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Expression of corticotropin-releasing factor in inflamed tissue is required for intrinsic peripheral opioid analgesia

(nociception/pain/neuropeptides/antisense oligonucleotides)

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ABSTRACT **Immune cell-derived opioid peptides can activate opioid receptors on peripheral sensory nerves to inhibit inflammatory pain. The intrinsic mechanisms triggering this neuroimmune interaction are unknown. This study investigates the involvement of endogenous corticotropin-releasing factor (CRF) and interleukin-1β (IL-1). A specific stress paradigm, cold water swim (CWS), produces potent opioid receptor-specific antinociception in inflamed paws of rats. This effect is dose-dependently attenuated by intraplantar but not by intravenous α-helical CRF. IL-1 receptor antagonist is ineffective. Similarly, local injection of antiserum against CRF, but not to IL-1, dose-dependently reverses this effect. Intravenous anti-CRF is only inhibitory at 10^4-fold higher concentrations and intravenous CRF does not produce analgesia. Pretreatment of inflamed paws with an 18-mer 3′-3′-end inverted CRF-antisense oligodeoxynucleotide abolishes CWS-induced antinociception. The same treatment significantly reduces the amount of CRF extracted from inflamed paws and the number of CRF-immunostained cells without affecting gross inflammatory signs. A mismatch oligodeoxynucleotide alters neither the CWS effect nor CRF immunoreactivity. These findings identify locally expressed CRF as the predominant agent to trigger opioid release within inflamed tissue. Endogenous IL-1, circulating CRF or antiinflammatory effects, are not involved. Thus, an intact immune system plays an essential role in pain control, which is important for the understanding of pain in immunosuppressed patients with cancer or AIDS.**

Pain, evoked by local injury and inflammation, can be controlled effectively by peripherally acting opioids (1). The local inflammatory response initiates the synthesis of opioid peptides within resident immune cells (2). Upon release, these peptides produce analgesia mediated by opioid receptors on peripheral sensory nerves (3). The endogenous impulse initiating this release is unknown. Corticotropin-releasing factor (CRF) and interleukin-1β (IL-1) are interesting candidates because both can liberate opioid peptides from the pituitary and from immune cells (3, 4). Furthermore, they are detectable in the circulation during stress and they are also present in inflamed tissue (5, 6). However, neither has been examined for its involvement in intrinsic pain inhibition.

In this study, we hypothesized that CRF or IL-1 might be the endogenous trigger for local opioid release and subsequent activation of peripheral opioid receptors in inflamed tissue. Previously, we have shown that a specific environmental stressful stimulus [cold water swim stress (CWS)] elicits potent peripheral opioid analgesia without involvement of central mechanisms (7). Using this paradigm, we now examined whether local or systemic blockade of endogenous CRF or IL-1 interferes with this stress-induced antinociception. This blockade was achieved by three different approaches. (*i*) We used the specific receptor antagonists, α-helical CRF and interleukin-1 receptor antagonist (IL-1ra). (*ii*) We applied specific antisera for *in vivo* immunoneutralization. (*iii*) We pretreated rats with antisense oligodeoxynucleotides (ODN) to inhibit peptide synthesis and evaluated the success of this treatment by immunocytochemistry and by radioimmunoassay (RIA).

MATERIALS AND METHODS

Subjects. Male Wistar rats weighing 180–225 g were purchased from Charles River Breeding Laboratories and housed individually in cages lined with ground corn cob bedding. Room temperature was maintained at 22 ± 0.5°C with a relative humidity between 40% and 60%. Standard laboratory rodent chow and tap water were available ad libitum. All experiments were conducted in the light phase of a 12 h/12 h (7 a.m./7 p.m.) light-dark cycle. Animals were handled three times before any testing was performed. The guidelines on ethical standards of the International Association for the Study of Pain were followed. Animal facilities were accredited by the American Association of Laboratory Animal Care and experiments were approved by the Institutional Animal Care and Use Committee of the Division of Intramural Research/National Institute on Drug Abuse/National Institutes of Health in accordance with Institute of Laboratory Animal Resources (National Research Council), Department of Health, Education and Welfare, Publication (NIH) 85–23, revised 1985.

Drugs and Immunoreagents. Drugs used were naloxone hydrochloride (Sigma), human/rat CRF (Sigma), CRF antagonist (α-helical CRF) (Sigma), recombinant IL-1ra (R & D Systems), Freund's complete adjuvant (Calbiochem), and halothane (Halocarbon Laboratories, Hackensack, NJ). Antisera used were the IgG fractions of rabbit anti-human/rat CRF (anti-CRF) (R & D Antibodies), and goat anti-murine interleukin-1β (anti-IL-1β) (R & D Systems); a different anti-human/rat CRF antiserum was employed in the RIA kit (Peninsula Laboratories); the anti-CRF had crossreactivites of < 0.5% for ovine CRF and did not crossreact with sauvagin, ACTH, or other related peptides, according to the manufacturers' specifications; anti-IL-1β had 25% crossreactivity for recombinant human IL-1β and none for other cytokines; and normal rabbit IgG (Sigma) was used as a control for nonspecific binding. Doses were calculated as the free base and drugs were dissolved in sterile isotonic saline (naloxone) and sterile water (CRF, α-helical CRF, IL-1ra, anti-CRF, anti-IL-1β, normal rabbit IgG). Routes and volumes of drug administration were intraplantar (i.pl.) (0.1 ml) or intravenous (i.v.) (0.2

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Abbreviations: IL-1, interleukin-1β; CRF, corticotropin-releasing factor; IL-1ra, interleukin-1 receptor antagonist; CWS, cold water swim stress; PPT, paw pressure threshold; ODN, oligodeoxynucleotide; i.pl., intraplantar; i.v., intravenous; ANOVA, analysis of variance; RIA, radioimmunoassay; s.c., subcutaneously.

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ml) into a tail vein through an indwelling 24-gauge Teflon catheter (Baxter Scientific Products, McGaw Park, IL). All substances were given 5 min before CWS.

ODNs. According to the nucleotide sequence of the rat CRF gene (8), an 18-mer 3′–3′ end inverted antisense ODN (5′-AGC-CGC-ATG-TTT-AGG-GG-3′–3′-C-5′) that corresponds to the translation initiation site of the CRF mRNA (nucleotides 1189–1206) was synthesized and purified by high performance liquid chromatography (HPLC) (Genosys, The Woodlands, TX). As a control, a mismatched 3′–3′ end inverted ODN (5′-AGC-CGG-TTG-ATT-AGC-GG-3′–3′-C-5′), containing the same base composition, was synthesized and HPLC purified (Genosys). Both the antisense and the mismatch construct had an average melting temperature of 63–65°C. They were compared to the GenBank database (FASTA search) and found not to be complementary to any registered nucleotide sequences. ODN were dissolved in saline at a final concentration of 50 μg per 100 μl for each injection. The half-life of 3′–3′-end inverted ODN is much longer (≈30 h) (9) than the half-life of CRF (≈30 min) (10). Based on these facts and on preliminary experiments, ODN were injected either i.pl. or subcutaneously (s.c.) 30 h, 18 h, and 6 h before CWS, RIA, or immunocytochemical experiments (i.e., on days 3 and 4 after induction of the inflammation).

Induction of Inflammation. Rats received an i.pl. injection of 0.15 ml of Freund's complete adjuvant into the right hindpaw under brief halothane anesthesia. Control animals were anesthetized but not injected. The paw volume was monitored using a plethysmometer (Ugo Basile, Varese, Italy). The inflammation remained confined to the right paw throughout the observation period. All experiments were conducted 4 days after inoculation.

Algesiometry. Animals (n = 6–8 per group) were gently restrained under paper wadding and nociceptive thresholds were evaluated using a modified Randall–Selitto paw pressure test, as described (7). At all times, the experimenter was blind to the condition employed. After baseline measurements, rats received an injection of either the test substance or vehicle. Five minutes later they were placed into a tub containing water at 1–2°C to swim for 1 min as described (7). At the end of this swim stress, they were removed from the water, immediatly dried with paper wadding, and paw pressure threshold (PPT) was reevaluated repeatedly thereafter.

Experiment 1. These experiments evaluated whether the receptor antagonists naloxone (18 μg), α-helical CRF (0.2–8 ng), IL-1ra (0.001–1 μg), or vehicle (saline), given i.pl. or i.v., could influence antinociceptive effects induced by CWS. These drugs were given alone or 5 min before CWS, and nociceptive thresholds were evaluated 7, 12, and 22 min after injection. Dose-response curves were constructed at the time of peak effects of α-helical CRF and IL-1ra.

Experiment 2. Next we examined whether passive immunization with i.pl. anti-CRF (0.0001–10 μg) or anti-IL-1β (0.0001–10 μg) alters CWS-induced antinociception. In control experiments we used anti-CRF (10 μg) that was heat denatured at 95°C for 45 min, normal rabbit IgG (10 μg), and antisera without CWS. In comparison, the effects of i.v. anti-CRF (0.2–400 μg) were examined. To mimic a possible systemic increase in circulating CRF, 10 ng of CRF were given i.v. in animals that were not subjected to CWS and antinociceptive effects were compared to those induced by CWS.

Experiment 3. These experiments assessed whether pretreatment of rats with i.pl. CRF antisense ODN (50 μg, injected at 30 h, 18 h, and 6 h before the experiments, respectively) could attenuate CWS-induced antinociceptive effects. Control animals received either 50 μg of mismatch ODN i.pl., 100 μl of vehicle i.pl., or 50 μg antisense ODN s.c. at the same time intervals. Nociceptive thresholds were determined immediately after CWS and paw volumes were measured separately.

Immunocytochemistry. Following the various pretreatments (vehicle i.pl., antisense i.pl., mismatch i.pl., antisense s.c.), rats were deeply anesthetized with pentobarbital (60 mg/kg i.p.) and perfused via the ascending aorta with 50 ml of normal saline, followed by 400 ml of ice-cold solution of 4% paraformaldehyde in 0.1 M PBS (pH 7.4). The skin and adjacent s.c. tissue were dissected from the plantar surface of both hind paws, immersed in the same fixative for an additional 2 h, and washed overnight in 0.1 M PBS containing 15% sucrose at 4°C. Longitudinal sections (20-μm thick) of subcutaneous tissue were mounted on chrome-alum subbed slides, briefly washed in PBS, and then immunostained using Vectastain ABC Kit (Vector Laboratories). Unless otherwise stated, all incubations were done at room temperature. PBS was used for washing after each step. Endogenous peroxidase was quenched with 0.3% H_2O_2 in 10% methanol/PBS for 30 min. Slides were then incubated with 5% normal goat serum for 1 h, blotted (without washing), and overlaid with the primary antiserum (1:1000 dilution of anti-CRF, R & D Antibodies) at 4°C overnight. Thereafter, they were incubated with the biotinylated secondary antibody (Vector Laboratories) for 1 h. Finally, the sections were washed and stained with 3′,3′-diaminobenzidine (DAB) tetrahydrochloride (Sigma) including 0.01% H_2O_2 in 0.05 M Tris-buffered saline (pH 7.6) and 0.8% nickel chloride for 3–5 min, then dehydrated, and mounted. To demonstrate specificity of staining, the following controls were included: (*i*) preadsorption of the primary antiserum with an excess of antigen (10^{-6} M CRF); (*ii*) omission of the primary or secondary antiserum. Cells that displayed a black/gray (specific) staining were identified in contrast to the brown/reddish (nonspecific) staining and were counted by a blinded observer. Four different groups (vehicle

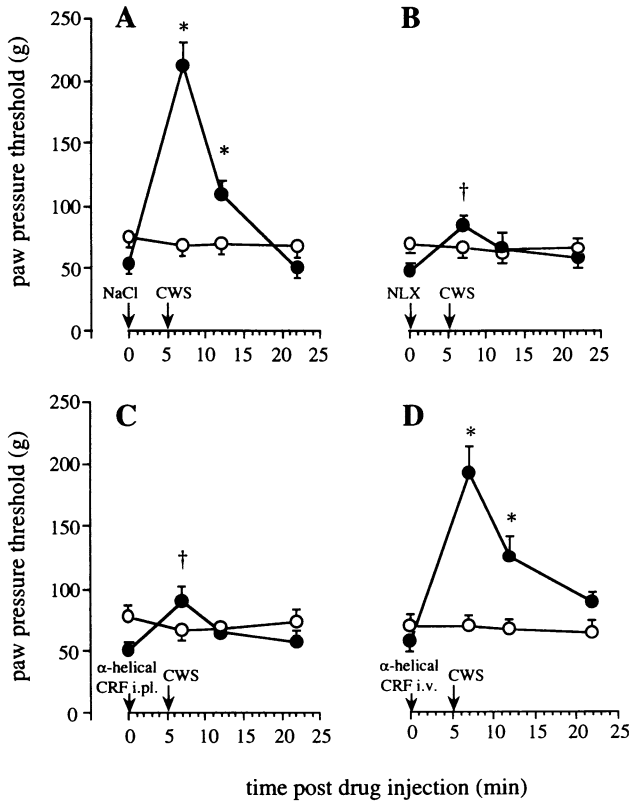

FIG. 1. Effect of i.pl. saline (*A*), i.pl. naloxone (NLX, 18 μg) (*B*), and of i.pl. and i.v. α-helical CRF (2 ng) (*C* and *D*, respectively) on CWS-induced PPT elevations (solid circles, inflamed paws; open circles, noninflamed paws). Baseline PPT were measured at 0 min. Drugs were injected 5 min before CWS. Values are means ± SEM. Asterisk denotes significant differences between baseline and post-CWS values ($P < 0.05$; Friedman and Wilcoxon tests); Dagger denotes significant differences compared to the peak effect of the control (saline) group ($P < 0.05$; Mann–Whitney U test).

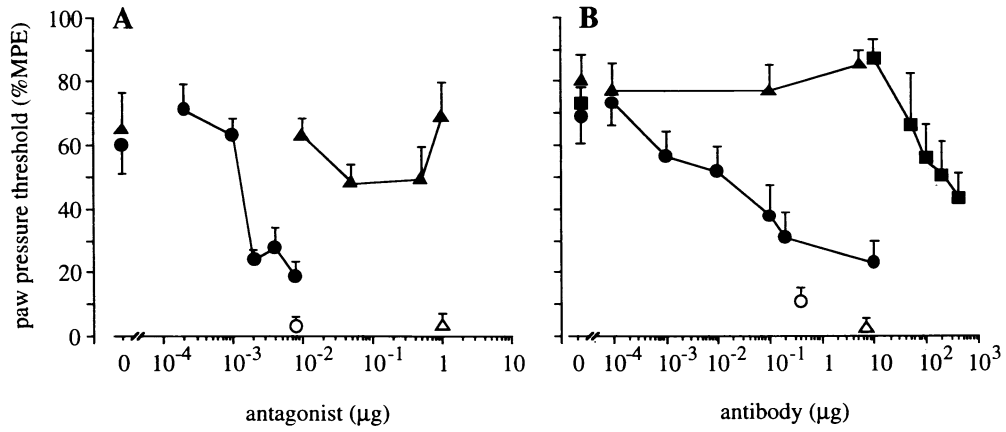

FIG. 2. (*A*) Effects of i.pl. α-helical CRF (solid circles) or i.pl. IL-1ra (solid triangles) on CWS-induced PPT elevations. α-helical CRF dose-dependently antagonized CWS-induced analgesia ($P < 0.05$; regression ANOVA). IL-1ra was inactive ($P > 0.05$; ANOVA). (*B*) Effects of i.pl. (solid circles) or i.v. (solid squares) anti-CRF and of i.pl. anti IL-1β (solid triangles) on CWS-induced PPT elevations. Both i.pl. and i.v. anti-CRF dose-dependently abolished CWS-induced analgesia ($P < 0.05$; regression ANOVA). Intravenous concentrations were 10^4-fold higher than i.pl. (i.pl. ED_{50} = 8.6 ng versus i.v. ED_{50} = 205 μg; the slopes were not significantly different; $P > 0.05$, Student's *t* test). Anti-IL1β was inactive ($P > 0.05$; ANOVA). Data (*A* and *B*) at 0 concentration represent effects of saline injections. Open symbols show the effects of the respective antagonists and antisera without CWS.

i.pl., antisense i.pl., mismatch i.pl., antisense s.c.) of three animals each were used. Two sections per animal and 10 areas of 100 μm^2 per section were counted using a ×40 objective on a Zeiss microscope equipped with a grid containing eyepiece.

RIA. After pretreatments (vehicle i.pl. or antisense i.pl.), animals were euthanized by CO_2 inhalation and subcutaneous tissue was dissected from the plantar surface of inflamed paws. The tissue was weighed and then incubated in 4–6 vol of 0.1 M HCl at 95°C for 10 min. Following homogenization, the samples were loaded on Microcon size 10 filters and centrifuged for 20 min at 12,700 rpm. The samples were then aliquoted, lyophilized, redissolved in RIA buffer, and assayed using a CRF-RIA kit (Peninsula Laboratories) as described (3). Independent assays (each in duplicate) were performed for each pretreatment group (n = 4–5 per group).

Data Analysis. PPT are given as raw values (means ± SEM) or (in dose-response curves) as percentage of maximum possible effect (% MPE) according to the following formula: $(PPT_{\text{post injection}} - PPT_{\text{basal}})/(250 - PPT_{\text{basal}})$. PPT changes over time within the same paw were analyzed by the Friedman test and (post hoc) by the Wilcoxon test. The Mann–Whitney *U* test was applied for comparisons between independent groups. Paw volume, immunocytochemical, and RIA findings in antisense experiments were analyzed by the Mann–Whitney *U* test and by analysis of variance (ANOVA). For dose-response curves an ANOVA and a subsequent linear regression ANOVA were performed to test the zero slope hypothesis. ED_{50} was calculated using the program by Tallarida and Murray (11). Slopes of the dose-response curves were compared by Student's *t* test. Differences were considered significant if $p < 0.05$ (two-tailed).

RESULTS

Algesiometry. *Experiment 1.* CWS produced a significant elevation of PPT in inflamed, but not in noninflamed paws (Fig. 1*A*) that reached its maximum at 2 min after CWS and returned to baseline within 10–17 min. This increase was not influenced by prior i.pl. injection of saline (Fig. 1*A*). However, it was antagonized by i.pl. naloxone (18 μg) (Fig. 1*B*) and by i.pl. (Fig. 1*C*), but not by i.v. α-helical CRF (2 ng) (Fig. 1*D*). The attenuation by i.pl. α-helical CRF was dose-dependent (Fig. 2*A*). IL-1ra did not inhibit CWS-induced PPT elevations at concentrations between 0.001 and 1 μg (Fig. 2*A*). Given alone, naloxone (18 μg), α-helical CRF (10 ng), or IL-1ra (1 μg) did not elicit any effects on PPT ($P > 0.05$; Friedman test) (Fig. 2*A*).

Experiment 2. CWS-induced PPT elevations were dose-dependently reversed by i.pl. anti-CRF (Fig. 2*B*). Neither heat-denatured anti-CRF (10 μg) nor normal rabbit IgG (10 μg) attenuated CWS effects (data not shown). Anti-CRF, given i.v., also inhibited CWS-induced effects (Fig. 2*B*), but the required concentrations were 10^4-fold higher compared to the i.pl. route (i.pl. ED_{50} = 8.6 ng versus i.v. ED_{50} = 205 μg). Intravenous injection of human/rat CRF (10 ng) at a 100-fold higher concentration than the average plasma CRF level (10 pg/ml) (12) did not produce any significant changes of PPT (data not shown). Anti-IL-1β did not alter CWS-induced PPT elevations (Fig. 2*B*). Administration of each antiserum alone had no effect ($P > 0.05$; Friedman test) (Fig. 2*B*).

Experiment 3. Pretreatment of inflamed paws with CRF antisense ODN significantly reduced PPT elevations elicited by CWS (Fig. 3). When antisense ODN were given s.c. or when mismatch ODN were used, CWS-induced PPT elevations were not attenuated (Table 1, Fig. 3). Paw volumes were not significantly different between groups treated with i.pl. saline, i.pl. antisense ODN, s.c. antisense ODN, or i.pl. mismatched ODN ($P > 0.05$, ANOVA) (Table 1).

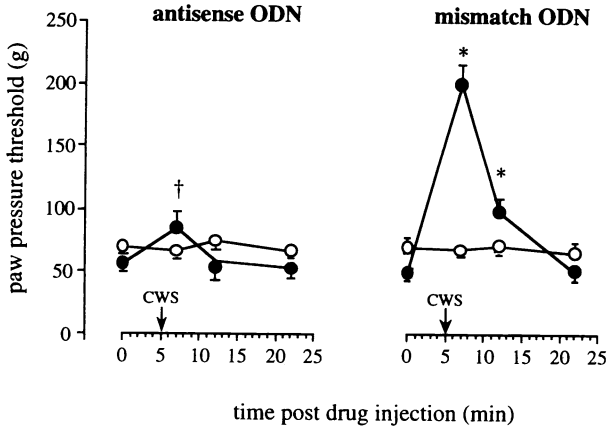

FIG. 3. Effects of i.pl. pretreatment with CRF antisense (50 μg) and mismatch (50 μg) ODNs on CWS-induced PPT elevations. Solid circles, inflamed paws; open circles, noninflamed paws. Similar to the saline group (see Fig. 1*A*), asterisks denote significant differences between baseline and post-CWS values ($P < 0.05$; Friedman and Wilcoxon tests) and the dagger denotes significant differences compared to the peak effect of the saline group ($P < 0.05$; Mann–Whitney *U* test).

Table 1. Changes in paw volume, CWS-induced PPT elevations (analgesia), and number of CRF-immunoreactive cells in inflamed paws after pretreatment with different ODN or saline (30, 18, and 6 h before experiments)

Treatment	PPT, g, after 1 min CWS	Paw volume, ml	CRF–immunoreactive cells/100 μm^2
Saline (i.pl.)	182.7 ± 16.9	4.61 ± 0.18	3.0 ± 0.14
Antisense ODN (s.c.)	170.0 ± 13.1	4.60 ± 0.14	3.0 ± 0.17
Antisense ODN (i.pl.)	84.7 ± 13.7*	4.60 ± 0.18	1.6 ± 0.02*
Mismatch ODN (i.pl.)	202.2 ± 16.1	4.61 ± 0.16	2.8 ± 0.07

*Statistical significances (Mann–Whitney U test; $P < 0.05$) in comparison with saline group. Means ± SEM are given.

Immunocytochemistry. Staining of healthy subcutaneous paw tissue with anti-CRF yielded immunoreactivity in vascular endothelial cells (not shown). CRF immunostaining of inflamed subcutaneous paw tissue of control (vehicle pretreated) animals revealed strong immunoreactivity in numerous inflammatory cells (apparently macrophages and lymphocytes), and in fibroblasts and vascular endothelial cells, as identified by their morphological appearances (Fig. 4*A*). These cells occurred singly or in groups in the periphery of inflammatory foci within the plantar subcutaneous tissue. This immunostaining was abolished after preadsorption or omission of the primary antiserum (Fig. 4*C*). In rats pretreated with i.pl. antisense ODN, CRF immunoreactivity was clearly diminished (Fig. 4*B*) and the number of CRF-labeled cells was significantly reduced (by 48%) compared with saline-treated control animals (Table 1). Treatment with i.pl. mismatch ODN or with s.c. antisense ODN did not alter the number of CRF-labeled cells compared to control animals (Table 1). Gross morphology (edema and infiltration of inflammatory cells into the subcutaneous tissue) was similar among the four groups.

RIA. In vehicle pretreated animals the amount of CRF extracted from inflamed paws was 1475.2 ± 136.1 pg/g of wet tissue. CRF-antisense pretreatment significantly reduced the CRF content to 937.4 ± 84.9 pg/g of wet tissue ($P < 0.01$; Mann–Whitney U test).

DISCUSSION

The stress paradigm used in the present experiments produces pronounced antinociception in inflamed but not in noninflamed tissue. This effect is reversible by local administration of naloxone, indicating that peripheral opioid receptors are activated by endogenous opioids. Opioid receptors are expressed in dorsal root ganglia and present on peripheral sensory nerve terminals both in inflamed and in healthy tissue (13–15), but opioid peptides, synthesized and released from immune cells, are found in inflamed tissue only (2, 13). This apparently accounts for the differential CWS effects in inflamed and noninflamed paws. Since minimal changes in stress severity can activate different pain control systems (16, 17), it is important to note that the present paradigm does not involve central pathways of stress-induced analgesia (7). Rather, it is a tool to selectively investigate intrinsic antinociceptive mechanisms at the inflammatory site, which are based on a release of immune cell-derived opioids and their interaction with opioid receptors on sensory nerves (2, 13).

What is the endogenous agent that induces the release of opioid peptides from immune cells? CRF and IL-1β are of particular interest because they stimulate such release *in vitro* and *in vivo* (3, 4). Local, but not i.v., administration of the antagonist α-helical CRF dose-dependently attenuated stress-induced antinociception, suggesting a peripheral receptor-specific action of endogenous CRF. Consistently, CRF receptors have been demonstrated on splenocytes, macrophages, B- and T-lymphocytes (18–20) and these receptors are upregulated in inflamed tissue (20). However, they are practically absent on peripheral nerve endings and in noninflamed tissue, indicating that the effects of CRF are mediated exclusively by its receptors on immune cells (20). IL-1 receptors are also present on inflammatory cells (5), but neither i.pl. administration of the antagonist IL-1ra nor anti-IL-1β altered the CWS effect, suggesting that IL-1β is not involved at this stage of the inflammatory process. Indeed, IL-1 gene expression has been shown to peak very early, i.e., 1–2 h after inflammatory stimuli (21, 22) and, therefore, it is conceivable that the amount of IL-1 present after 4 days of inflammation is not sufficient or functionally relevant.

Thus, the major endogenous stimulus for opioid release in this situation appears to be CRF. To identify the anatomical source of this peptide we used i.pl. and i.v. application of specific antisera. Passive immunization with i.pl. anti-CRF dose-dependently abolished antinociceptive effects following CWS. This neutralization by anti-CRF was specific, because heat-denatured anti-CRF and nonimmune serum were ineffective. Furthermore, the CWS-induced antinociceptive effect was only attenuated by 10^4-fold higher doses of i.v. anti-CRF and it was not mimicked by i.v. CRF, administered in a concentration that is 100-fold higher than usual plasma levels (12). Taken together, these results strongly suggest that endogenous CRF originates from the inflammatory site and that plasma-derived CRF is not involved in producing this localized stress-induced effect.

Indeed, we found significant amounts of immunoreactive CRF in the inflamed tissue, which is consistent with previous studies in different models of inflammation (6, 19). The concentration of local CRF was much higher than in plasma, supporting the notion that CRF might act in a paracrine manner (6). If local CRF plays a functional role, suppression of local synthesis of CRF should result in attenuation of the antinociceptive response. To this end, we used a CRF antisense–ODN targeted against the translation initiation site of the rat CRF mRNA. In view of the recently increased scrutiny toward *in vivo* antisense experiments (23), we chose a new class of ODN with a regular phosphodiester backbone and with a 3′–3′ end inversion to minimize unspecific effects and to prevent degradation by exonucleases, respectively (9). Such ODN have been shown to inhibit gene expression both *in vitro* and *in vivo* (9). This CRF–antisense pretreatment successfully reduced both CRF immunostaining and the amount of extracted CRF in inflamed paws. The specificity of this pretreatment is demonstrated by the fact that two different antisera detected a decrease in CRF immunoreactivity. In parallel, CRF–antisense ODN abolished CWS-induced analgesia. Pretreatment with mismatch ODN neither reduced CRF immunoreactivity nor the CWS effect, further supporting specificity of the antisense treatment. Gross inflammatory signs such as swelling, edema, and cellular infiltration remained unchanged, indicating that, in contrast to other models (6, 24), CRF does not influence Freund's adjuvant-induced paw inflammation. Together these findings indicate that locally produced CRF is essential for the generation of intrinsic peripheral opioid analgesia, but that it has no major influence on the development of this inflammatory process.

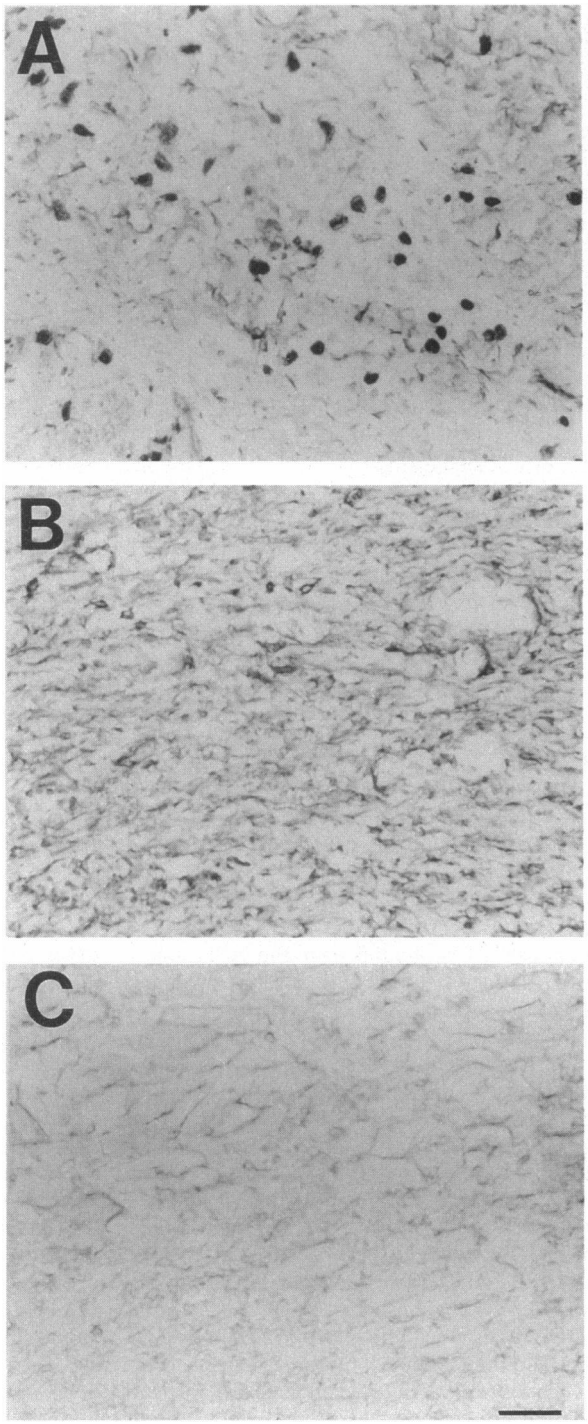

FIG. 4. Immunostaining of CRF in inflamed subcutaneous paw tissue after different pretreatments. (*A*) Vehicle pretreatment: CRF containing cells are discernible by black deposits. (*B*) CRF-antisense pretreatment reduces this staining in comparison to the vehicle group. (*C*) Preadsorption of anti-CRF with rat/human CRF abolishes specific staining. (Bar = 50 μm.)

In summary, we have found that local opioid analgesia is critically dependent on the expression of CRF in inflammatory cells. Upon a specific stress stimulus, CRF apparently triggers the release of opioid peptides within inflamed tissue and these peptides activate opioid receptors on sensory nerves resulting in the inhibition of pain. This mechanism does not involve endogenous IL-1β, circulating CRF, or antiinflammatory effects. Thus, pain can be effectively diminished by local paracrine interactions of the immune system with peripheral sensory nerves. It remains to be elucidated, though, how these peripheral defensive mechanisms are linked to the central perception of pain and stress. Nevertheless, these findings imply that (*i*) CRF may be the prototype of a novel generation of analgesics whose mechanism is based on the release of endogenous opioids within injured tissue, and (*ii*) a functional immune system is critical for pain control. Thus, these observations provide new insights into the pain occurring in immunosuppressed patients with cancer or AIDS.

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