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Interleukin 1β and corticotropin-releasing factor inhibit pain by releasing opioids from immune cells in inflamed tissue

(analgesia/nociception/cytokines/neuropeptides)

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ABSTRACT **Local analgesic effects of exogenous opioid agonists are particularly prominent in painful inflammatory conditions and are mediated by opioid receptors on peripheral sensory nerves. The endogenous ligands of these receptors, opioid peptides, have been demonstrated in resident immune cells within inflamed tissue of animals and humans. Here we examine *in vivo* and *in vitro* whether interleukin 1β (IL-1) or corticotropin-releasing factor (CRF) is capable of releasing these endogenous opioids and inhibiting pain. When injected into inflamed rat paws (but not intravenously), IL-1 and CRF produce antinociception, which is reversible by IL-1 receptor antagonist and α-helical CRF, respectively, and by the immunosuppressant cyclosporine A. *In vivo* administration of antibodies against opioid peptides indicates that the effects of IL-1 and CRF are mediated by β-endorphin and, in addition, by dynorphin A and [Met]enkephalin, respectively. Correspondingly, IL-1 effects are inhibited by μ-, δ-, and κ-opioid antagonists, whereas CRF effects are attenuated by all except a κ-antagonist. Finally, IL-1 and CRF produce acute release of immunoreactive β-endorphin in cell suspensions freshly prepared from inflamed lymph nodes. This effect is reversible by IL-1 receptor antagonist and α-helical CRF, respectively. These findings suggest that IL-1 and CRF activate their receptors on immune cells to release opioids that subsequently occupy multiple opioid receptors on sensory nerves and result in antinociception. β-Endorphin, μ- and δ-opioid receptors play a major role, but IL-1 and CRF appear to differentially release additional opioid peptides.**

An increasing number of experimental and clinical studies demonstrate that locally administered opioid agonists elicit potent analgesic effects in inflamed tissue (for review, see refs. 1–3). They interact with opioid receptors that are present on peripheral sensory nerves and are apparently up-regulated during the development of inflammation (4–6). Their endogenous ligands, opioid peptides, are found in resident immune cells within peripheral inflamed tissue (5, 7, 8). These findings are consistent with studies demonstrating that opioid peptides are produced within immune cells in culture (9, 10) and suggest a functional link between the immune and sensory nervous systems. Indeed, such immune-derived opioids [predominantly β-endorphin (β-END)] are apparently released during environmental stressful stimuli and result in the inhibition of pain (5, 8, 11). In view of the fact that opioid-containing cells have recently been demonstrated in human inflamed synovial tissue (11), it becomes most interesting to find tools to liberate these pools of potentially analgesic substances.

The aim of the present experiments was to identify agents that inhibit pain by releasing opioid peptides from resident immune cells within inflamed tissue *in vivo*. Two substances, interleukin 1β (IL-1) and corticotropin-releasing factor (CRF), were of particular interest because they are secretagogues of β-END in long-term cultured immune cells from healthy organisms (10). In our model and in the clinical situation, however, immunocytes sustain a chronic pathophysiological stimulation due to persistent inflammation *in vivo*. Parallel *ex vivo* studies were therefore performed to demonstrate that these substances indeed cause β-END release from inflamed immune cells with a kinetics similar to our algesiometric studies.

MATERIALS AND METHODS

Subjects. Experiments were conducted in male Wistar rats (Charles River Breeding Laboratories) (180–225 g) housed individually in cages lined with ground corn cob bedding. Standard laboratory rodent chow and tap water were available ad libitum. Room temperature was maintained at 22°C ± 0.5°C and a relative humidity between 40% and 60%. A 12/12 hr (7 a.m./7 p.m.) light/dark cycle was used. All testing was performed in the light phase. The ethical guidelines for investigations of experimental pain in animals were followed (12).

Drugs and Immunoreagents. The following drugs were used: recombinant human IL-1β (IL-1) (R & D Systems); human and rat CRF (Sigma); recombinant IL-1 receptor antagonist (IL-1ra) (R & D Systems); CRF antagonist (α-helical CRF) (Sigma); naloxone hydrochloride (Sigma); cyclosporine A (CsA) (Sandoz Pharmaceutical); (D-Phe)-Cys-Tyr-(D-Trp)-Orn-Thr-Pen-Thr-NH_2 (CTOP) (Peninsula Laboratories); *N,N*-diallyl-Tyr-Aib-Aib-Phe-Leu-OH (ICI 174,864) (RBI, Natick, MA); norbinaltorphimine (nor-BNI) (RBI); Freund's complete adjuvant (Calbiochem); halothane (Halocarbon Laboratories, North Augusta, SC). Antisera used were rabbit anti-β-endorphin (anti-β-END) (Peninsula Laboratories); rabbit anti-[Met]enkephalin (anti-ENK) (R & D Antibodies, Berkeley, CA); rabbit anti-dynorphin A (anti-DYN) (Peninsula Laboratories); according to the manufacturers' specifications, these antisera do not cross-react with each others' antigens; normal rabbit IgG (Sigma) was used as a control. Doses were calculated as the free base and drugs were dissolved in the following vehicles: sterile isotonic saline (CsA, naloxone), sterile water (IL-1, CRF, IL-1ra, α-helical CRF, anti-β-END, anti-ENK, anti-DYN, normal rabbit IgG, CTOP, ICI 174,864, nor-BNI). Routes and volumes of drug administration were intraplantar (i.pl.) (0.1 ml), i.p. (1 ml), or i.v. (0.2 ml) into a tail vein through an indwelling

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Abbreviations: IL-1, interleukin 1β; CRF, corticotropin-releasing factor; IL-1ra, interleukin 1 receptor antagonist; β-END, β-endorphin; ENK, [Met]enkephalin; DYN, dynorphin A; CsA, cyclosporine A; PPT, paw pressure threshold; i.pl., intraplantar; MPE, maximum possible effect; ir, immunoreactive; CTOP, (D-Phe)-Cys-Tyr-(D-Trp)-Orn-Thr-Pen-Thr-NH_2; nor-BNI, norbinaltorphimine.

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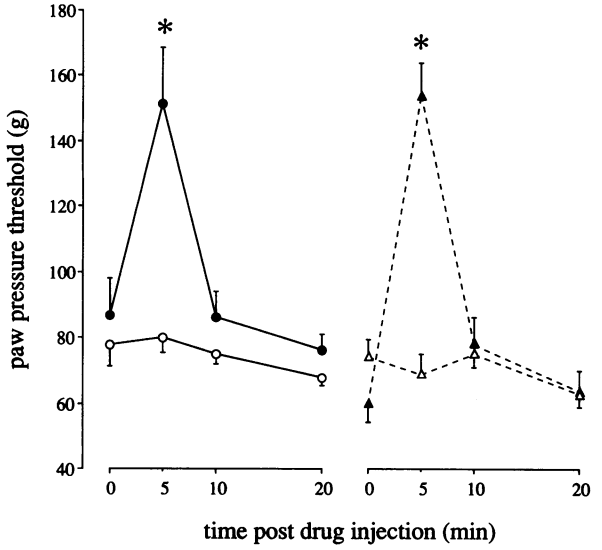

FIG. 1. Time course of PPT alterations after i.pl. injection of 2 ng of IL-1 (*Left*) and 1.5 ng of CRF (*Right*) in inflamed (closed symbols) and noninflamed (open symbols) rat paws. Data at 0 min represent PPT before drug injection. Asterisks denote significant differences between inflamed and noninflamed paws ($P < 0.05$; Wilcoxon test).

24-gauge Teflon catheter (Baxter, Deerfield, IL). Antagonists were given concomitantly with agonists in a total volume of 0.2 ml. All drugs were injected under brief halothane anesthesia.

Induction of Inflammation. Rats received an i.pl. injection of 0.15 ml of Freund's complete adjuvant into the right hindpaw. Control animals were anesthetized but not injected. The paw volume was monitored using a plethysmometer (Ugo Basile, Comerio, Italy). The inflammation remained confined to the right paw throughout the observation period. All experiments were conducted 4–5 days after inoculation.

Algesiometry. Nociceptive thresholds were evaluated using an Analgesy-meter (Ugo Basile) (13). Rats (five to seven per group) were handled twice before testing and then gently restrained under paper wadding and incremental pressure (maximum 250 g) applied onto the dorsal surface of the hindpaw. The pressure required to elicit paw withdrawal, the paw pressure threshold (PPT), was determined. The mean of three consecutive measurements, separated by 10 sec, was determined. The same procedure was then performed on the contralateral side; the sequence of sides was alternated between subjects to preclude "order" effects. After baseline measurements, drugs were injected and PPT was reevaluated 5, 10, and 20 min thereafter. The experimenter was blind to the substances administered. Doses and testing intervals were chosen based on pilot experiments.

Experiment 1. The time course and dose dependency of antinociceptive effects were examined after i.pl. administration of IL-1 (0.1–2 ng) or CRF (0.1–1.5 ng). Controls received NaCl (0.1 ml, i.pl.) or equivalent i.v. doses of IL-1 or CRF, respectively. Attenuation of these effects by IL-1ra (1–50 ng, i.pl.) or α-helical CRF (0.1–2 ng, i.pl.) or by pretreatment (at 48, 24, and 4 hr before testing) with i.p. CsA (0.75–3 mg per injection) or vehicle (1 ml) was examined in separate groups.

Experiment 2. Whether the antinociceptive effects of i.pl. IL-1 (2 ng) or CRF (1.5 ng) were mediated by opioid peptides was examined using i.pl. anti-β-END (0.025–0.2 μg), anti-ENK (0.5–8 μg), anti-DYN (0.1 ng–8 μg), or normal rabbit IgG (8 μg) as a control. Separate experiments assessed reversibility by the opioid antagonists naloxone (0.01–5 μg), CTOP (0.05–1 μg), ICI 174,864 (1–10 μg), or nor-BNI (6.25–50 μg) given i.pl.

***In Vitro* Release Experiments.** Four to 5 days after inoculation with Freund's complete adjuvant rats were euthanized by CO_2 inhalation. Popliteal lymph nodes were removed and ground using a cell dissociation sieve (size 60 mesh; Sigma). Cells were reconstituted in 5–15 ml of Hanks' balanced salt solution (HBSS) and centrifuged at 1300 rpm for 10 min at 20°C using a swinging bucket rotor. Cell pellets were then reconstituted in HBSS aiming at a concentration of 0.05–0.15 $\times 10^6$ cells per ml. Cell viability, as determined by the trypan blue exclusion method, was >95%. A 0.3-ml volume of this cell suspension was incubated with 0.1 ml of either HBSS, α-helical CRF (25–100 ng), or IL-1ra (25–100 ng) at 37°C in a shaking water bath. After 5 min 0.1 ml of either HBSS, CRF (25–100 ng), or IL-1 (25–100 ng) was added. Another 5 min later the suspension (total volume, 0.5 ml) was centrifuged at

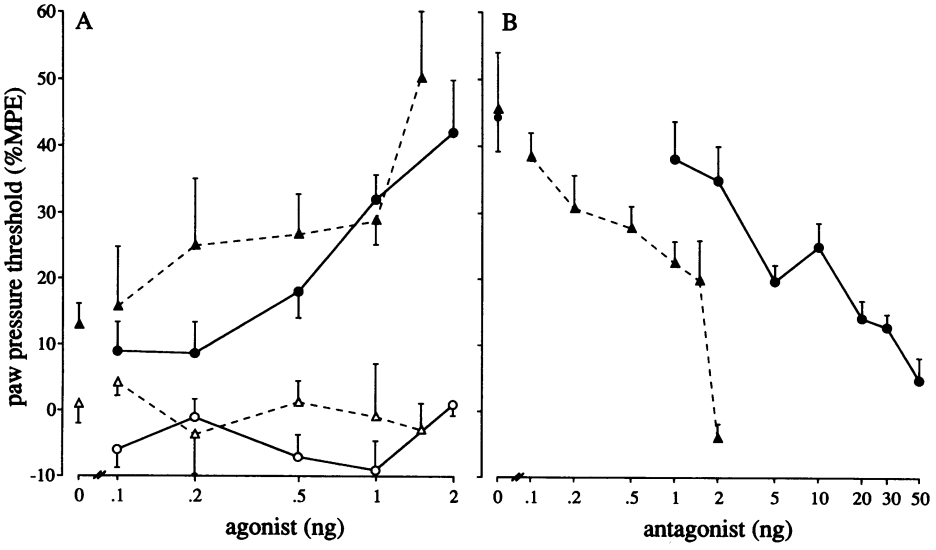

FIG. 2. (*A*) Effects of i.pl. IL-1 (circles) and CRF (triangles) in inflamed (closed symbols) and noninflamed (open symbols) paws. Data at 0 concentration represent the effects of saline injection. PPT elevations were maximal at 5 min postinjection and dose-dependent in inflamed paws (IL-1, $P < 0.001$; CRF, $P < 0.05$; linear regression ANOVA). In noninflamed paws PPT remained unchanged (IL-1, $P = 0.32$; CRF, $P = 0.86$; ANOVA). (*B*) Effects of IL-1ra (●) on IL-1 (2 ng)-induced PPT elevations and of α-helical CRF (▲) on CRF (1.5 ng)-induced PPT elevations. Antagonist effects were dose-dependent (IL-1ra, $P < 0.001$; α-helical CRF, $P < 0.001$; linear regression ANOVA).

Table 1. Influence of i.pl. IL-1 (2 ng) or CRF (1.5 ng) on paw volume before (0 min) and 5, 10, and 20 min after injection

Time after injection, min	Paw volume, ml			
	Inflamed		Noninflamed	
	IL-1β	CRF	IL-1β	CRF
0	5.93 ± 0.25	6.38 ± 0.43	3.15 ± 0.04	3.15 ± 0.06
5	5.70 ± 0.28	6.08 ± 0.37	3.19 ± 0.06	3.32 ± 0.05
10	5.85 ± 0.28	6.42 ± 0.33	3.15 ± 0.10	3.33 ± 0.06
20	5.86 ± 0.29	6.09 ± 0.29	3.20 ± 0.05	3.36 ± 0.07

Data are expressed as mean ± SEM.

5000 rpm for 10 min using a fixed angle rotor. Aliquots (0.3 ml) of the supernatants were lyophilized and stored at −20°C until further processing. The concentrations of cells and agents were chosen based on pilot experiments.

RIA. Assays were performed using a RIA kit (Peninsula Laboratories). Tubes were prepared in duplicate containing 0.1-ml standard concentrations of β-END or unknown samples (except total count, nonspecific binding, and total binding tubes) dissolved in RIA buffer (containing 0.1 M sodium phosphate, 0.05 M NaCl, 0.01% NaN_3, 0.1% bovine serum albumin, and 0.1% Triton X-100) and rabbit anti-β-END (0.1 ml) to be incubated overnight at 4°C. On day 2, 0.1 ml of ^{125}I-labeled β-END (12,000–15,000 cpm) was added and tubes were incubated overnight at 4°C. On day 3, 0.1 ml of goat anti-rabbit IgG and 0.1 ml of normal rabbit serum were added and incubated for 90 min at room temperature. Subsequently, 0.5 ml of RIA buffer was added and tubes were spun at 3000 rpm for 20 min at 4°C in a microcentrifuge (Beckman). After aspiration of supernatants (except total count tubes), radioactivity in the pellets was counted.

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 postinjection − basal PPT)/(250 − basal PPT). Values in right (inflamed) paws were compared to those in left paws by the Wilcoxon paired-sample test. Independent groups (control experiments) were compared by the Mann–Whitney U test. In *in vitro* experiments the mean ± SEM of four to eight experiments, using one lymph node each, is given. For dose–response curves an analysis of variance (ANOVA) and a subsequent linear regression ANOVA were performed to test the zero slope hypothesis. Differences were considered significant if $P < 0.05$ (two-tailed).

RESULTS

Algesiometry. *Experiment 1.* Both IL-1 and CRF produced significant elevations of PPT in inflamed but not in noninflamed paws (Fig. 1). PPT elevations were maximal at 5 min postinjection (Fig. 1) and they were dose-dependent (Fig. 2*A*). Equivalent doses given i.v. were ineffective (IL-1, $P = 0.27$; CRF, $P = 0.32$; Wilcoxon test) (data not shown). In noninflamed paws PPT remained unchanged (Fig. 2*A*) and was not different from that in untreated control animals (IL-1, $P = 0.40$; CRF, $P = 0.92$; Mann–Whitney U test) following either agent (not shown). The paw volume did not significantly change at any time of measurement (IL-1, $P = 0.32$; CRF, $P = 0.48$; ANOVA) (Table 1). IL-1 (2 ng)-induced PPT elevation was dose-dependently reversible by IL-1ra (Fig. 2*B*) but not by α-helical CRF (151.4 ± 17.5 g vs. 111.7 ± 15.7 g; $P = 0.11$; Mann–Whitney U test). The CRF (1.5 ng)-induced effect was dose-dependently antagonized by α-helical CRF (Fig. 2*B*) but not by IL-1ra (153.7 ± 21.4 g vs. 118.1 ± 9.1 g; $P = 0.22$; Mann–Whitney U test). CsA dose-dependently suppressed the antinociceptive effects of IL-1 and CRF ($P < 0.001$; linear regression ANOVA) but did not affect the volume of noninflamed ($P = 0.39$; ANOVA) or inflamed ($P = 0.30$; ANOVA) paws (Table 2).

Experiment 2. IL-1 effects on PPT were dose-dependently attenuated by anti-β-END ($P < 0.001$) and anti-DYN ($P < 0.05$, linear regression ANOVA) but not by anti-ENK ($P = 0.45$; ANOVA) (Fig. 3*A*). CRF effects on PPT were dose-dependently inhibited by anti-β-END ($P < 0.01$) and anti-ENK ($P < 0.05$; linear regression ANOVA) but not by anti-DYN ($P = 0.90$; ANOVA) (Fig. 3*B*). Normal rabbit IgG did not influence PPT elevations by IL-1 ($P = 0.54$) or CRF ($P = 0.47$; Mann–Whitney U test). All opioid receptor antagonists reversed the IL-1 effect dose-dependently (naloxone, $P < 0.01$; CTOP, $P < 0.01$; ICI 174,864, $P < 0.001$; nor-BNI, $P < 0.05$; linear regression ANOVA) (Fig. 3*C*). The CRF effect was reversed by naloxone ($P < 0.01$), CTOP ($P < 0.001$), and ICI 174,864 ($P < 0.001$; linear regression ANOVA) but not by nor-BNI ($P = 0.29$; ANOVA) (Fig. 3*D*).

***In Vitro* Release Experiments.** Both IL-1 and CRF produced dose-dependent release of immunoreactive (ir) β-END from cell suspensions (IL-1, $P < 0.005$; CRF, $P < 0.05$; linear regression ANOVA) (Fig. 4). IL-1 (100 ng)-induced release (4.45 ± 1.34 ng per 10^6 cells) was dose-dependently inhibited by IL-1ra ($P < 0.05$; linear regression ANOVA) but not by α-helical CRF (3.13 ± 0.59 ng per 10^6 cells; $P = 0.39$; Mann–Whitney U test). CRF (100 ng)-induced release (4.18 ± 0.95 ng per 10^6 cells) was dose-dependently attenuated by α-helical CRF ($P < 0.05$; linear regression ANOVA) but not by IL-1ra (4.50 ± 0.91 ng per 10^6 cells; $P = 0.81$; Mann–Whitney U test).

Table 2. Influence of CsA pretreatment (i.p. injections 48, 24, and 4 hr before algesiometry) on IL-1- and CRF-induced PPT elevation and on paw volume

CsA dose, mg	PPT, g				Paw volume, ml	
	Inflamed		Noninflamed			
	IL-1β (2 ng)*	CRF (1.5 ng)**	IL-1β (2 ng)	CRF (1.5 ng)	Inflamed	Noninflamed
0	137.6 ± 13.5	128.8 ± 11.4	74.8 ± 4.3	83.8 ± 5.9	6.23 ± 0.32	3.30 ± 0.06
2.25	153.3 ± 10.4	118.1 ± 7.0	72.7 ± 7.0	82.8 ± 4.4	5.98 ± 0.14	3.39 ± 0.04
4.5	106.7 ± 4.4	83.6 ± 4.5	78.9 ± 1.6	81.6 ± 7.7	5.67 ± 0.23	3.28 ± 0.07
9	89.9 ± 7.6	79.2 ± 8.5	78.8 ± 3.8	85.0 ± 5.0	6.22 ± 0.21	3.26 ± 0.04

Superscripts denote statistical significances as follows: *, ANOVA ($P < 0.001$); linear regression ANOVA ($P < 0.001$); **, ANOVA ($P < 0.001$); linear regression ANOVA ($P < 0.001$). Data are expressed as mean ± SEM.

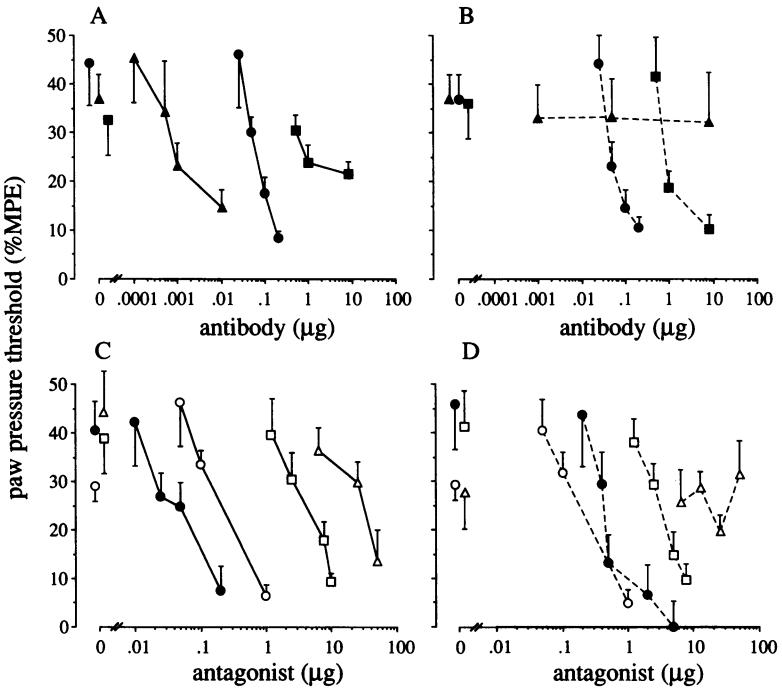

FIG. 3. Inhibition of IL-1 (*A* and *C*)- and CRF (*B* and *D*)-induced antinociception by anti-END (●), anti-ENK (■), and anti-DYN (▲) (*A* and *B*) and by naloxone (●), CTOP (○), ICI 174,864 (□), and nor-BNI (△) (*C* and *D*). Linear regression ANOVA was significant ($P < 0.05$) for all except anti-ENK in *A* ($P = 0.45$; ANOVA), anti-DYN in *B* ($P = 0.90$; ANOVA), and norBNI in *D* ($P = 0.29$; ANOVA).

DISCUSSION

The first set of experiments demonstrates that both IL-1 and CRF can potently inhibit nociception in inflamed tissue by a peripheral mechanism of action. The fact that IL-1 and CRF effects are dose-dependent and selectively reversible by their respective antagonists strongly suggests that these actions are mediated by IL-1 and CRF receptors, respectively. Within our observation period, neither agent alters nociceptive thresholds in noninflamed tissue or the swelling, indicating that the inflammatory process is necessary but that these antinociceptive effects do not occur via overt antiinflammatory actions. These findings extend previous reports of peripheral analgesic effects of CRF (14) but are in contrast to those of hyperalgesic effects induced by IL-1 (15). The difference between our situation and the latter, in which hyperalgesia occurred 3 hr after injection of IL-1 into noninflamed tissue (15), is clearly the presence of fully developed inflammation at the time of IL-1 application.

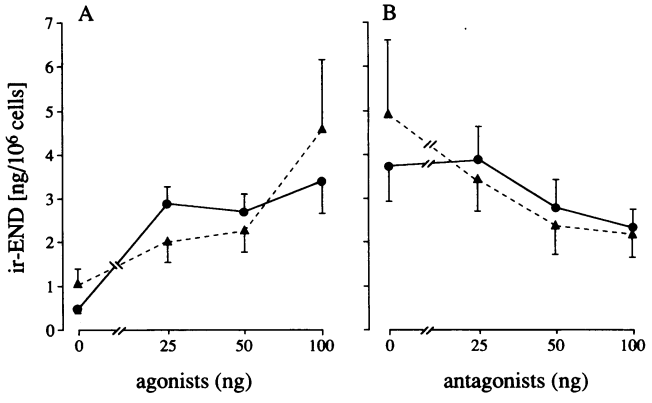

FIG. 4. IL-1 (●)- and CRF (▲)-induced release of ir-END (*A*) and antagonism by IL-1ra (●) and α-helical-CRF (▲) (*B*) in cell suspensions. Each point represents the mean ± SEM of four to eight experiments using one lymph node each.

What is the location of IL-1 and CRF receptors in peripheral inflamed tissue? Both IL-1 and CRF receptors have been demonstrated on various immune cells such as T and B cells or macrophages (16, 17). To investigate the role of such cells in the mediation of IL-1- and CRF-induced analgesia, we pretreated rats with CsA, an immunosuppressant that inhibits the transcription of early genes involved in the activation of T cells and other immunocytes (5, 18). Though not changing paw swelling, this pretreatment abolished IL-1 and CRF effects, suggesting that CsA does not significantly affect the inflammatory process (e.g., extravasation) but that the functional integrity of immune cells within inflamed tissue is crucial for the occurrence of those effects. Taken together, IL-1 and CRF receptors localized on immune cells appear to mediate the observed antinociceptive actions.

What are the mediators of these effects? Evidence has accumulated that immune cells produce opioid peptides under certain circumstances (9, 10, 19–25). We have detected β-END, ENK, and, recently, DYN, in lymphocytes and monocytic cells within inflamed tissue by immunocytochemistry (5, 7, 8). Investigations concerning the mechanisms of release of such peptides have shown that both IL-1 and CRF can stimulate release of β-END (10, 24, 26), an important endogenous ligand at peripheral opioid receptors (5, 27). Therefore, we hypothesized that both agents could release opioid peptides, which then produce analgesia via activation of opioid receptors on nociceptive nerve terminals (4–6). Indeed, our second set of experiments demonstrates that both IL-1- and CRF-induced antinociception is attenuated by anti-β-END. In addition, anti-DYN and anti-ENK inhibit the effects of IL-1 and CRF, respectively, indicating a differential release of opioids by the two agents. This is supported by the finding that different dose ranges of naloxone and different selective opioid antagonists reverse those agents' actions. Thus, while the effect of CRF is mediated by μ- and δ-, but not by κ-receptors, all three opioid receptor types are involved in IL-1's effect. Taken together, these results indicate that both agents release β-END, which activates μ- and δ-receptors, consistent with our previous studies that have

shown that β-END is the prevailing endogenous ligand of peripheral opioid receptors (5, 27). In addition, DYN (a κ-ligand) and ENK (a δ-ligand) appear to be liberated by IL-1 and CRF, respectively.

To further confirm our hypothesis that both agents release β-END from immune cells within the short time frame applied in our *in vivo* studies, we sought to mimic this situation *in vitro*. Indeed, both IL-1 and CRF produced release of ir-β-END in cell suspensions prepared freshly from inflamed lymph nodes. The fact that these effects are dose-dependent and selectively reversible by the respective antagonists strongly indicates that they are mediated by IL-1 and CRF receptors. These findings substantially extend previous reports of β-END release from immunocytes, since in those studies cells were obtained from healthy human volunteers and subjected to various long-term culture conditions (10, 24, 26). In contrast, our situation is one of a persistent pathophysiologically relevant *in vivo* stimulation of the immune system, which, we believe, resembles the clinical situation much more closely. The importance of distinguishing between immune cells from healthy organisms and those obtained under pathological conditions is further underscored by the recent demonstration of a truncated form of mRNA encoding proopiomelanocortin (POMC) (the β-END precursor) in normal cells, but of regular-length POMC mRNA (compared to that in the pituitary) in a cell line derived from a patient with lymphoma (19). Similarly, mRNA encoding proenkephalin (the ENK precursor) has been detected in activated but not in resting T lymphocytes (25). Finally, we have demonstrated that β-END can be released from lymphocytes within minutes of stimulation *in vitro*, consistent with the time course of our effects *in vivo*. Taken together, our data indicate that, in fully established inflammation, IL-1 and CRF are able to acutely release β-END via activation of their specific receptors on immune cells *in vitro* and *in vivo*.

Considering that these results implicate β-END as a predominant mediator of the analgesic effects triggered by IL-1 and CRF, the short duration of these effects, though consistent with our former studies (27), is noteworthy. Although β-END is relatively resistant to degradation at physiologic pH values in plasma and in the central nervous system, virtually nothing is known about its fate in the milieu of peripheral inflamed tissue. Particular to this milieu are, for example, a low pH and a high proteolytic activity (28). However, the fact that the antinociception following a single injection of an exogenous peptide is short-lasting does not exclude the possibility that endogenous peptides, which may be continuously released *in vivo*, have effects of longer duration. Indeed, our clinical studies indicate that under postoperative conditions, locally mediated endogenous opioid analgesia is effective for several hours (11).

In summary, we have found that IL-1 and CRF liberate β-END and, in addition, DYN and ENK within inflamed tissue. These opioid peptides interact with multiple opioid receptors on nociceptive nerve terminals to result in potent analgesia. These findings have several interesting implications: (*i*) Beyond its widely accepted role as a proinflammatory agent, IL-1 can locally generate analgesia, thus maintaining a delicate homeostasis during the healing process of injured tissue. (*ii*) We have discovered a mechanism for the previously unexplained peripheral antinociceptive actions of CRF (14) and a possible function of CRF produced in inflammatory sites (29). (*iii*) Two possible mediators for our formerly described stress-induced analgesia in inflammation (5, 8, 27) are now identified. Thus, it is conceivable that stress causes release of CRF and/or IL-1, which then liberate opioids within inflamed tissue. (*iv*) Our findings provide an incentive for the development of a novel generation of analgesics, the mechanism of which is based on the local release of endogenous opioids within injured tissue.

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