by CRF-1 receptors located in the hypophysis [10], the decrease in the basal plasma corticosteroid content after administration of NBI 27914 supports blockade of CRF-1 receptors in our experiments. At the same time, we did not observe that NBI 27914 had any effect on corticosterone levels induced by injection of physiological saline or CRF (see Table 1). The action of NBI 27914 in this case may have been transient and apparent at other time points.

The involvement of CRF-2 receptors in mediating the analgesic effect of CRF is evidence that the analgesic effect of CRF is prevented after administration of astressin 2B. The results are in good agreement with results from our previous experiments on the involvement of CRF-2 receptors in supporting the analgesic action of CRF using electrical stimuli in anesthetized rats [2].

Astressin 2B, unlike NBI 27914, had no effect on basal somatic pain sensitivity. The lack of any effect of astressin 2B on the basal latent period of the pain reaction is evidence that CRF-2 receptors are not involved in regulating the basal pain sensitivity level. Analogous data have also been obtained by other investigators studying both somatic [8] and visceral [25] pain sensitivity.

Published data indicate that astressin 2B, in contrast to NBI 27914, does not cross the blood-brain barrier [30]. These data suggest that the action of astressin 2B may be mediated by peripheral CRF-2 receptors located in the skin, muscles, or other peripheral organs [10, 27, 29]. The inability of astressin 2B to cross the blood-brain barrier may also be related to its lack of influence on the basal latent period.

We did not see any effect of astressin 2B on the plasma corticosterone level. This is consistent both with the results of our previous studies [2] and with published data [26] indicating that although CRF-2 receptors may have a role in controlling the functional activity of the HHACS in stress, they are not involved in its activation.

Our data provide evidence that both types of receptor are involved in mediating the actions of CRF on somatic pain sensitivity; activation of CRF-1 receptors may contribute to the development of pain reactions, while CRF-2 receptors are involved in their suppression. This suggestion is consistent with the view discussed in the literature [24, 28]. It can be suggested that the absence of any analgesic effect in conditions of blockade of CRF-1 and CRF-2 receptors may have different causes. The fact that CRF had no effect on somatic pain sensitivity on the background of NBI 27914 may be due to blockade of the transmission of pain information via CRF-1 receptors, while the absence of any effect on the background of astressin 2B may be due to the inability to activate CRF-2 receptors, as needed for suppression of the pain effect induced by activation of CRF-1 receptors.

Thus, the analgesic effect of CRF given systemically may be mediated by both CRF-1 and CRF-2 receptors, CRF-1 rats, unlike CRF-1 receptors, being involved not only in mediating the analgesic effect of CRF, but also in regulating basal pain sensitivity.

This work was supported by the Program No. 7 of the Presidium of the Russian Academy of Sciences, the Russian Foundation for Basic Research (Grant No. 13-04-01680a), and the Russian Science Foundation (Grant No. 14-15-00790).

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