BIOPHYSICS AND BIOCHEMISTRY

Effect of Glucocorticoid Receptor Blockade on Analgesic Effect of Corticotropin-Releasing Factor

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The role of glucocorticoid receptors in the analgesic effect of corticotropin-releasing factor in rats was studied after glucocorticoid receptor blockade with antagonist RU 38486. Glucocorticoid hormones can potentiate the analgesic effect of corticotropin-releasing factor or modulate the mechanisms of this effect, which depends on the type of painful stimulus.

Key Words: *corticotropin-releasing factor; corticosterone; glucocorticoid receptors; pain sensitivity; rats*

Published data and results of our previous studies [4,8,10,13] show that various hormones of the hypothalamic—pituitary—adrenal system (HPAS) are involved in the regulation of pain sensitivity. Treatment with the key hormone of HPAS, corticotropin-releasing factor (CRF), produces an analgesic effect in animals and humans [5,9,11]. Our previous experiments with pharmacological blockade of HPAS [2,3] showed that the hormonal axis of HPAS plays a role in the analgesic effect of CRF. The involvement of endogenous glucocorticoids in the analgesic effect of ACTH [1] provides support to the fact that the analgesic effect of CRF is mediated by glucocorticoids. However, there is no direct evidence that glucocorticoids (hormones of the final stage in HPAS) play a role in the analgesic effect of CRF. Here we studied the role of glucocorticoid hormones in the analgesic effect of CRF. The study was conducted on rats with glucocorticoid receptor blockade.

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MATERIALS AND METHODS

Experiments were performed on Sprague–Dawley rats weighing 200-300 g. Glucocorticoid receptors were blocked with a specific antagonist RU 38486 (Sigma; 20 mg/kg in 5 ml/kg 1,2-propylene glycol, Vekton). The antagonist was injected subcutaneously 140 min before administration of CRF [6]. Control animals received the solvent instead of RU 38486.

CRF (Sigma) was injected intraperitoneally (40 μg/kg in 2 ml/kg physiological saline) or intracerebroventricularly (2 μg, 7 μl per rat) [2]. Control animals received the solvent instead of CRF. In each experimental series, the preparation was injected to four groups of rats: group 1, RU 38486 solvent+CRF solvent; group 2, RU 38486 solvent+CRF; group 3, RU 38486+CRF solvent; and group 4, RU 38486+CRF.

The analgesic effect was evaluated by the increase in the threshold level or latency of the pain response to electrical or thermal stimulation of the tail, respectively. The strength of current inducing the tail-flick response under conditions of electrostimulation (sinusoidal current, 500 Hz), was taken as the pain thresh-

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old. The current varied from 0.07 to 2 mA with an increment of 70 μA [1-3]. The latency was estimated as the interval from the start of exposure of the ventral surface of the tail to a focused light beam [14] to the tail-flick reaction.

To evaluate the relationship between pain sensitivity and functional activity of HPAS after CRF treatment, the pain response was recorded in anesthetized rats. Nembutal in a dose of 40 mg/kg was injected intraperitoneally 20 min before studying the baseline level of pain sensitivity [1-3,12]. The pain threshold or latency of the nociceptive reaction in anesthetized rats was measured 3, 8, 15, 20, and 30 min after CRF injection. Studying the pain sensitivity under conditions of thermal stimulation was conducted on anesthetized and awake animals (preadapted to experimental conditions for 1 week). The latency of the pain response in awake rats was measured before and 8 min after CRF injection. The rats were decapitated immediately after studying the pain sensitivity (by the 30th and 8th minutes for anesthetized and awake animals, respectively). The blood was taken from trunk vessels. Serum corticosterone concentration was measured by the spectrophotometric micromethod [6].

The differences in serum corticosterone concentration were evaluated by *t* test or modified *t* test for different dispersions. The pain thresholds were compared by Mann–Whitney test.

RESULTS

Systemic and central administration of CRF to control animals (receiving the solvent instead of antagonist) was followed by an increase in serum corticosterone concentration and development of the analgesic effect. It was manifested in an increase in the pain threshold (Figs. 1 and 2) or latency of the pain response (Fig. 3, Table 1). The analgesic effect was observed 3 min after CRF administration. The analgesic effect of CRF during electrostimulation (from the 3rd to the 30th minute) was more prolonged than that observed under conditions of thermal stimulation (from the 3rd to the 8th minute). Administration of physiological saline instead of CRF had no effect on the pain threshold and latency of the pain response in control animals (as compared to the baseline values before injection of physiological saline; Figs. 1-3, Table 1).

Administration of RU 38486 caused an increase in serum corticosterone concentration (Figs. 1-3, Table 1), which is consistent with published data [6,7] and illustrates the blockade of glucocorticoid receptors under these experimental conditions. Combined treatment with CRF and RU 38486 was followed by a greater increase in serum corticosterone concentration (as compared to the effects of each of these agents; Figs. 1-3, Table 1).

Glucocorticoid receptor blockade in anesthetized rats did not modulate the baseline pain sensitivity under conditions of electrostimulation. The analgesic effect of systemic (Fig. 1) or central administration of CRF (Fig. 2) was partly or completely abolished under these conditions, respectively. Hence, RU 38486 abolishes the analgesic effect of CRF. We conclude that this effect is mediated by glucocorticoid receptors and, therefore, endogenous glucocorticoids. The analgesic effect persisted for 3 min after systemic administration of CRF under conditions of glucocorticoid receptor blockade (Fig. 1). These data indicate that

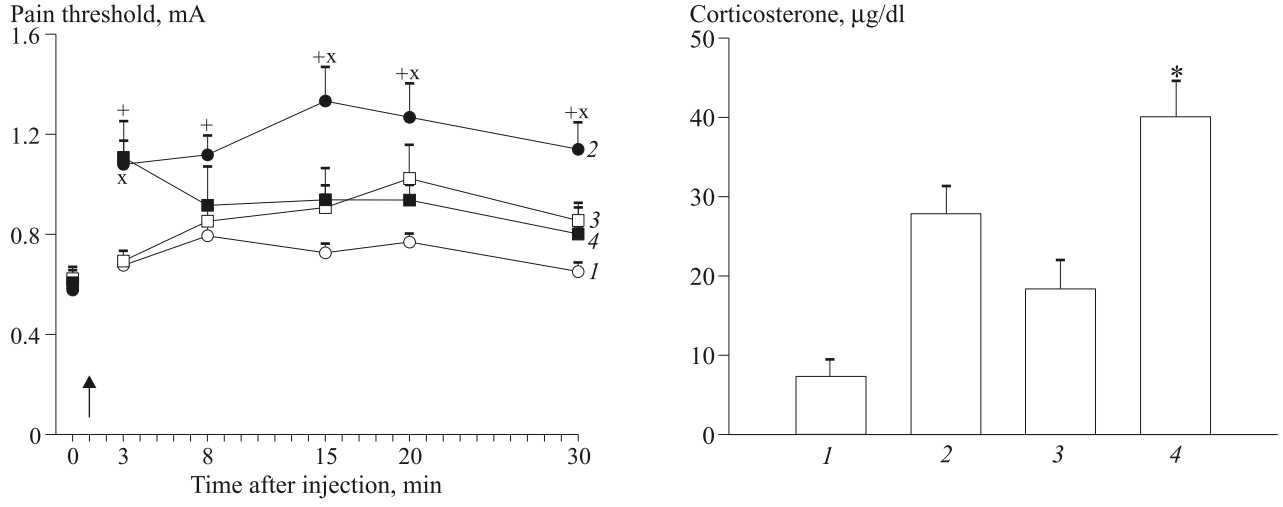

Fig. 1. Influence of RU 38486 on the analgesic effect of systemic treatment with CRF and serum corticosterone concentration in anesthetized rats after electrostimulation. Here and in Figs. 2 and 3: RU 38486 solvent and CRF solvent (*1*); RU 38486 solvent and CRF (*2*); RU 38486 and CRF solvent (*3*); RU 38486 and CRF (*4*). $p<0.05$: *compared to *1*, *2*, and *3*; +compared to *1*; ×compared to *4*. Number of measurements in each group is 7-10. Arrow: administration of CRF or physiological saline.

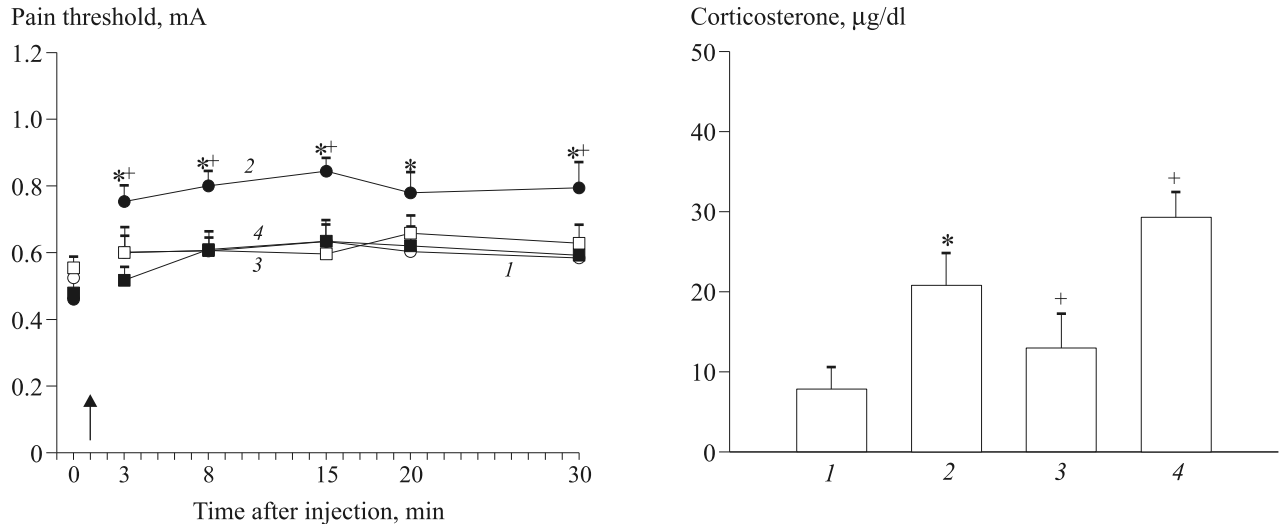

Fig. 2. Influence of RU 38486 on the analgesic effect of central treatment with CRF and serum corticosterone concentration in anesthetized rats after electrostimulation. $p<0.05$: *compared to *1*; +compared to *4*. Number of measurements in each group is 9-11.

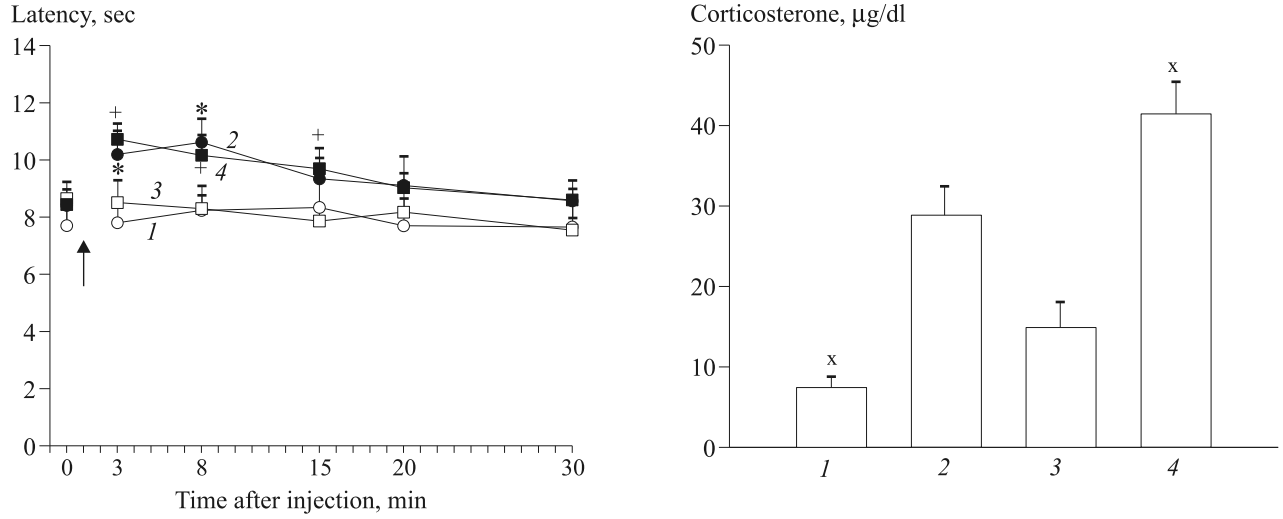

Fig. 3. Influence of RU 38486 on the analgesic effect of systemic treatment with CRF and serum corticosterone concentration in anesthetized rats after thermal stimulation. $p<0.05$: *compared to *1*; +compared to *4*; xcompared to other groups. Number of measurements in each group is 8-14.

the analgesic effect of CRF also involves some other mechanisms, which are not realized via glucocorticoid receptors.

Glucocorticoid receptor blockade in anesthetized rats did not influence the baseline pain sensitivity and analgesic effect of systemic treatment with CRF under conditions of thermal stimulation (Fig. 3). It was interesting to evaluate why RU 38486 does not modulate analgesic activity of CRF. It could be related to the nature of stimulation of effect of anesthesia. We studied the effect of RU 38486 on analgesic activity of CRF in awake rats during thermal stimulation (Table 1). The analgesic effect was observed 140 min after injection of RU 38486 to awake animals. The initial latency of the pain response (6.10±0.22 sec, n=43) was increased after RU 38486 administration (8.07±0.59 sec, n=22). The response latency in animals of this group was higher ($p<0.05$) than in rats receiving RU 38486 solvent (6.28±0.42 sec, n=21). A similar increase in the pain response latency was observed in animals receiving RU 38486 and CRF solvent. Our findings confirm intrinsic analgesic activity of this antagonist (Table 1). Combined treatment of awake rats with RU 38486 and CRF was followed by a greater increase in the pain response latency (as compared to the effect of CRF solvent; Table 1). The CRF-induced increase in the pain response latency (as compared to that in animals of the CRF solvent group) practically did not differ in specimens with (125±7%, n=9) or without blockade of glucocorticoid receptors (128±9%, n=8). Hence, RU

TABLE 1. Influence of RU 38486 on the Analgesic Effect of Systemic Treatment with CRF and Serum Corticosterone Concentration in Awake Rats by the 8th Minute after Thermal Stimulation (*M*±*m*)

Groups (study agents)	Latency, sec	Corticosterone, μg/dl
1 (RU 38486 solvent+CRF solvent)	5.89±0.39	19.4±2.2
2 (RU 38486 solvent+CRF)	7.24±0.41*+	31.5±2.9*
3 (RU 38486+CRF solvent)	7.53±0.49*+	28.8±3.2*+
4 (RU 38486+CRF)	9.67±0.7*	42.4±2.8*

Note. $p<0.05$: *compared to group 1; +compared to group 4. Number of measurements in each group is 8-15.

38486 did not modulate the analgesic effect of CRF in awake rats. These data suggest that the analgesic effect of CRF under conditions of thermal stimulation is mediated by the glucocorticoid-independent mechanisms. However, the increase in the analgesic effect of CRF during glucocorticoid receptor blockade (as compared to that in control rats; Table 1) suggests that the involvement of glucocorticoids in the analgesic effect of CRF is related to changes in the mechanisms for action of CRF on pain sensitivity.

Our results indicate that the analgesic effect of CRF on pain sensitivity is mediated by mechanisms involving or not involving endogenous glucocorticoids. Glucocorticoid hormones can potentiate the analgesic effect of CRF (during electrostimulation) or modulate the mechanisms of analgesic activity of CRF (during thermal stimulation). Hence, the role of glucocorticoids depends on the type of pain stimulation.

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