Intrinsic Mechanisms of Antinociception in Inflammation: Local Opioid Receptors and $\beta$-Endorphin

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This study examined antinociception induced through the activation of local opioid receptors in inflammation by endogenous opioids. Rats developed a unilateral localized inflammation upon injection of Freund’s adjuvant into one hindpaw. Four to 6 d later they were subjected to cold water swim (CWS), an environmental stimulus known to activate intrinsic opioid systems. Following CWS (1 min) the animals’ withdrawal threshold to noxious pressure applied onto the paws increased significantly more on the inflamed paw than on the noninflamed paw. This unilateral antinociceptive effect in inflamed paws was dose-dependently and stereospecifically reversible by intraplantar (i.pl.) but not systemic (i.v. or s.c.) administration of the opioid antagonist naloxone (18 $\mu$g). This suggested that CWS-induced antinociception in inflamed tissue was brought about by the activation of local opioid receptors. Antiinflammatory or vasoconstrictive events, as measured by paw volume and temperature, did not contribute to this unilateral antinociception. Receptor-selective antagonists indicated the involvement of $\mu$- and $\delta$- but not $\kappa$-receptors. Intravenous application of a universal antibody to endogenous opioid peptides (3-ET) and a specific antibody to $\beta$-endorphin, but not antiserum against met-enkephalin or dynorphin, abolished the CWS effect. Finally, the i.pl. injection of synthetic $\beta$-endorphin (1-31) produced an antinociceptive effect in inflamed paws which was reversible by i.pl. naloxone and selective $\mu$- and $\delta$-receptor antagonists. These findings suggest that antinociception in inflamed tissue can be induced through the activation of local opioid receptors by endogenous $\beta$-endorphin released during CWS.

Traditionally, antinociception produced by exogenous as well as endogenous opioids has been associated with activation of opioid receptors in the central nervous system. Recently, however, we and others have demonstrated that exogenous opioid agonists can exert antinociceptive effects by interacting with local opioid receptors in inflamed tissue of the rat (Ferreira and Nakamura, 1979; Joris et al., 1987; Stein et al., 1988b, 1989a). Furthermore, we have been able to differentiate the types of opioid receptors involved (Stein et al., 1989a). The physiological significance of these receptors, however, is yet to be elucidated. In particular, the question arises as to which are the endogenous ligands for these receptors and what stimuli call them into play.

Three families of endogenous opioid peptides derived from 3 precursor peptides are known to date: the pro-opiomelanocortin (POMC), the pro-enkephalin, and the pro-dynorphin system. These precursors undergo differential processing in the various regions of the central and peripheral nervous systems (Höltt, 1986; Lewis et al., 1987) and the major cleavage products have differing affinities to the 3 opioid receptor types $\mu$, $\delta$, and $\kappa$. Intrinsic opioid antinociceptive systems can be activated by certain environmental stimuli (Terman et al., 1984; Millan and Herz, 1985; Bodnar, 1986). Out of a wide variety of models we chose cold water swim (CWS) and examined its ability to elicit antinociception through local opioid receptor specific mechanisms in inflammation.

Specifically, this study investigated (1) the antinociceptive effect of different durations of CWS in unilateral hindpaw inflammation, (2) whether this effect is reversible by locally, compared to systemically, applied naloxone and its stereoisomer, (3) whether antiinflammatory and/or vasoconstrictive events contribute to this antinociception, (4) which types of opioid receptors are involved, (5) whether this effect can be abolished by antibodies against endogenous opioid peptides, and (6) whether this effect can be mimicked by local administration of the opioid peptide $\beta$-endorphin.

Sterespecific and dose-dependent reversibility by local, but not systemic, application of naloxone provides evidence for an involvement of peripherally located opioid receptors. Differential abolition of an effect by antagonists that are highly selective for particular receptor types and by specific antibodies will point to the identity of the endogenous ligand(s) involved. Imulation of this effect by exogenous administration of the putative ligand will confirm its functional significance. Using these criteria we have concluded that antinociception in inflamed tissue can be induced through activation of local $\mu$- and $\delta$-opioid receptors by endogenous $\beta$-endorphin released during CWS.

Materials and Methods

Subjects. Experiments were carried out in male Wistar rats (Ivanovas, Kisslegg, FRG) (180-200 gm) housed individually in cages lined with sawdust. Standard laboratory rodent chow and water were available ad libitum. Room temperature and relative humidity were maintained at 22 $\pm$ 0.5°C and 60%, respectively. A 12 hr/12 hr (8 A.M./8 P.M.) light-dark cycle was used. All testing was conducted in the light phase. The guidelines on ethical standards for investigations of experimental pain in animals (Zimmermann, 1983) were followed.
Induction of inflammation. The inflammatory agent used was modified Preud‘s complete adjuvant (FCA), containing 0.1% heat-killed and dried Mycobacterium butyricum in 85% Maroc! 52 and 15% Aracel A mannide monoooleate emulsifier (Calbiochem, La Jolla, CA). Rats received an intraplantar injection of 0.15 ml of this suspension into the right hindfoot under brief ether anesthesia. Control animals were anesthetized but not injected. Inflammation of the injected paws was apparent within 12 hr following treatment with FCA. A detailed description of the time course and magnitude of the inflammatory reaction is given elsewhere (Stein et al., 1988a). In all rats studied, the inflammation remained confined to the inoculated paw. All testing was conducted between 4 and 6 days postinoculation.

Parameters of inflammation. Paw volume was measured by submerging the hindpaw to the tibiotarsal joint into the water-filled Perspex cell of a plethysmograph (Ugo Basile, Comerio, Italy). The volume of displacement, which is equal to the paw volume, was indicated on a digital display. The surface temperature of the plantar skin was measured with an Infrared radiation thermometer (Ultrakust, Ruhmannsfelden, FRG).

Algesiometric testing. Antinoceptive effects were evaluated using the paw pressure test. The animal was gently restrained under paper wadding and incremental pressure applied via a wedge-shaped, blunt piston onto an area of 1.75 mm² of the dorsal surface of the hindpaw by means of an automated gauge (Ugo Basile, Comerio, Italy). The pressure required to elicit paw withdrawal was recorded (PPT), and the paw pressure threshold (PPT) was determined. A cutoff of 250 gm was employed. Three consecutive trials, separated by intervals of 10 sec, were conducted and the average determined. The same procedure was then carried out on the contralateral side; the sequence of sides was alternated between subjects to preclude "order" effects. Separate groups of animals were used for each treatment, with the observer blind to the experimental condition employed.

Drugs and their administration. The following drugs were used: naloxone-HCl (DuPont, Geneva, Switzerland), (+)-naloxone-HCl (National Institute on Drug Abuse), (D-Phe)-Cys-Tyr-(D-Trp)-Orn-Thr-Val-NH₂ (CTOP) (Peninsula Laboratories Inc., Belmont, CA), N, N-diisyl-

Tyr-Ala-Abp-Phe-Leu (ICI 174,864) (Cambridge Research Biochemicals, Ltd., Cambridge, UK), nor-binaltorphimine (nor-BNI) (courtesy of Dr. A. W. Lipkowski, University of Minnesota), β-endorphin (1-31) (β-EP) (Novabiochem AG, Läufelfingen, Switzerland), halothane and ether (Hoechst AG, Frankfurt a.M., FRG). Doses were calculated as the dose per body weight by injecting saline, (+)-, and (-)-naloxone (18 μg) into the inflamed paw; baseline values were then taken and 10 min later the animals were subjected to 1 min CWS. Both parameters were determined immediately thereafter.

Changes in paw volume and temperature were expressed as percentage of baseline values (100%). Left and right paws were then compared using the Wilcoxon test for paired samples. The null hypothesis was that there is no change in PPT over time.

To construct a dose-response curve, PPT elevation (in % MPE) was plotted against the dose of (+)-naloxone. A linear regression analysis of variance (ANOVA) was performed to test the zero slope hypothesis (Kirk, 1982).

Experiment 3. This experiment examined the effect of CWS on PPT elevation (in % MPE) in inflamed paws. Two groups of rats (n = 6) received either saline or (+)-naloxone (18 μg) into the inflamed paw; baseline values were then taken and 10 min later the animals were subjected to 1 min CWS. Both parameters were determined immediately thereafter.

Changes in paw volume and temperature were expressed as percentage of baseline values (100%). Left and right paws were then compared using the Wilcoxon test for paired samples. The null hypothesis was that there is no change in PPT over time.

Experiment 4. In this experiment we sought to clarify which types of opioid receptors are involved in the mediation of the antinociceptive effect of CWS in inflammation. To examine this question, μ-, δ-, and κ-selective antagonists (Cotton et al., 1984; Gulwa et al., 1986; Portoghese et al., 1987) were administered prior to CWS to inflamed paws. The protocol was analogous to the foregoing: 3 groups (n = 5-6) received either saline (0.1 ml) or (-)-naloxone (18 μg) into the inflamed paw; baseline values were then taken and 10 min later the animals were subjected to 1 min CWS. Both parameters were determined immediately thereafter.

Changes in paw volume and temperature were expressed as percentage of baseline values (100%). Left and right paws were then compared using the Wilcoxon test for paired samples. The null hypothesis was that there is no change in PPT over time.

Table 1. Relative cross-reactivities of antibodies with opioid peptides in radioimmunoassay

| Peptides          | Cross-reactivities (%) |
|-------------------|------------------------|
|                   | 3-E7               | β-EP         | ME          | DYN         |
| β-endorphin (1-31)| 100                 | 0.5          | <0.001      | 0           |
| α-endorphin       | 100                 | <0.001       | 0           | 0           |
| γ-endorphin       | 100                 | 0            | 0           | 0           |
| Met-enkephalin    | 80                  | 0.05         | 100         | <0.005      |
| Leu-enkephalin    | 80                  | 10           | <0.05       | 100         |
| DYN A (1-17)      | 0                   | 0            | <0.05       | 100         |
| DYN A (1-13)      | 16                  | 0            | 0           | <0.005      |
| DYN A (1-8)       | 0                   | 0            | 0           | <0.005      |

IC₅₀ is taken as 100%. 0 = not tested.

Data on 3-E7 from Gramsch et al., 1983.
Figure 1. Effect of i.pl. saline, (-)-naloxone (NLX), and (+)-naloxone and of i.v. and s.c. (-)-naloxone on CWS (1 min) induced PPT elevation. Injections were made 10 min before CWS. Closed symbols, inflamed paw; open symbols, noninflamed paw. Values are means ± SE (n = 5-6).

*Significance of differences between PPT elevations in right and left paws: p < 0.05 (Wilcoxon test).

100, 200, and 400 µg) 5 min before CWS. PPT were recorded 1 min post CWS. All doses and times of administration were chosen based on pilot experiments (see also Stein et al., 1989a). The effects of i.pl. CTOP (1 µg), ICI 174,864 (20 µg), and nor-BNI (400 µg) without CWS were determined in separate groups of rats.

Increases in PPT (in % MPE) were plotted against the dose and a linear regression ANOVA was used to test the zero slope hypothesis. Data obtained in control experiments without CWS were analyzed by the Friedman test, the hypothesis being that there is no change in PPT.

Experiment 5. This experiment examined whether the antinociception induced by CWS in inflamed paws could be abolished by antibodies against endogenous opioid peptides. Analogous to the above protocols, separate groups of rats (n = 5-6) received i.v. injections of pan-opioid antibodies (3-E7) (0.05, 0.15, 0.25, 0.5, and 1.0 µg) as well as specific antibodies to β-EP (0.25, 0.5, and 1.0 µg) to ME (1, 4, and 8 µg) and to DYN (1, 4, and 8 µg) 5 min before CWS. In control experiments for nonspecific binding, normal rabbit IgG (5 µg) was administered. PPT were measured 1 min post CWS. All doses and time intervals for testing were chosen based on pilot experiments. The effects of the respective maximum doses of antisera without CWS were determined in separate groups of FCA-treated rats.

Elevations of PPT (in % MPE) were plotted against dose and the data were subjected to linear regression ANOVA. Data obtained in control experiments were analyzed by the Friedman test.

Experiment 6. This experiment examined whether the antinociceptive effect of CWS could be mimicked by i.pl. administration of β-EP. After determination of baseline PPT, 3 groups of rats (n = 5-6) received β-EP into both hindpaws (0.25, 0.5, and 1 µg per paw) under brief halothane anesthesia. PPT were reevaluated at 5, 10, and 20 min post-injection. Doses and testing intervals were chosen based on pilot experiments.

Analogously to the previous experiments, PPT elevation (in % MPE) in the right paw was compared to that in the left paw using Wilcoxon's test. Dose-response curves were constructed by plotting the increase of PPT (% MPE) at the time of peak effect (5 min) against the dose and were then subjected to a linear regression ANOVA.

Table 2. Effect of CWS on the paw pressure threshold (PPT) in control animals and in rats with inflammation of the right hindpaw (treated)

| CWS duration (min) | 0.5 | 1 | 2 |
|-------------------|-----|---|---|
|                   | Pre | Post | Pre | Post | Pre | Post |
| Controls          |     |     |     |      |     |      |
| Right             | 73 ± 6 | 93 ± 7 | 58 ± 3 | 100 ± 11 | 71 ± 4 | 147 ± 16 |
| Left              | 60 ± 4 | 85 ± 5 | 57 ± 4 | 94 ± 16 | 60 ± 3 | 181 ± 15 |
| Treated           |     |     |     |      |     |      |
| Inflamed          | 58 ± 6 | 71 ± 7 | 52 ± 10 | 160 ± 18 | 46 ± 4 | 250 ± 0 |
| Noninflamed       | 74 ± 8 | 70 ± 12 | 70 ± 6 | 72 ± 8 | 69 ± 4 | 132 ± 23 |

PPT (in gm) at time of maximum effect (1 min post CWS). Means ± SE (n = 6 per group).

Pre = baseline; Post = after CWS.

* Significance of differences between PTT elevations in inflamed and noninflamed paws: p < 0.05 (Wilcoxon test).
Whether the antinociceptive effect of i.pl. β-EP could be reversed by the same antagonists as used in the foregoing experiments was finally examined. To assess this question, the same doses of (-)-naloxone, CTOP, ICI 174,864, nor-BNI, and NaCl were injected concomitantly with β-EP (1 μg) into inflamed paws. PPT were measured 5 min postinjection. Fourteen separate groups (n = 5-6) of rats were used. Data analysis was analogous to experiment 4.

Results

Experiment 1. CWS produced an elevation of PPT that reached its maximum at 1 min post CWS and returned to baseline values within 10-15 min post CWS (see Fig. 1). The magnitude of this elevation increased with increasing CWS duration. In control animals, the increases in PPT produced by CWS were not different between left and right hindpaws (Wilcoxon), nor were they different from those of noninflamed (left) paws of treated animals (Mann-Whitney; Table 2). In FCA-treated animals, the elevation of PPT was significantly greater in inflamed paws than in contralateral, noninflamed paws (p < 0.05, Wilcoxon) or in controls (p < 0.05, Mann-Whitney) at all CWS durations except 0.5 min (Table 2).

Experiment 2. One minute CWS resulted in a significantly (p < 0.05, 1-tailed Wilcoxon test) higher elevation of PPT in inflamed compared with noninflamed paws in the 4 groups receiving either i.pl. saline, i.pl. (+)-naloxone, or systemic (i.v. or s.c.) (-)-naloxone (Fig. 1). This effect was antagonized dose-dependently by i.pl. (-)-naloxone (Fig. 2), being completely abolished at a dose of 18 μg (Fig. 1) (Friedman test). Trend analysis revealed. PPT by dose of (-)-naloxone, F(3,20) = 3.4, p < 0.05, linear regression, F(1,20) = 10.1, p < 0.005.

Experiment 3. Paw volume did not change significantly during CWS in either saline- or (-)-naloxone-pretreated animals (Table 3). Paw temperature dropped to the same extent (ca. 50%) in both inflamed and noninflamed paws (Wilcoxon). This decrease was not significantly different between saline- and (-)-naloxone-pretreated groups (Mann-Whitney, Table 3).

Experiment 4. The increase of PPT in inflamed paws produced by CWS was dose dependently reversed by specific μ- (CTOP) and δ- (ICI 174,864) but not κ- (nor-BNI) antagonists (Fig. 2). Trend analysis was as follows. PPT by dose of CTOP: F(2,15) = 8.5, p < 0.005, linear regression, F(1,15) = 16.3, p < 0.005; ICI 174,864: F(2,14) = 5.7, p < 0.05, linear regression, F(1,14) = 11.1, p < 0.01; nor-BNI: F(3,18) = 0.03, p > 0.05. Neither antagonist given alone produced significant changes in PPT (Friedman test, Table 4).

Experiment 5. The elevation of PPT induced by CWS (1 min) was dose-dependently antagonized by 3-E7 and the antibody against β-EP but not by antibodies to ME or DYN or by IgG (Fig. 3). Trend analysis was: PPT by dose of 3-E7: F(4,22) = 4.8, p < 0.01, linear regression, F(1,22) = 18.9, p < 0.001; β-EP antibody: F(2,13) = 24.3, p < 0.001, linear regression.
Experiment 6. Intraplantar administration of β-EP (0.25–1 μg) produced dose-dependent PPT elevations in inflamed but not in contralateral noninflamed paws (Fig. 4). Trend analysis of data on inflamed paws revealed: PPT by dose, $F(2,17) = 5.2$, $p < 0.05$, linear regression, $F(1,17) = 9.4$, $p < 0.01$.

The increase in PPT produced by β-EP (1 μg i.pl.) in inflamed paws was dose-dependently antagonized by (-)-naloxone, CTOP, and ICI 174,864 but not by nor-BNI (Fig. 5). ANOVA was as follows: PPT by dose of naloxone: $F(3,19) = 6.3$, $p < 0.005$, linear regression, $F(1,19) = 18.4$, $p < 0.001$; CTOP: $F(2,15) = 7.2$, $p < 0.01$, linear regression, $F(1,15) = 14.0$, $p < 0.005$; ICI 174,864: $F(2,13) = 6.4$, $p < 0.05$, linear regression, $F(1,13) = 12.8$, $p < 0.005$; nor-BNI: $F(3,19) = 0.3$, $p > 0.05$.

Discussion

Experiment 1 demonstrates that CWS can produce antinociceptive effects against noxious pressure in both normal and FCA-treated rats. These effects are clearly enhanced in inflamed paws compared to contralateral noninflamed paws or to control animals. The similarity of this phenomenon to that observed after systemic administration of exogenous opioid agonists in this model (Stein et al., 1988b) prompted us to put forth the hypothesis that the recruitment of peripheral opioid receptor-specific mechanisms accounts for the augmented antinociceptive effect of CWS in inflamed tissue. We tested this hypothesis by examining whether this effect was reversible by naloxone injected locally into the inflamed paw.

Experiment 2 demonstrates that the antinociceptive effect produced by CWS in inflamed paws can be reversed by locally applied naloxone but not by equivalent doses given systemically. Moreover, this antagonism is stereospecific and dose-dependent. Taken together, these findings are consistent with the notion that CWS can activate local opioid receptor-specific mechanisms in inflamed tissue.

The question arises as to whether antiinflammatory and/or vasoconstrictive effects contribute to this unilateral antinociception. Both of these are unlikely, the former since paw volume did not change during CWS and the latter since temperature decreased to the same extent in both hindpaws and, in contrast to the antinociceptive effect, this drop was not reversible by naloxone. Therefore, we are inclined to invoke a neural mechanism. As discussed extensively in a previous report (Stein et al., 1989a), the most likely location of these peripheral receptors seems to be the primary afferent neuron. This contention is
supported both by biochemical studies demonstrating opioid binding (LaMotte et al., 1976; Fields et al., 1980; Ninkovic et al., 1982) or opioid modulation of substance P release (Yaksh, 1988) and by electrophysiological data demonstrating opioid-specific effects (Werz and Macdonald, 1982; Frank, 1985; Russell et al., 1987) on sensory neurons. It should be borne in mind, however, that by varying the specific parameters of CWS (e.g., temperature, duration) it may well be possible to activate central opioid and/or nonopioid antinociceptive systems in addition to these peripheral mechanisms.

Previously, we have shown that it is possible to differentiate between the types of peripheral opioid receptors mediating antinociception in this model (Stein et al., 1989a). Therefore, experiment 4 sought to identify the receptor type(s) involved. The results demonstrate that the antinociceptive effect of CWS on inflamed paws can be reversed dose-dependently by antagonists selective for \( \mu \)- and \( \delta \)-, but not for \( \kappa \)-receptors. Exogenous \( \kappa \)-agonists, however, can produce peripheral antinociceptive effects in inflammation pointing to the presence of \( \kappa \)-receptors (Joris et al., 1987; Stein et al., 1988b, 1989a). Thus, it is conceivable that endogenous \( \kappa \)-ligands may be activated by other environmental stimuli.

Nevertheless, the above findings indicate that opioid agonists with activity at \( \mu \)- and/or \( \delta \)- but not at \( \kappa \)-receptors mediate the CWS effect. Inferring from the well-documented binding affinities of the endogenous opioid peptides (Höllt, 1986), one would consider cleavage products from either the POMC or the proenkephalin A system, such as \( \beta \)-EP or the enkephalins, as the most likely candidates. To further delimit the possibilities, we added antisera against the major representatives of the 3 opioid families in experiment 5. The results show that both the specific antibody to \( \beta \)-EP and 3-E7, which exhibits complete immunoreactivity with \( \beta \)-EP (Gramsch et al., 1983), but not antisera against ME or DYN, can abolish CWS-induced antinociception in inflamed paws.

These findings support the contention that \( \mu \)- and \( \delta \)-receptors in the inflamed paw may be activated by \( \beta \)-EP released during CWS. This hypothesis, however, is based on the assumption that both \( \beta \)-EP and opioid receptors display similar binding characteristics in inflamed tissue as in conventional assays (Höllt, 1986). That this may not be correct was already discussed in a previous report (Stein et al., 1989a): First, the anatomical circumstances in peripheral tissue must be considered. Before reaching its presumed locus of action, the peripheral sensory nerve axon or its terminal, an opioid molecule has to traverse several connective tissue and/or lipid membranous barriers. Each peripheral nerve axon is ensheathed in a Schwann cell and, in the case of A\( \delta \) fibers, in several layers of lipid myelin. The nerve fiber lies embedded in the surrounding subcutaneous connective tissue. As in the case of local anesthetics, the milieu (pH) of this

Table 4. Effects of opioid antagonists and antisera on PPT in rats not subjected to CWS

| Time (min) | CTOP (1 \( \mu \)g) | ICI 174,864 (20 \( \mu \)g) | nor-BNI (400 \( \mu \)g) | 3-E7 (1 \( \mu \)g) | \( \beta \)-EP (1 \( \mu \)g) | ME (8 \( \mu \)g) | DYN (8 \( \mu \)g) |
|-----------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| 0         | 36 ± 3          | 29 ± 6          | 62 ± 6          | 57 ± 5          | 47 ± 3          | 48 ± 8          | 32 ± 2          | 28 ± 2          | 45 ± 9          | 29 ± 5          | 43 ± 5          | 47 ± 9          |
| 5         | 40 ± 3          | 35 ± 3          | 47 ± 6          | 64 ± 18         | 56 ± 10         | 63 ± 9          | 56 ± 7          | 42 ± 4          | 38 ± 8          | 23 ± 8          | 36 ± 4          | 34 ± 4          | 45 ± 4          | 60 ± 11         |
| 15        | 35 ± 2          | 29 ± 4          | 53 ± 6          | 58 ± 10         | 45 ± 3          | 34 ± 3          | 29 ± 2          | 21 ± 4          | 32 ± 2          | 22 ± 2          | 43 ± 3          | 43 ± 3          | 48 ± 12         |
| 30        | 34 ± 2          | 26 ± 4          | 48 ± 8          | 53 ± 9          | 82 ± 21         | 62 ± 16         | 48 ± 5          | 42 ± 6          | 32 ± 2          | 23 ± 6          | 26 ± 1          | 28 ± 2          | 44 ± 3          | 36 ± 5          |

Antagonists were injected into the inflamed paw and antisera intravenously at 0 min. PPT in gm (means ± SE; \( n = 3-6 \) per group). L = left, noninflamed paw; R = right, inflamed paw.
environment may crucially influence activity and penetration of the agonist through these layers by altering the relative fraction of agent present in its charged or uncharged forms (Savarese and Covino, 1986). This is of special importance in the acidic conditions of inflamed tissue. Second, changes in tissue pH may result in alteration of receptor conformation which may entail a transformation from desensitized to active state, or vice versa. Thus, several critical factors have to be considered and one has to be careful in drawing parallels to more customary assays.

For these reasons, we carried out the last experiment investigating the actions of exogenously applied β-EP. The data clearly demonstrate that intraplantar administration of β-EP can produce an antinociceptive effect in inflamed paws. This effect is dose-dependent and reversible by naloxone, CTOP, and ICI 174,864 but not by nor-BNI. Moreover, the dose ranges for the antagonists to block the effect of β-EP are very similar to those required to abolish the effect of CWS on inflamed paws. Apparently, β-EP displays activity at both μ- and δ-receptors, which is in line with studies on its central application in vivo (Shook et al., 1988; Bals-Kubik et al., 1990) as well as on in vitro assays (Wüster et al., 1979; Shook et al., 1988).

Taken together, the present evidence strongly indicates that the endogenous ligand that is released during CWS and activates μ- and δ-receptors in inflamed paws is most likely β-EP.

One important question remains unanswered: What is the anatomical source for the endogenous opioids released during CWS? The pituitary is the richest source of β-EP and various stimuli can cause the release of pituitary opioids (Guillemin et al., 1977). Beyond that, however, opioid peptides have been detected in primary sensory neurons (Botticelli et al., 1981; Przewlocki et al., 1983; Weihe et al., 1988). Studies addressing this issue have commenced in our laboratory (Stein et al., 1989b; Parsons et al., 1990).

In summary, we have demonstrated that peripherally located opioid receptors in inflamed tissue can be activated by endogenous opioids released during exposure to environmental stimuli and mediate a decrease of nociception. One of the most likely endogenous ligands appears to be β-EP.

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