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Short communication

Antisera against endogenous opioids increase the nocifensive response to formalin: demonstration of inhibitory β-endorphinergic control

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Abstract

The roles of endogenous opioid peptides in the brain in the modulation of nocifensive responses to formalin in ICR mice were studied. Mice were pretreated intracerebroventricularly (i.c.v.) with rabbit antiserum against β-endorphin, [Leu^5]enkephalin, [Met^5]enkephalin or dynorphin A-(1–17) 1 h prior to intraplantar injection of formalin (0.5%, 25 μl) and the nocifensive licking responses were then observed. Pretreatment of mice with antiserum against β-endorphin enhanced the second phase, but not the first phase of the nocifensive responses to formalin. Pretreatment with antiserum against [Leu^5]enkephalin also caused a small but statistically significant enhancement of the second phase, but not the first phase of nocifensive responses to formalin. On the other hand, pretreatment with antiserum against [Met^5]enkephalin or dynorphin A-(1–17) did not affect the nocifensive response to formalin. Our results indicate that β-endorphinergic, and to a lesser extent, [Leu^5]enkephalinergic systems are activated at the supraspinal sites to attenuate the nocifensive responses to formalin stimulation.

Keywords: Tonic pain; Nocifensive response; Formalin-induced; Antiserum against β-endorphin; Pain control descending; (Mouse)

1. Introduction

Intraplantar injection of a dilute formalin solution causes nocifensive licking in the mouse and flinching in the rat, which closely resembles human responses to painful stimuli (Dubuisson and Dennis, 1977; Abbott and Franklin, 1986; Wheeler-Aceto et al., 1990). These nocifensive responses to formalin appear in two phases, an initial period of acute nocifensive behavior lasting for 10 min from the time of injection, and a second tonic phase occurring 15 min after injection and lasting 50–60 min (Tjølsen et al., 1992). The initial acute phase may represent a direct stimulation of nociceptors, whereas the second phase may represent an enhanced response of sensitized central neurons resulting from a low-level neuronal input due to peripheral inflammatory insult (Hunskaar and Hole, 1987; Dubner and Ren, 1999). Dickenson and Sullivan (1987) demonstrated, in electrophysiological studies, that formalin application to a hind paw excited primary afferent c-fibers in a biphasic manner and followed a time course similar to that observed in behavioral studies, suggesting that formalin-induced nocifension was mediated by the small diameter unmyelinated nociceptors.

Peripheral inflammatory processes may elicit changes in brain endogenous opioids. Porro et al. (1991) demonstrated a marked increase in β-endorphin immunoreactivity in ventral periaqueductal gray matter, ventromedial hypothalamus and some other brain regions resulting from formalin-induced inflammation (Facchinetti et al., 1992). Zangen et al. (1998) demonstrated that upon induction of a nociceptive stimulus by injection of formalin into the hind paws of rats, the extracellular levels of β-endorphin in the arcuate nucleus increased markedly, corresponding to their nocifensive response. Furthermore, pretreatment with antiserum against β-endorphin markedly increased the nocifensive response to formalin in the rat (Porro et al., 1991). The present experiments were therefore designed to systemically investigate the importance of the various endogenous opioid peptides at supraspinal sites, which might be involved in the modulation of nocifensive responses to formalin stimulation.

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2. Materials and methods

2.1. Animals

Male ICR mice, weighing 25–30 g (Charles River Breeding Laboratory, Wilmington, MA) were used. The animals were housed five per cage in a room maintained at 22 ± 0.5°C with an alternating 12-h light/dark cycle. Food and water were available ad libitum. The animals were used only once. All experiments were approved by and conformed to the guidelines of the Medical College of Wisconsin Animal Care Committee.

2.2. Nocifensive testing

Mice were placed into translucent plastic observation chambers (12 × 12 × 25 cm) for adaptation, 1 h prior to the experiments. The mice were then pretreated intracerebroventricularly (i.c.v.) with various doses of antiserum against β-endorphin, other endogenous opioid peptides or

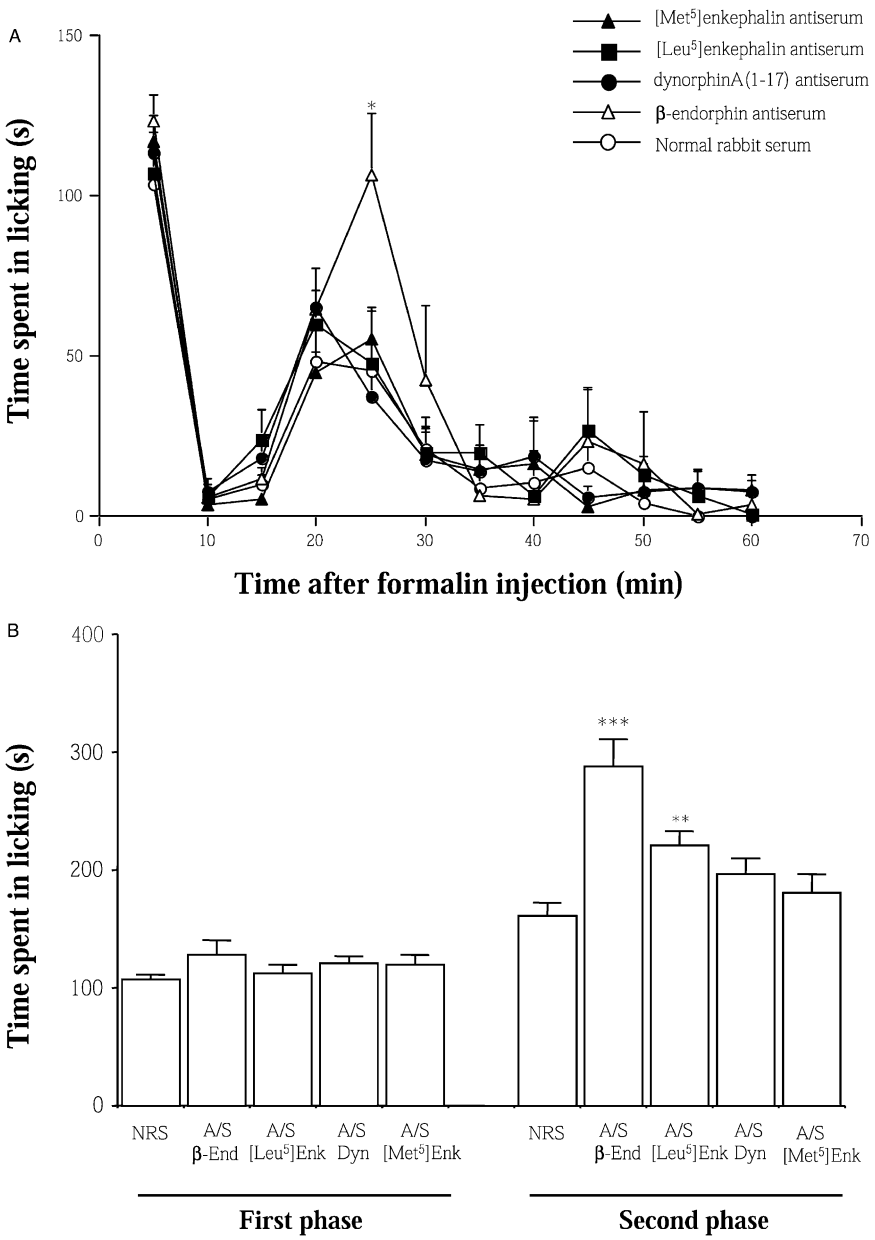

Fig. 1. Effect of i.c.v. pretreatment with normal rabbit serum (NRS) or antiserum (A/S) against β-endorphin, [Leu^5]enkephalin, [Met^5]enkephalin and dynorphin A-(1–17) on paw-licking responses induced by formalin in mice. (A) Time course of paw-licking responses following formalin injection. (B) Cumulative paw-licking responses to formalin during first and second phases. Groups of mice were pretreated with antiserum against β-endorphin, [Leu^5]enkephalin, [Met^5]enkephalin or dynorphin A-(1–17) 1 h before subcutaneous intraplantar injection of 0.5% 25 μl formalin. Paw-licking responses were then observed. The data were expressed as means ± SEM; error bars indicate SEM; the unpaired Student's *t*-test and ANOVA followed by Dunnett's test were used to test the difference between and among groups, respectively; $^{*}P < 0.05$, $^{**}P < 0.005$, $^{***}P < 0.001$ vs. normal rabbit serum; each group contained eight mice.

normal rabbit serum 1 h prior to intraplantar injection of formalin. The mice received a s.c. injection of 25 μl of 0.5% formalin solution into the plantar surface of the right hindpaw (Shibata et al., 1989) with a microsyringe and 26-gauge needle and were observed for licking behavior. Time spent licking the injected paw was counted in 5-min bins, starting with the formalin injection and ending 60 min later (Hunskaar et al., 1985; Wheeler-Aceto et al., 1990). To quantify the licking response over the first and second phases, total times spent in licking between 0 and 10 min, and between 10 and 60 min were added up, giving a cumulative distribution over time.

2.3. Antisera and intracerebroventricular injection

The antisera against β-endorphin, [Leu^5]enkephalin, [Met^5]enkephalin and dynorphin A-(1–17) were produced by repeated intradermal injection of male New Zealand rabbits with opioid peptide, coupled to bovine thyroglobulin according to the method described previously (Hollt et al., 1978). The specificities of the antisera were characterized by radioimmunoassay or enzyme-linked immunosorbent assay. The antiserum against β-endorphin does not cross-react with [Leu^5]enkephalin, [Met^5]enkephalin or dynorphin A-(1–17). The anti-[Leu^5]enkephalin serum does not immunoreact with β-endorphin or dynorphin A-(1–17). It shows a 14% cross-immunoreactivity with [Met^5]enkephalin. The anti-[Met^5]enkephalin antiserum does not cross-react with dynorphin A-(1–17) or β-endorphin. However, it shows a 29.4% cross-immunoreactivity with [Leu^5]enkephalin. The antiserum to dynorphin A-(1–17) does not show cross-immunoreactivity with β-endorphin, [Leu^5]enkephalin or [Met^5]enkephalin.

Intracerebroventricular injection was performed according to the method described by Haley and McCormick (1957), using a 25-μl Hamilton syringe with a 26-gauge needle. The injection volume was 4 μl and contained doses of antiserum ranging from 30 to 200 μg.

2.4. Statistical analysis

The behavioral test data are presented as the means ± SEM for different 5-min time bins during the first and the second phases. An unpaired Student's *t*-test was applied to assess the difference between groups. Analysis of variance (ANOVA) followed by Dunnett's test was performed to test the difference among groups. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effects of i.c.v. pretreatment with antisera against β-endorphin, [Leu^5]enkephalin, [Met^5]enkephalin and dynorphin A-(1–17) on the nocifensive responses induced by formalin injection

As shown in Fig. 1(A), the administration of 0.5% formalin produced typical biphasic nocifensive paw-licking with an initial acute phase over 0–10 min and a prolonged tonic phase from 10 to 60 min. Mice pretreated with β-endorphin antiserum (200 μg/4 μl) showed a significant enhancement at one time point of the second phase, but not of the first phase, of the nociceptive responses to formalin stimulation compared with mice pretreated with normal rabbit serum. This increase of the nocifensive responses to formalin was only significant at the 20–25-min period after formalin injection. A small, but statistically significant, increase of the second phase of nocifensive response to formalin was also observed in mice pretreated with antiserum against [Leu^5]enkephalin (200 μg/4 μl) (Fig. 1(B)). However, pretreatment with [Met^5]enkephalin (200 μg/4 μl) or dynorphin A-(1–17) (200 μg/4 μl) did not affect nocifensive responses to formalin stimulation (Fig. 1(A), (B)).

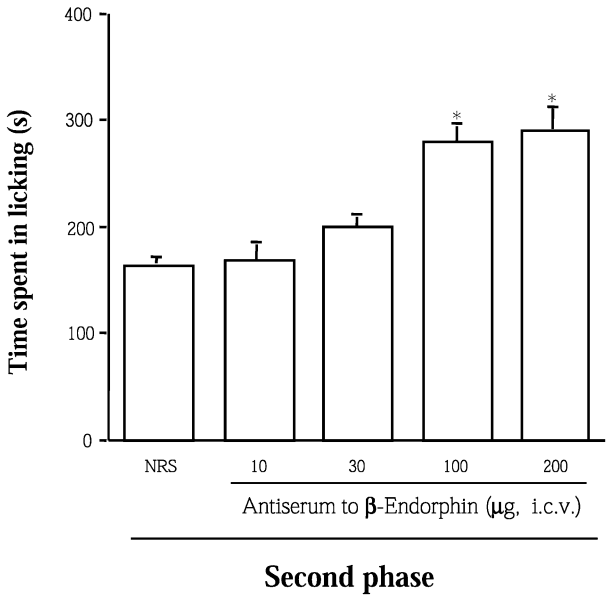

Fig. 2. Effect of different doses of antiserum against β-endorphin on second phase of paw-licking responses to formalin. Groups of mice were pretreated i.c.v. with 10, 30, 100, and 200 μg of antiserum against β-endorphin 1 h before intraplantar injections of 0.5% 25 μl formalin and the paw-licking responses were then observed. The data were expressed as means ± SEM; error bars indicate SEM; the unpaired Student's *t*-test and ANOVA followed by Dunnett's test were used to test the difference between and among groups, respectively; $^*P < 0.001$ vs. normal rabbit serum; each group contained seven to eight mice.

Pretreatment with 100 and 200 μg of antiserum against β-endorphin significantly enhanced the second phase of the nocifensive responses to formalin (Fig. 2). None of these doses of β-endorphin antiserum affected the first phase of the formalin-induced nocifension (data not shown).

4. Discussions

The results of our present studies clearly demonstrate that pretreatment with antiserum against β-endorphin,

which binds the released β-endorphin at the extracellular sites, markedly enhanced the second phase, but not the first phase of the nocifensive response to formalin stimulation in mice. These observations confirm and extend those of Porro et al. (1991), who showed an increase in formalin-induced flinching after pretreatment with antiserum against β-endorphin in the rat. Furthermore, Hamba (1988) earlier reported that rats displayed increased behavioral responses to formalin injection after lesion of the arcuate nucleus of the hypothalamus, the main site of β-endorphin-producing cells. These findings strongly indicate that formalin stimulation activates a central β-endorphinergic system and induces the release of β-endorphin, which, in turn, exerts an inhibitory effect on the nocifensive response. Since the first phase of the nocifensive response to formalin stimulation was not affected by the β-endorphin antiserum pretreatment, it is most likely that the central β-endorphinergic system is not tonically active in normal circumstances, but is stimulated initially by formalin during the first phase of the nocifensive response. This view is supported by the findings that immunoreactive β-endorphin is increased in periaqueductal gray and other brain regions important for pain and pain control, and that there is a release of immunoreactive β-endorphin from the hypothalamic arcuate nucleus at times corresponding to the second phase of nociceptive responses to formalin stimulation (Porro et al., 1988; Facchinetti et al., 1992; Zangen et al., 1998).

We found that i.c.v. pretreatment with antiserum against [Leu^5]enkephalin, but not [Met^5]enkephalin or dynorphin A-(1–17), also enhanced the paw-licking induced by formalin injection, suggesting that [Leu^5]enkephalin, but not [Met^5]enkephalin or dynorphin A-(1–17), was also released during attenuation of formalin-induced nocifension. Similarly, Ossipov et al. (1996) reported that intrathecal pretreatment with antiserum against [Leu^5]enkephalin, but not [Met^5]enkephalin, potentiated formalin-induced nocifension, suggesting that [Leu^5]enkephalin, but not [Met^5]enkephalin, is also released from the spinal cord. However, administration of antiserum against dynorphin A-(1–17), given spinally, was also found to potentiate the formalin-induced nociception.

The question arises as to the source of the [Leu^5]enkephalin. [Leu^5]enkephalin and [Met^5]enkephalin share the same precursor, preproenkephalin, and are supposed to stimulate the same δ-opioid receptors. However, pretreatment with antiserum against [Met^5]enkephalin did not affect formalin-induced nocifension. Thus, it is not likely that the source of [Leu^5]enkephalin is preproenkephalin. Silberring et al. (1992) described a dynorphin convertase, which degrades dynorphins into [Leu^5]enkephalin[Arg^6]. A carboxypeptidase then acts on [Leu^5]enkephalin[Arg^6] to form [Leu^5]enkephalin. Thus, [Leu^5]enkephalin could have come from preprodynorphin.

The 200-μg dose of antiserum against [Met^5]enkephalin or dynorphin A-(1–17) used for pretreatment in the present study is judged to be sufficient to bind any [Met^5]enkephalin or dynorphin A-(1–17) released extracellularly. We have previously demonstrated that the antinociception induced by etorphine, β-endorphin or bremazocine given i.c.v. was blocked by the intrathecal pretreatment with even smaller doses of the same antiserum against [Met^5]enkephalin or dynorphin A-(1–17). This indicated that the antinociceptive effects induced by etorphine or β-endorphin are mediated by the release of [Met^5]enkephalin and those induced by bremazocine involve the release of dynorphin A-(1–17) (Tseng and Collins, 1993; Xu and Tseng, 1997). The failure of the pretreatment with these two antisera to affect the formalin response indicates that [Met^5]enkephalin and dynorphin A-(1–17) at the supraspinal sites are not involved in modulation of the formalin responses.

It is concluded that pretreatment with antiserum against β-endorphin, or to a lesser extent, that against [Leu^5]enkephalin, but not with antiserum against [Met^5]enkephalin or dynorphin A-(1–17) enhanced the formalin-induced nocifension. Our findings suggest that formalin stimulation activates the central β-endorphinergic and [Leu^5]enkephalinergic systems and causes the release of β-endorphin and [Leu^5]enkephalin, which, in turn, attenuate the nocifensive response to formalin in mice.

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