JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2016, 67, 4, 595-603
www.jpp.krakow.pl

N.I. YARUSHKINA, T.R. BAGAEVA, L.P. FILARETOVA

INVOLVEMENT OF CORTICOTROPIN-RELEASING FACTOR RECEPTORS TYPE 2, LOCATED IN PERIAQUADUCTAL GRAY MATTER, IN CENTRAL AND PERIPHERAL CRF-INDUCED ANALGESIC EFFECT ON SOMATIC PAIN SENSITIVITY IN RATS

Laboratory of Experimental Endocrinology, Pavlov Institute of Physiology, Russian Academy of Sciences, St. Petersburg, Russia

Corticotropin-releasing factor (CRF) is involved in the regulation of pain sensitivity and can induce an analgesic effect in animals and humans. The periaqueductal gray matter (PAGM) of the midbrain is one of the key structures of the antinociceptive system. The aim of the study was to investigate the involvement of CRF receptor type 2 (CRF-R2 receptors), localized in the PAGM, in the analgesic effect caused by central or systemic CRF on somatic pain sensitivity in conscious rats. Somatic pain sensitivity was tested by a tail flick test (measuring tail flick latency induced by tail's thermal stimulation). The involvement of CRF-R2 receptors was studied by administering the selective antagonist astressin$_2$-B into the PAGM. Both peripheral and central CRF administration caused an increase in tail flick latencies (analgesic effect). Administration of astressin2-B into the PAGM attenuated the analgesic effect induced by the central as well as systemic CRF administration. The results suggest that one of the mechanisms of the CRF-induced analgesic effect may be mediated by CRF-R2 receptors located in PAGM.

Key words: *corticotropin-releasing factor, somatic pain sensitivity, analgesic effect, corticotropin-releasing factor receptors type 2, periaqueductal gray matter of the midbrain, corticosterone*

INTRODUCTION

CRF is an important regulator of physiological functions and behavior in stress. Exogenous CRF may cause a decrease in somatic pain sensitivity (analgesic effect) in animals (1, 2) and humans (3, 4). However, CRF may also exert a hyperalgesic effect on somatic pain sensitivity (5).

CRF action is mediated through CRF receptors of type 1 and 2 (CRF-R1 and CRF-R2 receptors) (6). CRF-R1 and CRF-R2 receptors may mediate opposite (hyper- or hypoalgesic) effects of CRF on pain sensitivity (7, 8). It has been shown that an increase in visceral pain sensitivity might be mediated by CRF-1 receptors and decreased by CRF-R2 receptors (8).

In the present study we focused on the CRF-induced analgesic effect on somatic pain sensitivity. Because CRF-R2 receptors are involved in suppression of pain sensitivity (5), it was reasonable to propose that one of the mechanisms of CRF-induced analgesic action on somatic pain sensitivity is provided through involving CRF-R2 receptors. It has been shown that peripheral CRF-R2 receptors are involved in a local CRF-induced analgesic effect on tonic pain, caused by inflammation in somatic areas (9). In our previous studies we had demonstrated that peripheral CRF-R2 receptors are involved in the peripheral CRF-induced analgesic effect on acute somatic pain in rats (10, 11). However, there is little data on the participation of central CRF-R2 receptors in the CRF-induced analgesic effect on somatic pain sensitivity. It is well documented that the CRF-R1 and the CRF-R2 receptors located within the amygdala are involved in somatic pain regulation, and may mediate opposite effects on somatic pain sensitivity (7). Pronociceptive effect of intra-amygdala CRF administration has been shown to be mediated by CRF-R1 receptors, while the antinociceptive effect of CRF is mediated by CRF-R2 receptors within the amygdala (7). The contribution of CRF-R2 receptors to somatic pain inhibition in other brain structures remains unclear.

The periaqueductal gray matter (PAGM) is a mesencephalic structure that is involved in multiple behavioral and physiological processes, including nociception (12), fear and anxiety (13, 14), cardiovascular control (15) and respiration (16), sexual (17) and maternal behavior (18), vocalization (19). Anatomically, the PAGM can be divided into four columns along its rostrocaudal axis: the dorsomedial, dorsolateral, lateral and ventrolateral columns (20). Both CRF receptor subtypes have been shown within the PAGM in rats (6). CRF-R1 and particularly CRF-R2 receptors are expressed in dorsal and dorsolateral parts of the PAGM of the midbrain (21, 22), and their localization corresponds to the distribution of CRF and urocortin terminals within the PAGM (23, 24).

The dorsal part of the PAGM is a common component for both modulation of anxiety-related defensive reactions and inhibition of pain sensitivity (20). Intra-dorsal PAGM administration of CRF enhances the expression of anxiety-related behavior, and alters autonomic reactions (blood pressure and heart rate) through the CRF receptors located within the PAGM (13, 25-27). According to these findings, the CRF-R1 receptors in the dorsal PAGM are involved in regulation of

defensive behavior associated with both anxiety and panic, whereas the CRF-R2 receptors are involved in the type of behaviors, leading to an effect, opposite to that caused by the CRF-R1 receptors (26-28). At the same time the dorsolateral PAGM plays a crucial role in mediating nonopiod analgesia induced by an electric foot shock (29) or fear (14). Intra-dorsal PAGM administration of CRF causes pain inhibition (25, 30). However, there is only one study on the role of CRF-R1 and CRF-R2 receptors located within PAGM in pain regulation (25). It has been demonstrated that the intra-dorsal PAGM CRF-R1 receptor antagonist NBI 27914 prevents intra-PAGM CRF-induced analgesic effect on tonic pain induced by formalin injection, suggesting the involvement of CRF-1 receptors within the PAGM in the analgesia. However, intra-dorsal PAGM administration of the CRF-R2 receptor antagonist antisauvagine 30 did not influence the analgesic effect (25). This data suggests that further studies are necessary to elucidate the role of CRF-R2 receptors within the PAGM in the CRF-induced analgesic effect.

Although peripheral CRF-induced analgesic effects are well known in animals and humans (1, 4), there still remains a controversial question about the involvement of central mechanisms in systemic CRF-induced analgesic effect. To answer this question we studied the contribution of CRF-R2 receptors located in the PAGM to not only the central, but also in peripheral CRF-induced effect.

The aim of the present study was to investigate the involvement of CRF-R2 receptors located in the PAGM in peripheral and central CRF-induced analgesic effect on somatic pain sensitivity.

MATERIALS AND METHODS

Animals

Adult male Sprague-Dawley rats (Stolbovoe, Moscow, Russia) weighing 220 – 300g 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 the European Communities Council guidelines on animal research (86/609/EEC) and the local animal care committee at the Pavlov Institute of Physiology RAS.

Drugs

We used rat/human (r/h) CRF (Sigma, Saint Louis, USA), and astressin2-B, a selective CRF-R2 receptor antagonist (Sigma, Saint Louis, USA). Immediately before use the compounds were dissolved in sterile saline (r/h CRF) or double-distilled water (astressin$_2$-B).

Administration of CRF and CRF-R2 receptor antagonist astressin$_2$-B

CRF was administered peripherally (intraperitoneally), as well as centrally (intra-PAGM). CRF was injected intraperitoneally at a dose of 40 µg/kg (in the volume of 2 ml/kg). The dose was chosen so as to produce an increase in glucocorticoids levels (10, 31). CRF was administered intra-PAGM at doses of 0.007; 0.07 or 0.7 µg/rat (in the volume of 0.5 µl/rat). Injection area within the PAGM was limited by the dorsomedial, dorsolateral and lateral subdivisions of the left PAGM in the midbrain (6.8 mm posterior to bregma) (32) (*Fig. 1*). Control animals were given a vehicle of CRF.

The involvement of CRF-R2 receptors in the CRF-induced analgesia was studied by administering CRF-R2 receptor selective antagonist astressin$_2$-B (33). Astressin$_2$-B was administered intra-PAGM (in the same area within the PAGM as CRF), at a dose of 1 µg/rat in volume 0.5 µl/rat, 30 min before CRF or vehicle of CRF were given either centrally or peripherally. The dose was selected based on data of literature (34, 35).

Surgery

The rats were anesthetized with Zoletil®50 (Virbac, France, 15 mg/kg (0.5 ml/kg), intraperitoneally) and Rometar (Bioveta, Czech Republic, 10 mg/kg, 0.5 ml/kg, intraperitoneally) and placed in a stereotaxic frame (David Korf, USA). A stainless steel guide cannula (23-gauge, length 5 mm) was implanted unilaterally (at the left side) at the coordinates (32): 6.8 mm posterior from bregma, 0.5 mm left of the midline, and 4.5 mm ventral from the skull surface (*Fig. 1*). The guide cannula was fixed to the skull with dental acryl. Dummy cannulas (stylet, 27-gauge) of the same length as the guide cannula were inserted into the guide cannula immediately after the surgery to prevent obstruction. The animals were then placed per in individual cages and allowed to recover from the surgery for 1 week. During the second week after the surgery the rats were habituated to experimental conditions.

Microinjection

On a day of testing the animals were gently held and their stylets were removed. A stainless steel cannula-injector (27-gauge) was lowered into the guide cannula, so that it extended 2 mm below the tip of the guide cannula. The injector was attached to a 1 µl Hamilton microsyringe *via* polyethylene tubing, and, then the rats were hand-injected through injector with solution of volume 0.5 µl. Each drug was infused over a 60 s period and the injector remained in situ for a further 2 min following the injection. One day before experiment the injector was lowered but no solution was delivered to acclimate rats to the infusion procedure and to minimize stress induced by injection on the test day.

Nociceptive testing

A tail-flick latency test was used for measurement of somatic pain sensitivity in conscious rats (36). This pain test conforms to the ethics of study of pain sensitivity in awake animals, and it is widely used in experimental studies (37, 38). Pain reaction was induced by thermal stimulation of a tail by using a light beam analgesia meter. Radiant heat was applied to the ventral surface of the tail at 6 – 7 cm from the tip. Tail flick latency time was measured as the time from the onset of the heat exposure to the withdrawal of the tail. The intensity of the radiant heat was adjusted to yield the baseline latencies of 4 – 6 s. The heat stimulus was discontinued after 15 s to avoid tissue damages (the cutoff point was 15 s). For each animal, the tail flick latency was obtained as the mean of five measurements. Analgesia was defined as an increase of tail flick latencies after the CRF injection compared to the baseline and tail flick latencies after its vehicle injection. The animals had been allowed to habituate to experimental conditions for 1 week before the tail flick test.

Collection of blood samples and estimation of plasma corticosterone levels

After completion of nociceptive testing the rats were decapitated (for details see also *Experimental protocol*) and blood samples were collected from the trunk vessels. The samples were then centrifuged at 4°C, and the plasma was frozen at –20°C. Plasma corticosterone levels were determined by spectrofluorometric micromethod (39).

Histology

After completion of experiments, cannula-injector placements were marked with microinjections of methylene blue dye in the volume of 0.5 µl/rat. Brains were extracted and placed in 10% formalin. One week later the brains were cut with a microtome-cryostat and frozen sections 30 – 40 µm thick were placed on a slide to identify the injection site using the atlas (32). Only those rats that had injection sites within the PAGM were chosen for statistical analysis.

Experimental protocol

In all experiments rats were transported to the experimental room and left undisturbed for 30 min prior to testing.

Experiment 1: effect of peripheral corticotropin-releasing factor on tail flick latencies and on corticosterone plasma levels

After measurement of the baseline tail flick latencies (Bas1) the rats were administered intraperitoneally by CRF (n = 9) or its vehicle (n = 4). Tail flick latencies were tested 5, 10, 15 and 20 min after the CRF (or its vehicle) administration (*Fig. 2*). After completion of nociceptive testing at 20 min after the CRF administration the animals were decapitated and blood samples were collected from the trunk vessels.

Experiment 2: effect of central corticotropin-releasing factor on tail flick latencies and on corticosterone plasma levels

In preliminary experiments the rats were given one of the doses of CRF (0.007 µg/rat (n = 6); 0.07 µg/rat (n = 4); 0.7 µg/rat (n = 9)) or its vehicle (n = 14) and the time-courses of the effect of CRF (or its vehicle) on tail flick latencies were examined. For each of doses the tail flick latencies were measured before and for 30 min after the intra-PAGM administration of CRF (or vehicle). A significant increase in tail flick latencies was detected 15 min after the intra-PAGM CRF administration. Based on these results we built a dose-response curve (*Fig. 3A*). For each dose, tail flick latencies were given at 15 min after CRF administration as percent of baseline tail flick latencies (*Fig. 3B*). The dose of 0.7 µg/rat was chosen for further experiments.

Corticosterone levels in the plasma were measured at 15 min after the intra-PAGM CRF administration at the dose of 0.7 µg/rat. A group of rats was subjected to intra-PAGM administration of CRF (or its vehicle) under anesthesia (Zoletil®50, 15 mg/kg (0.5 ml/kg), intraperitoneally) and Rometar (10 mg/kg (0.5 ml/kg), intraperitoneally). Then, the anesthetized rats were decapitated 15 min after the intra-PAGM administration and blood samples were collected from the trunk vessels.

Experiments 3 and 4: effect of astressin$_2$-B on peripheral (experiment 3) or central (experiment 4) corticotropin-releasing factor-induced analgesia

Immediately after measurement of the baseline tail flick latencies (Bas1) all animals were subjected to the intra-PAGM administration of antagonist of CRF-R2 receptors astressin$_2$-B or its vehicle. After the intra-PAGM injection the rats were returned to their home cages and 30 min later were tested for tail flick latencies (Bas2). The rats were then given CRF (or its vehicle) peripherally (intraperitoneally) at the dose of 40 µg/kg (experiment 3) or centrally at the dose of 0.7 µg/rat (experiment 4). Tail flick latencies were measured for 30 min after the intraperitoneal or intra-PAGM administration with 5 min intervals. The drugs were administered in following combinations: vehicle of astressin$_2$-B + vehicle of CRF; vehicle of astressin$_2$-B + CRF; astressin$_2$-B + vehicle of CRF; astressin$_2$-B + CRF.

Data and statistical analysis

Data was expressed as mean ± S.E.M. Data was analyzed with ANOVA module of the MedCalc Version 12.7.0.0. (Statictics for biomedical research, MedCalc Software, Belgium). Statistical significances were tested by one or two-way repeated measures ANOVA (factors: treatment (group) and time), followed by a post hoc Turkey-Kramer test. A student t-test was applied to analyze corticosterone levels. In each case, the required level for significance was considered to be $P < 0.05$.

Percentage of suppression compared to that obtained when CRF was injected i.p. and PAGM. It was calculated using following formula: % suppression = $\{(TFL_{CRF+Vehicle} - TFL_{CRF+antagonist}) / TFL_{CRF+Vehicle}\} \times 100$, where, $TFL_{CRF+Vehicle}$ = CRF-induced tail flick latencies in rats pretreated with vehicle of antagonist; $TFL_{CRF+antagonist}$ = CRF-induced tail flick latencies in rats pretreated with antagonist (astressin$_2$-B).

RESULTS

Effect of peripheral corticotropin-releasing factor on tail flick latencies and plasma corticosterone levels (experiment 1)

Peripheral CRF administration produces an increase in tail flick latencies. An increase in CRF-induced tail flick latencies was observed at 5, 10 and 15 min after injection compared to basal tail flick latency ($P < 0.05$) and corresponding tail flick latencies in vehicle treated animals (*Fig. 2a*). The CRF-induced analgesic effect was accompanied by an elevation of plasma corticosterone levels at 20 min after injection (*Fig. 2b*).

Effect of central corticotropin-releasing factor on tail flick latencies and on corticosterone plasma levels (experiment 2)

Histological examination revealed that 33 of the 37 implanted animals had injection sites within the dorsal, dorsolateral and lateral parts of the left PAGM (*Fig. 3A*). Additional 4 animals had injection sites outside the PAGM that included deep gray and white layers of the superior colliculus (n = 3) and dorsal tegmentum bundle (n = 1) and were used as additional controls.

Central (intra-PAGM) CRF administration caused dose-dependent analgesic effect at 15 min after injection ($F(3,32) = 13.25$; $P < 0.001$) (*Fig. 3A*). Intra-PAGM CRF injection at a dose of 0.007 µg/rat did not affect tail flick latencies compared with vehicle of CRF. CRF at the dose of 0.07 µg/rat resulted in an increase of tail flick latencies compared to the dose of 0.007 µg/rat and vehicle of CRF ($P < 0.05$). Increasing the dose of CRF up to 0.7 µg/rat led to a further increase in tail flick latencies ($P < 0.05$). Since the increase of tail flick latencies after CRF administration at a dose of 0.7 µg/rat constituted more than 50% compared with baseline tail flick latencies, this dose was selected for further investigation. *Fig. 3Ba* demonstrates absolute tail flick latencies induced by this dose of CRF, at 15 min after injection. Intra-PAGM CRF administration resulted an increase in tail flick latencies compared to baseline tail flick latencies and tail flick latencies after injection of saline ($F(2,17) = 5.26$; $P < 0.05$) (*Fig. 3Ba*). Intra-PAGM CRF-induced increase in tail flick latencies was accompanied by an increase in corticosterone plasma level at 15 min after injection (*Fig. 3Bb*).

CRF administration into areas outside the PAGM (superior colliculus and midbrain tegmentum) did not cause any changes in tail flick latencies compared to baseline tail flick latencies and

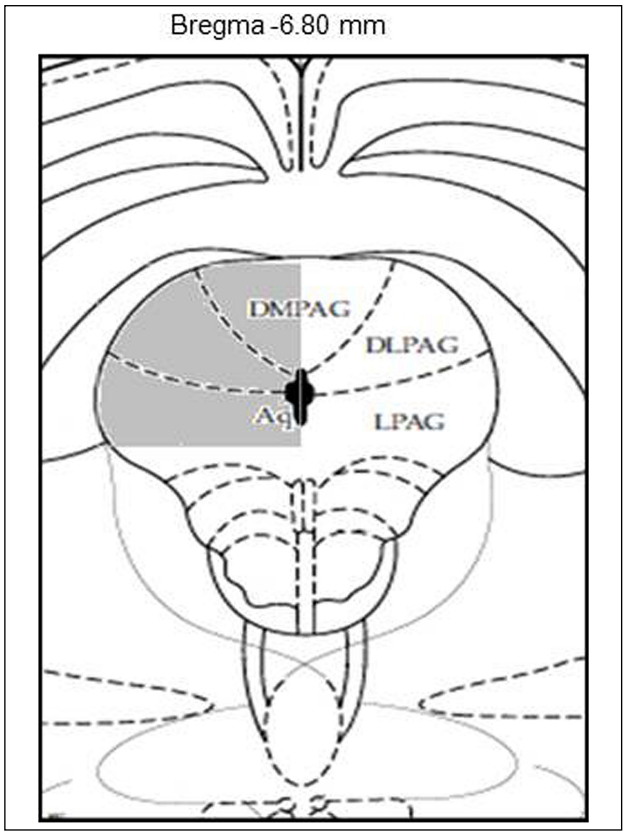

Fig. 1. Schematic diagram of the rat brain section showing the injection sites within the midbrain periaqueductal gray. The brain section was given 6.8 mm posterior to bregma based on the stereotaxic atlas (32). Filled area corresponds to the whole area where the microinjections were placed. The injection area included dorsomedial (DMPAG), dorsolateral (DLPAG) and lateral (LPAG) periaqueductal gray. Aq, aqueduct (Sylvius).

saline-induced tail flick latencies (*Fig. 3Ba*), which indicates the specificity of CRF action on pain sensitivity within the dorsal, dorsolateral and lateral parts of the PAGM.

Effect of astressin$_2$-B on peripheral corticotropin-releasing factor-induced analgesic effect (experiment 3).

In this experiment 39 of 50 implanted rats had injection sites within the dorsal, dorsolateral and lateral parts of PAGM. 11 rats had injection sites outside the PAGM (superior colliculus (n = 8); aqueduct (Sylvius) (n = 2), lateral ventricle (n = 1) were excluded from analysis.

CRF caused the analgesic effect in animals that were given antagonist vehicle before CRF injection. The CRF-induced analgesic effect manifested at 10 and 15 min compared to the corresponding tail flick latencies after saline administration ($P < 0.05$) and tail flick latencies before CRF administration (Bas2) ($P < 0.05$) (*Fig. 4*).

Astressin$_2$-B itself did not influence the baseline somatic pain sensitivity. Tail flick latencies in rats pretreated with astressin$_2$-B and rats pretreated with vehicle of astressin$_2$-B were not significantly different from their baseline tail flick latencies and between themselves (*Fig. 4*). Pretreatment with astressin$_2$-B affected peripheral CRF-induced analgesic effect. Effects of treatment ($F(3,50) = 5.41$), time ($F(3,50) = 3.92$) and their interaction ($F(9,50) = 4.68$) were significant ($P < 0.01$) (*Fig. 4*). Intra-PAGM administration of astressin$_2$-B attenuated peripheral CRF-induced analgesic effect at 10 min after CRF injection ($P < 0.05$). The percentage of suppression of intraperitoneal CRF-induced analgesic effect by astressin$_2$-B made up 20 ± 6% (n = 9).

Effect of astressin2-B on central corticotropin-releasing factor -induced analgesic effect (experiment 4)

In this experiment 27 of 33 implanted rats had injection sites within the dorsal, dorsolateral and lateral parts of the PAGM and only those rats were chosen for analysis. 6 of the 33 animals had

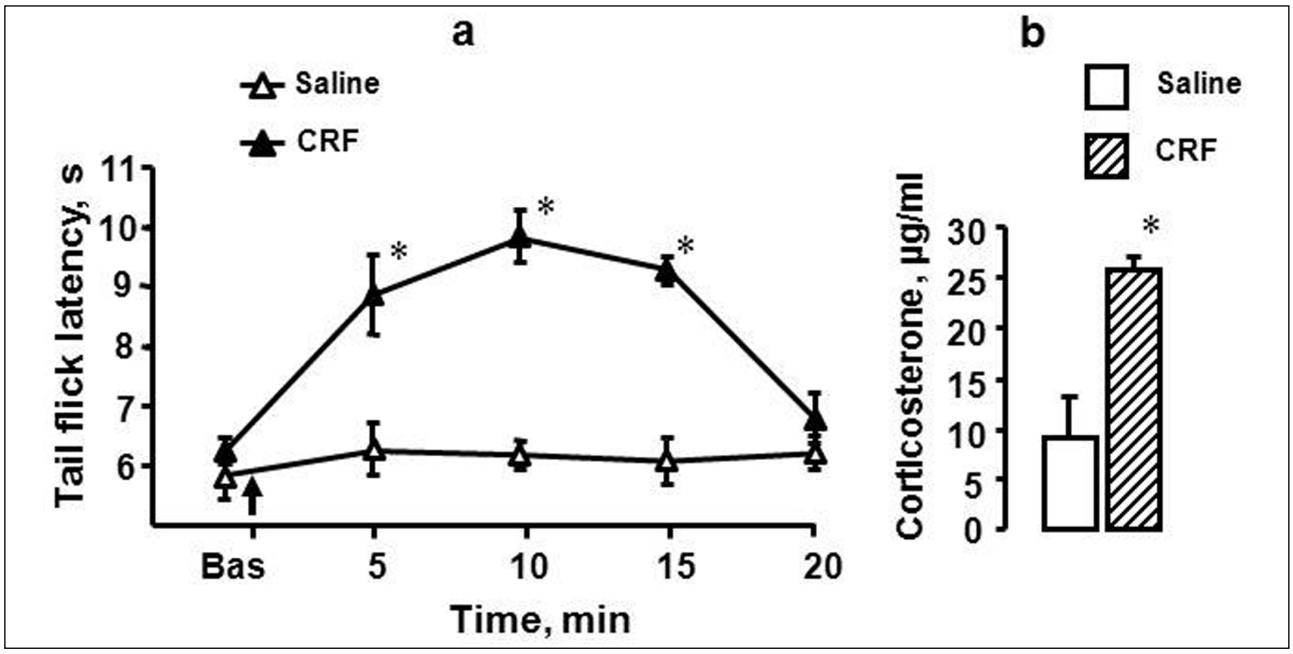

Fig. 2. Effect of peripheral CRF administration on tail flick latencies (a) and plasma corticosterone levels (b) in conscious rats. CRF was injected intraperitoneally at a dose of 40 µg/kg. Plasma corticosterone levels were measured immediately after completion of tail flick latency testing (at 20 min after CRF or saline injection). Significant differences at $P < 0.05$* verus baseline tail flick latency (Bas) or saline. Arrow indicates a moment of CRF or saline injection; n = 7 – 10 per group.

injection site outside the PAGM (in the superior colliculus) and were excluded from analysis.

CRF produced the analgesic effect in the animals pretreated with vehicle. These rats showed an increase in the tail flick latencies at the 10 and 15 min compared to tail flick latencies before CRF administration (Bas2) ($P < 0.05$) and compared with the corresponding tail flick latencies after saline administration ($P < 0.05$) (*Fig. 5*).

As in experiment 3, intra-PAGM astressin2-B did not cause changes in baseline pain sensitivity, but affected the central CRF-induced analgesic effect ($F(3,24) = 3.10$; $P < 0.05$) (*Fig. 5*). Pretreatment with astressin$_2$-B eliminated CRF-induced analgesic effect at 10 and 15 min after CRF administration. CRF-induced tail flick latencies after astressin2-B injection did not differ from the corresponding tail flick latencies after saline administration, but were significantly lower than CRF-induced tail flick latencies in the rats without antagonist (vehicle + CRF versus astressin$_2$-B + CRF, $P < 0.05$). percentage of suppression of intra-PAGM CRF-induced analgesic effect by astressin$_2$-B made up 27 ± 6% (n = 6) and 36 ± 11% (n = 5) at 10 and 15 min, respectively.

DISCUSSION

In the present study we have shown that intra-dorsal PAGM astressin$_2$-B had not effect on baseline somatic pain sensitivity, but attenuated the analgesic effect induced by central as well as peripheral CRF administration. The obtained results suggest that the CRF-R2 receptors located in the PAGM are involved in peripheral, as well as central CRF-induced analgesic effect on somatic pain sensitivity in rats.

The present results confirmed our previous data on the role of CRF in somatic pain regulation (10, 31). Here we found that intra-PAGM CRF administration produces analgesic effect, which is mediated by CRF-R2 receptors within the PAGM. At the same time CRF-induced analgesic effect was not prevented by

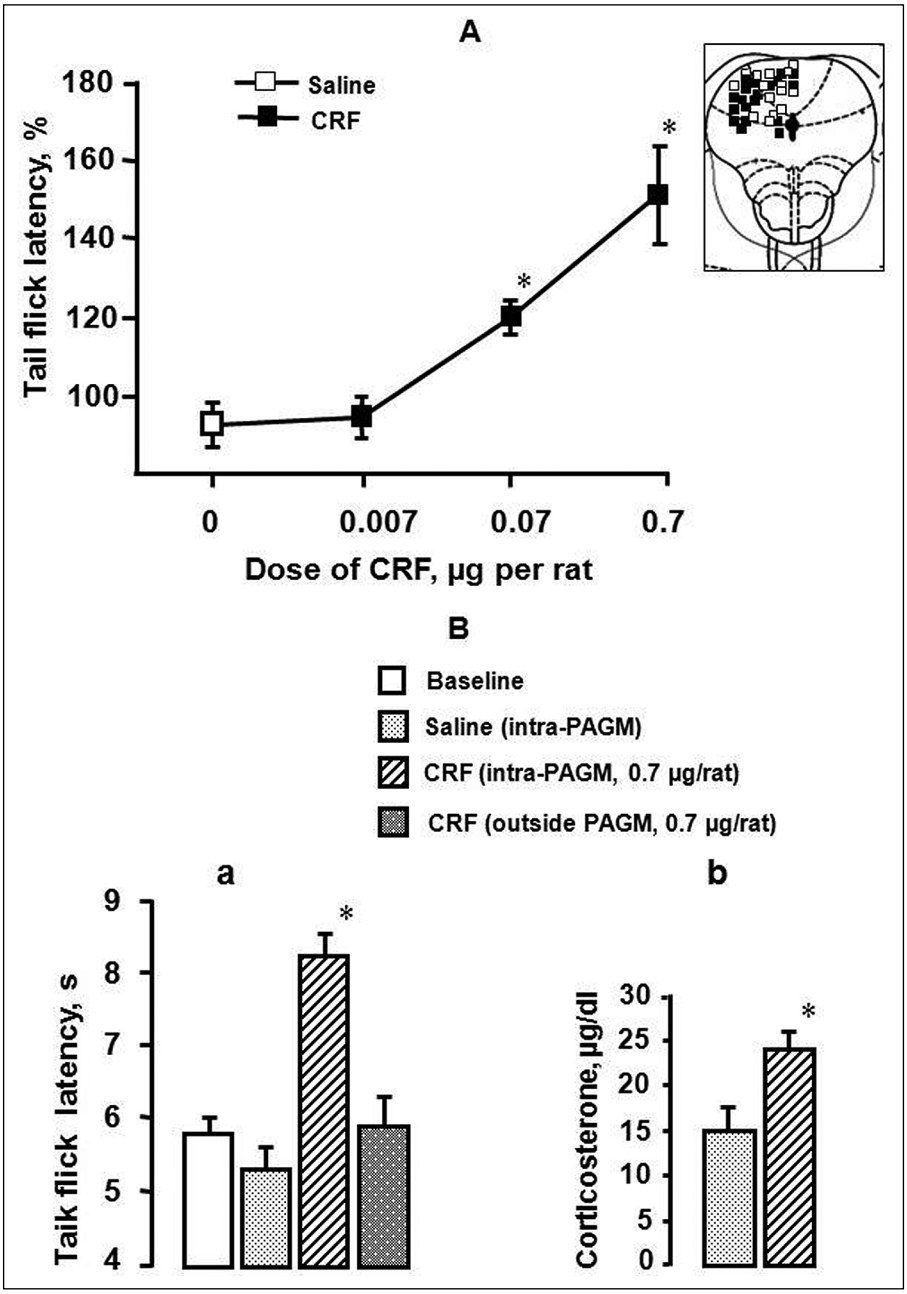

Fig. 3. Effect of central administration of CRF on tail flick latencies and plasma corticosterone levels in conscious rats.
(A) Dose-dependent effect of intra-PAGM administration of CRF on tail flick latencies measured at 15 min after intra-PAGM CRF administration. Tail flick latencies were expressed as percent of corresponding baseline tail flick latencies. Significant difference at $P < 0.05$* - from all groups; n = 4 – 14 per group. The diagram of brain section shows the injection sites within the PAGM: filled squares - CRF group; open squares - saline group.
(B) Effect of intra-PAGM administration of the CRF at the dose of 0.7 µg/rat on the tail flick latencies (a) and corticosterone plasma levels (b) at 15 min after injection. Tail flick latencies were given in absolute units (seconds). Significant difference at *$P < 0.05$: from all groups; n = 4 – 9 per group.

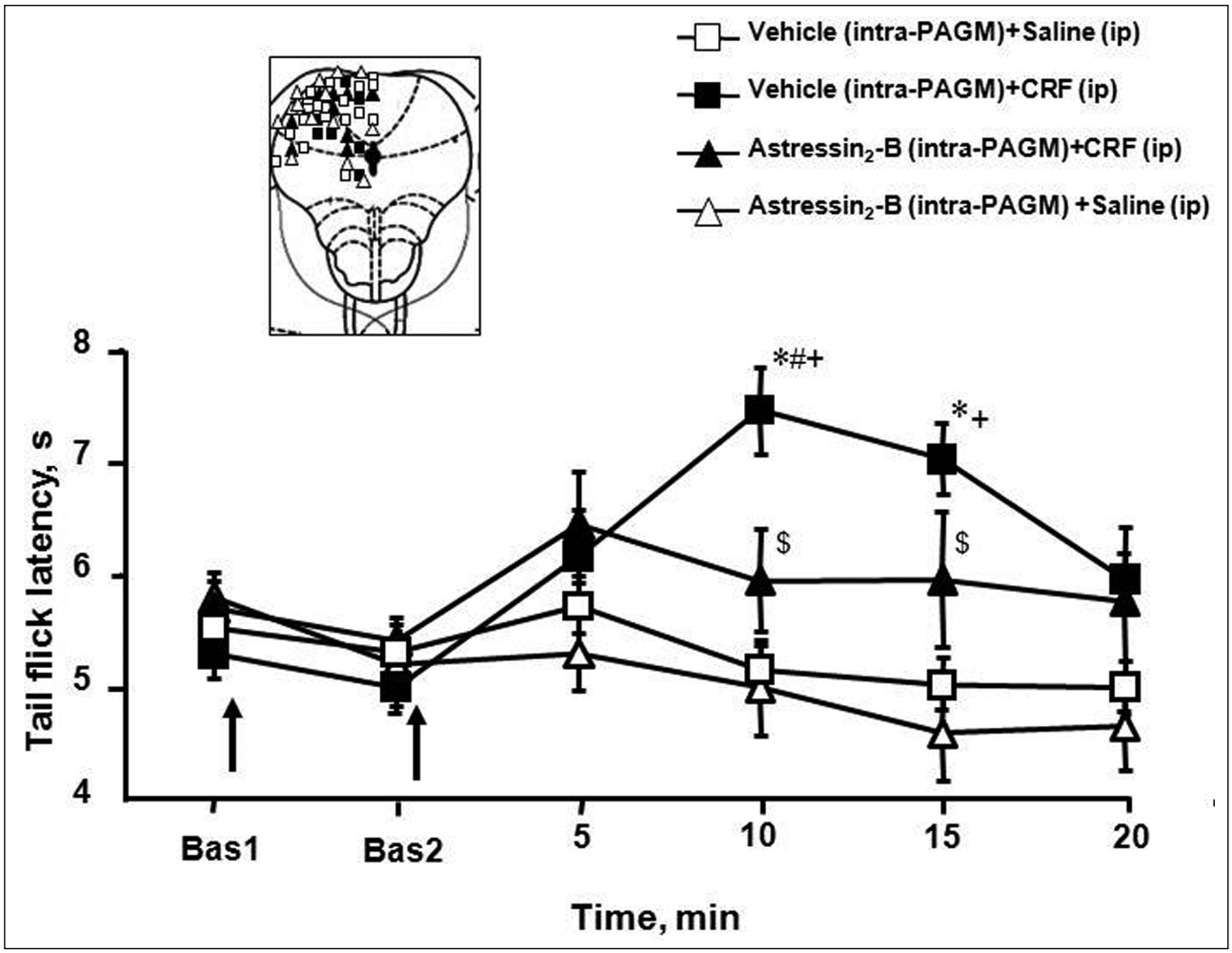

Fig. 4. Effect of central pretreatment with CRF-R2 receptor antagonist astressin$_2$-B on peripheral CRF-induced analgesia. Astressin$_2$-B (1 µg/rat, intra-PAGM) or its vehicle was injected 30 min before CRF (40 µg/kg, i.p.) or saline administration. Significant difference at $P < 0.05$*: versus Bas1 (tail flick latency before astressin$_2$-B or its vehicle administration) and Bas2 (tail flick latency before CRF or its vehicle (saline) administration); # versus <<astressin$_2$-B + CRF>> group; + versus <<vehicle + saline>> or <<astressin$_2$-B + saline>> groups; $ versus <<astressin$_2$-B + saline>> group. Arrows indicate a moment of astressin$_2$-B (vehicle) or CRF (vehicle) injection, respectively. The diagram of brain section shows the injection sites within PAGM; n = 9 – 18 per group.

intra-PAGM CRF-R2 receptor antagonist. The finding suggests that other mechanisms within PAGM, including CRF-R1 receptors, may be also involved in CRF-induced analgesia. Our results support the idea regarding the involvement of the PAGM in the pain inhibiting pathways that are related to CRF. One of the sources of endogenous CRF to the PAGM is the amygdala (40). The amygdala-PAGM pathways are involved in stress-induced analgesia and pain inhibition (41). It might be hypothesized that one of the mechanisms of stress-induced analgesia may be mediated by involving CRF-R2 receptors within the dorsal PAGM. Unfortunately, the type of neurons that express CRF-R2 receptors is unknown. However, it has been shown that CRF receptors are presented in the PAGM cells containing opioid peptides (9). It might be assumed that CRF binds CRF receptors within the PAGM for modulation of opioid action that can contribute to CRF-induced analgesia. This suggestion is supported by our previous data (30) on the role of opioid receptors located in the PAGM in CRF-induced analgesic effect. We showed that intra-PAGM naltrexone pretreatment resulted in reduction of intra-PAGM CRF-induced analgesia (30).

It should be noted that the involvement of CRF in pain inhibition is apparently not dependent on the type of pain, but the mechanisms underlying the intra-PAGM CRF-induced analgesia may vary. In our experiments the involvement of CRF-R2 receptors in CRF-induced analgesia was shown under circumstances of acute (short-term) pain, but their participation failed to be detected under circumstances of tonic (long-term) pain caused by formalin injection in another study (25). It is possible that CRF-R2 receptors are involved in inhibition of acute pain rather than tonic pain.

New fact obtained in the present study concerns the role of central CRF-R2 receptors in peripheral CRF-induced analgesia. According to our previous data the analgesic effect induced by peripheral CRF is mediated by peripheral CRF-R2 receptors (11). Here we have shown that CRF-R2 receptors within the PAGM may be also involved in the CRF-induced analgesic effect. However, it remains unclear how peripherally injected CRF may reach the PAGM and activate CRF receptors into this area. According to the data of literature radiolabeled CRF has not been detected in the brain for 30 min after its intraperitoneal injection at a dose of 50 µg/kg (8).

One of mechanisms explaining the PAGM involvement in peripheral CRF-induced analgesia may be mediated by through alterations of blood pressure that can activate endogenous CRF in the brain. Peripheral CRF is involved in the regulation of cardiovascular functions (42). It is well documented that

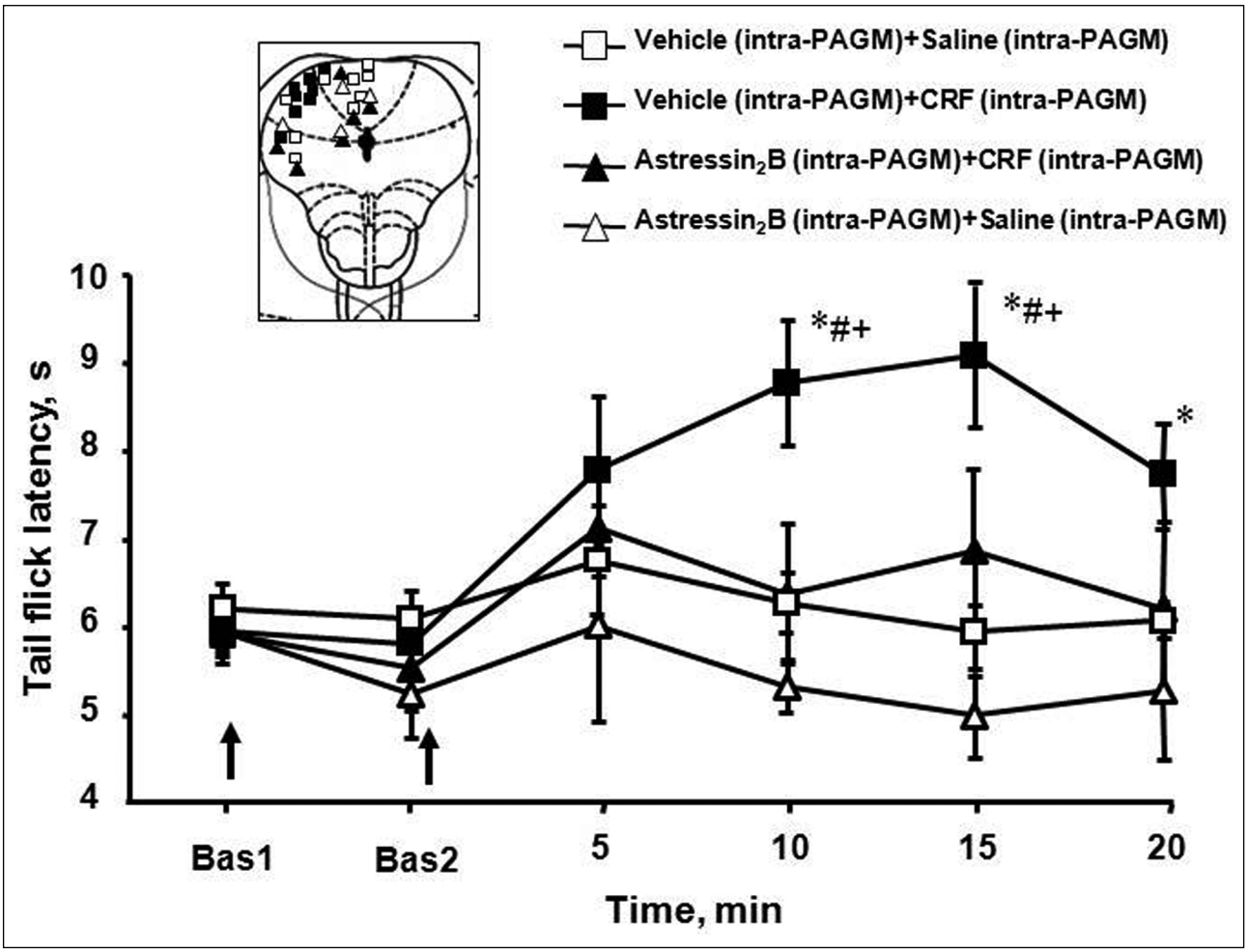

Fig. 5. Effect of central pretreatment with CRF-R2 receptor antagonist astressin$_2$-B on intra-PAGM CRF-induced analgesia. Astressin$_2$-B (1 µg/rat, intra-PAGM) or its vehicle was injected 30 min before CRF (0.7 µg/rat, intra-PAGM) or its vehicle administration. Significant difference at P < 0.05*: versus Bas1 (tail flick latency before astressin$_2$-B or its vehicle administration) and Bas2 (tail flick latency before CRF or its vehicle (saline) administration); # versus <<astressin$_2$-B + CRF>> group; + versus <<astressin$_2$-B + saline>> or <<vehicle + saline>> groups. Arrows indicate a moment of astressin$_2$-B (or vehicle) or CRF (or vehicle) injection, respectively. The diagram of brain section shows the injection sites within the PAGM; n = 4 – 9 per group.

peripherally (intravenously) injected CRF causes a dose-dependent decrease in blood pressure (hypotension) and tachycardia in rats (43-45). The hypotensive effect has been caused by intravenous CRF at a doses of 7.5 – 750 µg/kg with a latency of 5 – 10 s and lasted for 1 hour in conscious rats (45). It would be expected that intraperitoneal administration of CRF at dose of 40 µg/kg is also effective for hypotension production. Hypotension is a stressor that stimulates the endogenous CRF release in the brain structures including paraventricular nucleus of the hypothalamus, central nucleus of the amygdala, locus coeruleus (46, 47). The activation of the locus coeruleus neurons by hypotensive stress is mediated by CRF coming from the central amygdala (47). PAGM is known as one of the brain regions which are sensitive to fluctuations in blood pressure (48, 49). It is possible that CRF is released within PAGM (in response to changes in blood pressure) and binds CRF-R2 receptors which may be involved in pain regulation. Thus, the involvement of CRF-R2 receptors within PAGM in peripheral CRF-induced analgesia might be due to peripheral CRF effect on cardiovascular functions. Nevertheless we cannot exclude completely that peripherally administered CRF may also act *via* CRF receptors at circumventricular organs that are relatively unprotected by the blood-brain barrier. However, the verification of these assumptions is a task for future research.

Central, as well as peripheral CRF-induced analgesic effects were accompanied by an increase in plasma corticosterone suggesting activation of the hypothalamic-pituitary-adrenocortical (HPA) axis. The HPA axis hormones, glucocorticoids and ACTH, participate in regulation of somatic pain sensitivity (50, 51). Therefore, they may contribute to peripheral as well as central CRF-induced analgesic effect. This idea is supported by our previous data. It has been shown that glucocorticoids produced in response to the PAGM stimulation at the 15 min, are involved in analgesia caused by the PAGM stimulation (50). The results of the present study confirmed the PAGM role in the HPA axis activation.

The involvement of glucocorticoids in peripheral CRF-induced analgesia in conscious rats remains to be elucidated. We had shown previously that peripheral CRF causes plasma corticosterone rise at time interval from 8 to 20 min after CRF injection (31). Our present results confirmed an increase in corticosterone levels at 20 min after CRF administration. According to our data, CRF caused an increase in both tail flick latencies and corticosterone levels at 10 – 15 min after injection. CRF-induced increase in tail flick latencies then disappeared, while corticosterone level was elevated at 20 min. Thus, we did not find a correlation (r = 0.31; P = 0.096) between CRF-induced analgesic effect and an increase in corticosterone levels. It might be explained by the fact that

glucocorticoids act in concert with other mechanisms that are also involved in the peripheral CRF-induced analgesic effect. Our previous data (31) and data of literature (52) demonstrate a crucial role of opioids in peripheral CRF-induced analgesic effect in conscious rats. We showed that peripheral (intraperitoneally) CRF-induced effect is eliminated by peripheral (intraperitoneally) naltrexone pretreatment in conscious rats (31). Glucocorticoids are also involved in CRF-induced analgesia, however, their role is not likely to be significant compared to opioids, since, according to our data, the pretreatment with antagonist of glucocorticoid receptors RU 38486 only modulates the peripheral CRF-induced effect, but doesn't eliminate it, like naltrexone (31). It should be noted that corticosterone production may be also dependent on other physiological systems, acting within adrenal glands and brain during stress such as IL-1β and NO systems (53). Glucocorticoids exerting their action through glucocorticoid receptors are involved in the different physiological processes in brain and peripheral organs that allow survival and adaptation during stress (54). Change of somatic pain sensitivity is one of aspects of the stress-induced adaptive response.

It is known that both peripheral and central (intra-PAGM) administration of ACTH cause analgesic effects (50, 55). Peripheral ACTH-induced analgesic effect appears at 8 min and is mediated by opioid receptors in conscious rats (50). We have demonstrated that opioid receptors are involved in peripheral CRF-induced effect. These facts allow us to suggest that ACTH, as well as opioid receptors can be involved in peripheral CRF-induced effects in conscious rats. It is unlikely, however, that ACTH might be involved in central CRF-induced analgesic effect, because analgesia caused by intra-PAGM administration of ACTH is mediated by non-opioid mechanisms (55).

Pharmacological treatment of pain, anxiety and depression continues to be an important clinical problem (56). Data obtained can be a useful approach for uncovering new therapeutic targets for the treatment of pain and stress-related disorders.

In conclusion, the results of the present study suggest that peripheral, as well as central analgesic effects of CRF on somatic pain sensitivity may be mediated by CRF-R2 receptors located in the PAGM.

Acknowledgements: Study was supported by Russian Science Foundation (RSF), No 14-15-00790. All co-authors Natalia I. Yarushkina, Tatiana R. Bagaeva, and Ludmila P. Filaretova are supported by the grant of Russian Science Foundation.

Conflict of interests: None declared.

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Received: October 26, 2015
Accepted: July 19, 2016

Author's address: Dr. Natalia Yarushkina, Laboratory of Experimental Endocrinology, Pavlov Institute of Physiology, Russian Academy of Sciences, 6 nab. Makarova, St. Petersburg 199034, Russia.
E-mail: yarni60@mail.ru