Br. J. Pharmacol. (1993), **108**, 1137–1142

Inhibition of spinal opioid antinociception by intrathecal β-endorphin$_{1-27}$ in the rat

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1 The effects of intrathecal (i.t.) administration of β-endorphin and two shorter fragments, human and ovine β-endorphin$_{1-27}$, were examined for antinociceptive activity in the tail-flick and paw-pressure tests in the rat. Additionally, the ability of ovine β-endorphin$_{1-27}$ to influence the action of i.t. β-endorphin, morphine and [D-Pen^2-D-Pen^5]enkephalin (DPDPE) was also examined in these tests.

2 After i.t. injection, β-endorphin produced potent dose-related antinociception in the tail-flick and paw-pressure tests. Shorter endorphins produced much weaker effects. The order of antinociceptive efficacy was β-endorphin > human β-endorphin$_{1-27}$ > ovine β-endorphin$_{1-27}$.

3 Administration of ovine β-endorphin$_{1-27}$ (0.72, 1.44 nmol, i.t.) significantly attenuated the antinociceptive effect of β-endorphin (2.88 nmol, i.t.) in the tail-flick and paw-pressure tests.

4 Both i.t. morphine and DPDPE produced dose-related antinociception in the tail-flick and paw-pressure tests. The potency of DPDPE was lower than that of morphine in both tests; however, the effect of DPDPE was weaker in the paw-pressure test.

5 Administration of ovine β-endorphin$_{1-27}$ (1.44 nmol, i.t.) significantly attenuated the antinociceptive effect of morphine (14.9 nmol, i.t.) in both tests and the effect of DPDPE (38.7 nmol) in the tail-flick test.

6 The results show that β-endorphin$_{1-27}$ acts as an opioid antagonist at the spinal level in the rat. Its ability to inhibit the action of morphine and DPDPE suggests that it may attenuate β-endorphin action by an interaction with μ- and/or δ-opioid receptors.

Keywords: β-Endorphin; β-endorphin$_{1-27}$; antinociception; tail-flick test; paw-pressure test; antagonist; intrathecal administration

Introduction

The presence of β-endorphin, a 31-amino acid endogenous opioid peptide of the proopiomelanocortin family in the pituitary and brain is associated with the existence of shorter carboxy terminal fragments, β-endorphin$_{1-26}$ and β-endorphin$_{1-27}$, in acetyl and non-acetyl forms (Zakarian & Smyth, 1982; Dores *et al.*, 1986). These peptide fragments have also been detected in the external medium following incubation of rat hypothalamic slices under physiological conditions (Hong *et al.*, 1989). Pharmacological studies involving the use of opioid-sensitive isolated tissue preparations have demonstrated that β-endorphin$_{1-27}$ behaves as an agonist and that in certain preparations its potency is comparable to that of the parent peptide, β-endorphin (McKnight *et al.*, 1983). However, several *in vivo* studies have demonstrated that β-endorphin$_{1-27}$ also has the potential to inhibit certain effects of β-endorphin. In mice, intracerebroventricular (i.c.v.) injections of β-endorphin$_{1-27}$ have been reported to inhibit the antinociceptive (Hammonds *et al.*, 1984), hypothermic (Suh *et al.*, 1987) and motivational effects (Bals-Kubik *et al.*, 1988) of β-endorphin administered by the same route. However, i.c.v. β-endorphin$_{1-27}$ was found not to influence the antinociceptive effects of centrally administered morphine or [D-Pen^2-D-Pen^5]enkephalin (DPDPE), agonists which selectively activate μ- and δ-receptors, respectively (Suh *et al.*, 1988). In contrast, i.c.v. β-endorphin$_{1-27}$ was found to block the motivational effect of β-endorphin, DPDPE and D-Ala^2,N-Me-Phe^4,Gly-ol-enkephalin (DAMGO), a μ-receptor selective agonist. These observations on the opioid antagonism by β-endorphin$_{1-27}$ have led to the suggestion that fragments derived from β-endorphin in the brain may act as endogenous inhibitors of endorphin-mediated functions (Hammonds *et al.*, 1984; Nicolas & Li, 1985; Bals-Kubik *et al.*, 1988).

Until recently, the existence of β-endorphin in the spinal cord tissue was not recognized, although spinal injection of this peptide has been shown to produce potent antinociceptive action in the rat (Yaksh & Henry, 1978). Evidence from anatomical studies has revealed the presence of β-endorphin-immunoreactive fibres, but not cell bodies in the rat spinal cord (Tsou *et al.*, 1986). A recent biochemical study demonstrated that β-endorphin immunoreactive material is present in extracts of the rat spinal cord, and chromatographic analysis of these extracts showed the presence of acetyl and non-acetyl forms of β-endorphin, β-endorphin$_{1-27}$ and β-endorphin$_{1-26}$ (Gianoulakis & Angelogianni, 1989). Thus, these anatomical and biochemical findings have raised important questions regarding the role of different β-endorphin-derived peptides in functions of the spinal cord.

Since the spinal actions of short fragments of β-endorphin have not been evaluated, the present investigation was undertaken to study effects of human β-endorphin as well as the effects of ovine and human forms of β-endorphin$_{1-27}$ in tests of spinal nociception. This paper describes the actions of these peptides in the tail-flick and paw-pressure tests after acute intrathecal administration in the rat. It also describes the influence of ovine β-endorphin$_{1-27}$ on the antinociceptive actions of spinally administered β-endorphin, morphine and DPDPE in these tests.

Methods

Surgical procedure

All experiments were performed on male, Sprague-Dawley rats weighing 270–300 g. Animals were housed separately, given full access to food and water, and acclimatized to the environment three to four days before experimentation. Room temperature was maintained at 21–22°C under a 12-h

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light/12-h dark cycle.

The technique of intrathecal drug administration, originally described by Yaksh & Rudy (1976), was used to test the spinal actions of opioids on thermally and mechanically-induced nociceptive reactions. Briefly, the animals were anaesthetized under halothane (4% induction, 2% maintenance) and placed in a rat stereotaxic frame. A PE-10 intramedic polyethylene catheter (i.d. 0.28 mm × o.d. 0.61 mm) (Clay Adams; Becton, Dickinson and Company, Parsippany, NJ, U.S.A.) was inserted into the intrathecal space through a transverse slit made in the cisternal membrane and carefully passed 7.5 cm into the lumbar subarachnoid space. The free end of the catheter was exteriorized through the skin over the skull and the protruding segment of the catheter was plugged with a short piece of stainless steel wire. The skin over the cisternal opening was closed by a suture, anaesthesia discontinued, and the animal returned to its home cage. Animals showing neurological deficits (hind limb flexion, rigidity or paralysis) following recovery from surgery were excluded from experiments.

Experimental protocol

Following a three-day recovery period from surgery, catheter-implanted animals were used only once for antinociception tests (tail-flick and paw-pressure) performed between 09 h 00 min and 12 h 00 min. Saline or drugs were injected via the intrathecal catheter with a 50-μl Hamilton syringe in a 10 μl volume followed by a 10 μl 0.9% saline injection to flush the catheter.

The tail-flick test (D'Amour & Smith, 1941) was used to evaluate the response to thermal nociceptive stimuli. Thermal heat was applied to the base of the tail using an analgesia meter (Owen *et al.*, 1981) with the heat source intensity adjusted to provide a baseline response latency of 2–3 s and the cut-off time set at 10 s.

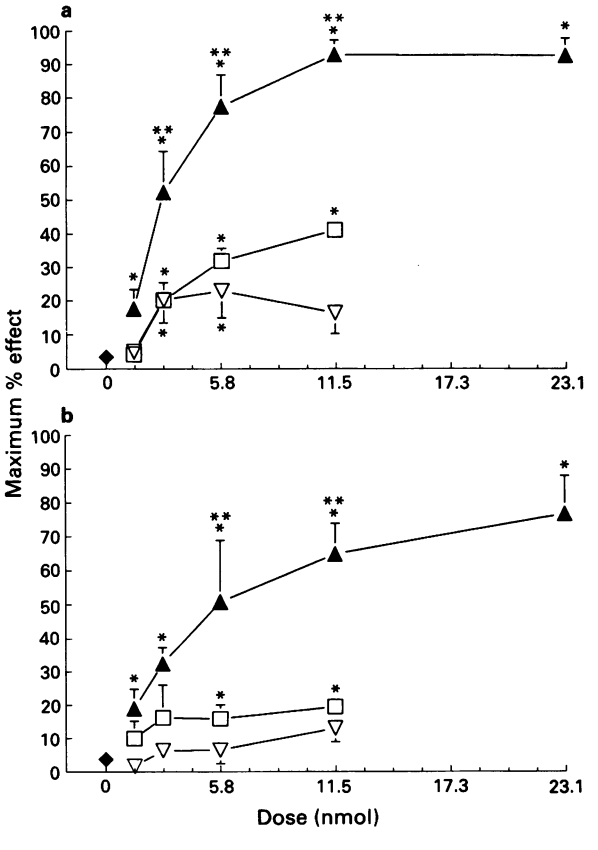

Figure 1 Dose-response curves showing the antinociceptive effects of intrathecally injected saline (◆), human β-endorphin (▲), human β-endorphin$_{1-27}$ (□), and ovine β-endorphin$_{1-27}$ (▽) in the (a) tail-flick test and (b) paw-pressure test. The values shown are the peak responses elicited after injection of each dose. Each point represents the mean ± s.e. of 5–6 animals. *$P<0.05$ versus saline-injected; **$P<0.05$ versus human and ovine β-endorphin$_{1-27}$.

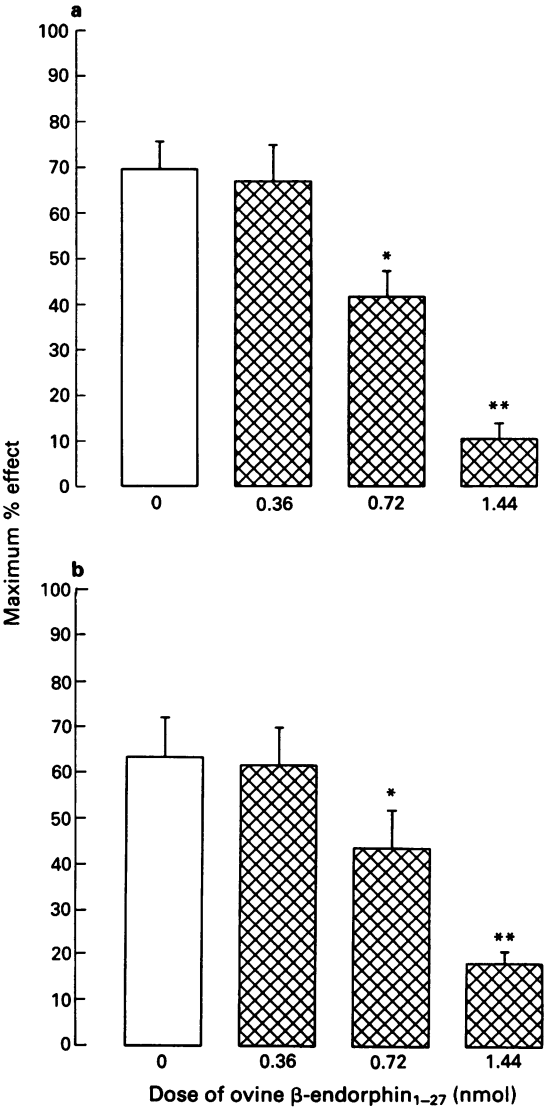

Figure 2 The effect of different intrathecal doses of ovine β-endorphin$_{1-27}$ (0.36, 0.72, 1.44 nmol) (cross-hatched columns) on the antinociceptive response produced by β-endorphin (2.88 nmol, i.t.) (open columns) in the (a) tail-flick test and (b) paw-pressure test. Each column represents the peak response elicited by the treatment. Values shown are a mean ± s.e. of 5–6 animals. *$P<0.05$; **$P<0.01$ (versus β-endorphin alone).

The paw-pressure test, previously described by Loomis *et al.* (1985), was used to evaluate the response to mechanical nociceptive stimuli. Briefly, mechanical pressure was applied to the surface of a non-inflamed hind paw using an air-filled syringe coupled to a pressure gauge until a withdrawal response was observed and the pressure immediately recorded and released. A cut-off pressure of 300 mmHg was used in this test.

Control responses in both tests for each rat were determined before i.t. injections. Following an i.t. injection of saline or drug, the response to each stimulus was determined at 10-min intervals for a period of 60–90 min. The measurement of the paw-pressure withdrawal response was interspersed with tail-flick latency measurements. Previous experiments have demonstrated that no interactions occur between responses in the two tests (Loomis *et al.*, 1985). Results of the tail-flick and paw-pressure tests at each time

point were expressed as the maximum percentage effect (MPE):

$$\frac{\text{Post drug response} - \text{baseline response}}{\text{Cut-off value} - \text{baseline response}} \times 100$$

At the end of each experiment, the placement of the catheter in the lumbar region was verified by dye injections.

Drugs and solutions

β-Endorphin, human and ovine β-endorphin$_{1-27}$, and DPDPE were obtained from Peninsula Laboratories, Inc. (California, U.S.A.). Morphine sulphate was obtained from BDH Pharmaceuticals (Toronto, Canada). All drugs were dissolved in 0.9% saline immediately prior to injection and expressed as quantity injected in nmol. In all experiments, the total volume injected into the intrathecal space did not exceed 20 μl.

Statistical analysis

All data reported represent the mean MPE ± s.e. (standard error). Significant difference at individual time points between two groups was determined by unpaired, two-tailed Student's *t* test where appropriate. Statistical analysis of the time-course effect or dose-response effect between groups was conducted by analysis of variance (ANOVA), followed by Newman-Keuls multiple comparison test. Differences were considered significant at $P \leqslant 0.05$.

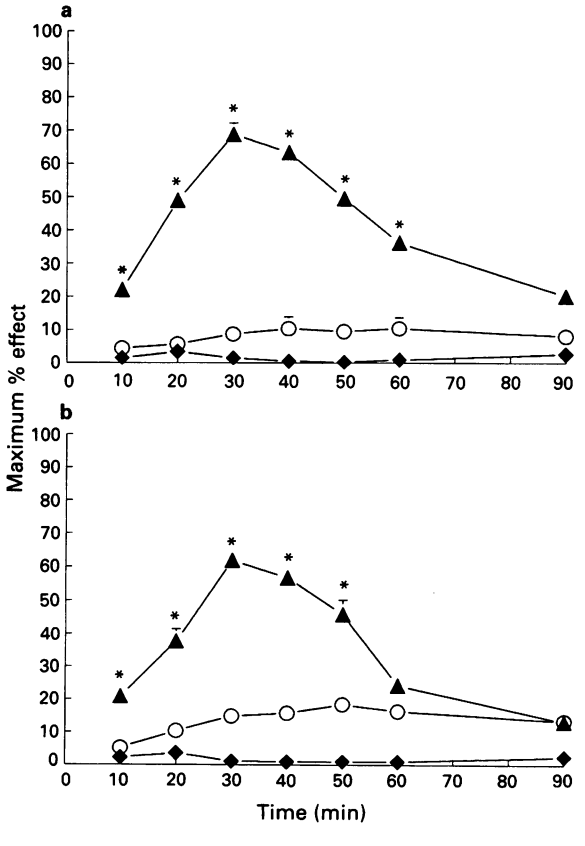

Figure 3 The time-course of antinociceptive effect of intrathecal human β-endorphin (2.88 nmol) alone (▲) and in combination (○) with ovine β-endorphin$_{1-27}$ (1.44 nmol) in the (a) tail-flick test and (b) paw-pressure test; (◆) effect of saline injection. Each point represents mean ± s.e. of 5–6 animals. *$P<0.05$, significantly different from saline-injected animals and drug combination injected animals.

Results

In control experiments ($n = 5$), i.t. administration of saline had no effect in the tail-flick or paw-pressure tests. Ten minutes after i.t. saline, the tail-flick latency was 2.32 ± 0.25 s compared to the pre-injection latency of 2.18 ± 0.17 s, and the threshold pressure for eliciting a hind paw withdrawal response was 99.3 ± 8.2 mmHg compared to the pre-injection threshold pressure of 97.5 ± 7.4 mmHg. These values remained stable over the 60–90 min testing period following the saline injection.

Effect of i.t. β-endorphin and β-endorphin$_{1-27}$

Figure 1 illustrates comparative effects of intrathecally injected β-endorphin, human β-endorphin$_{1-27}$, and ovine β-endorphin$_{1-27}$ in the tail-flick and paw-pressure tests. In both tests, β-endorphin produce a dose-related antinociceptive effect represented by an increased response latency in the tail-flick test and elevated threshold pressure for eliciting a paw withdrawal response in the paw-pressure test. In both tests, the peak effect occurred at 30 min post injection and the response returned to near baseline level 60–90 min post injection (see below). The dose-response curve for the effect of β-endorphin (Figure 1) was characterized by a very steep rise in the response with an increase in the injected dose (1.44, 2.88, 5.8, 11.5, 23.1 nmol, i.t.). The values (MPE) of maximum responses obtained in the tail-flick and paw-pressure tests were 92.9 ± 4.4 and 76.6 ± 11.3, respectively. In contrast, i.t. injections of the two forms of β-endorphin$_{1-27}$ (1.44, 2.88, 5.8, 11.5 nmol, i.t.) produced considerably weaker antinociceptive effects. The maximum responses (MPE) produced by human β-endorphin$_{1-27}$ were 41.2 ± 2.1 (tail-flick test) and 19.5 ± 2.6 (paw-pressure test), and corresponding values produced by the ovine peptide were 23.1 ± 7.9 and 13.0 ± 4.2. Thus, all three peptides tested here produced antinociceptive activity in the tail-flick and paw-pressure tests, the rank order of their apparent efficacy being: β-endorphin > human β-endorphin$_{1-27}$ > ovine β-endorphin$_{1-27}$. None of the three peptides produced signs indicative of an impairment of motor function.

Effect of ovine β-endorphin$_{1-27}$ on the response to i.t. β-endorphin

To examine the interaction between β-endorphin$_{1-27}$ and β-endorphin, i.t. injection of a submaximal dose (2.88 nmol) of β-endorphin was delivered in combination with each of three doses (0.36, 0.72 and 1.44 nmol) of ovine β-endorphin$_{1-27}$. These doses of β-endorphin$_{1-27}$ produced no apparent antinociceptive effect when injected alone, but in combination experiments, two doses (0.72 and 1.44 mol) of the peptide significantly attenuated the effect of β-endorphin in both tests (Figure 2). Administration of 0.72 nmol of β-endorphin$_{1-27}$ reduced the antinociceptive effect of β-endorphin by about 40% in the tail-flick test and 31% in the paw-pressure test. A higher dose (1.44 nmol) reduced this effect by 85% and 71% in the tail-flick and paw-pressure tests, respectively. Thus, spinal administration of the shorter peptide exerted an inhibitory action on the antinociceptive effect elicited by β-endorphin in both tests. The time-course of the antinociceptive effect of β-endorphin, administered alone or in combination with β-endorphin$_{1-27}$, is depicted in Figure 3. As shown, the peak effect in the tail-flick test occurred at 30 min and recovered to near baseline level 90 min post injection. The attenuation of the β-endorphin-induced effect by β-endorphin$_{1-27}$ (1.44 nmol) was apparent 10 min after the injection and this reduction was sustained over the 60–90 min observation period. A similar time-course of effect was seen in the paw-pressure test.

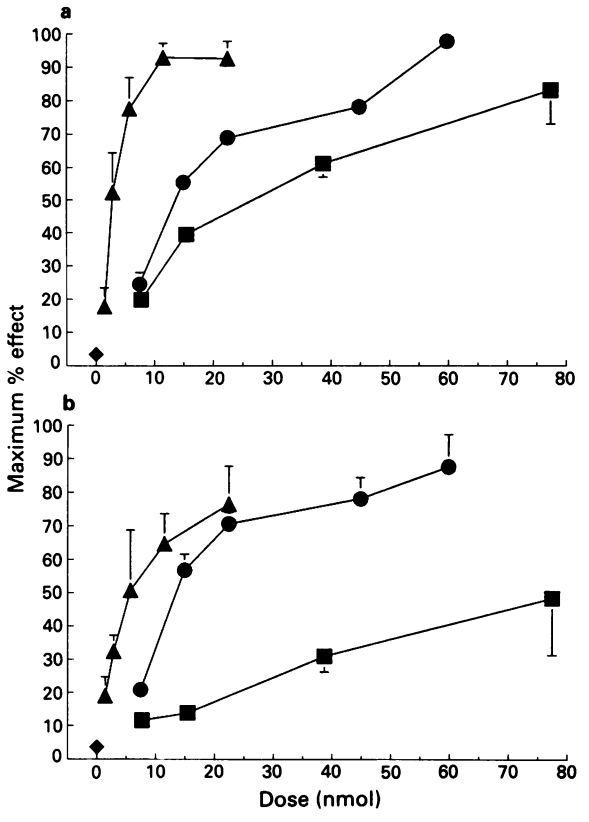

Figure 4 Dose-response curves showing the antinociceptive effects of intrathecal β-endorphin (▲), morphine (●) and [D-Pen^2-D-Pen^5]-enkephalin (DPDPE) (■) in the (a) rat tail-flick test and (b) paw-pressure test; (◆) saline injection (20 μl, i.t.). The values shown are peak responses elicited by each agent. Each point represents the mean ± s.e. of 5–6 animals. The effects shown at all doses, except DPDPE (7.7 nmol) in paw-pressure test, are significantly different ($P<0.05$) from saline-injected.

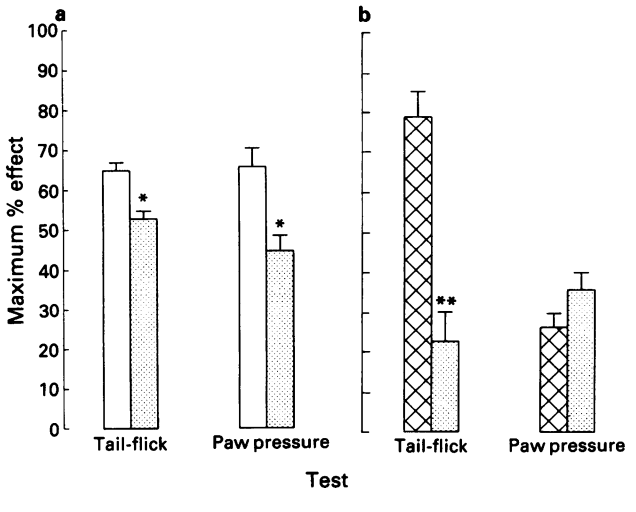

Figure 5 The effect of intrathecal β-endorphin$_{1-27}$ (stippled column, 1.44 nmol) on the antinociceptive effect of (a) morphine (open column, 14.9 nmol, i.t.) and (b) [D-Pen^2-D-Pen^5]enkephalin (DPDPE) (cross hatched column, 38.7 nmol, i.t.) in the tail-flick test and paw-pressure test. Each column represents peak response elicited by the treatment. Values shown are a mean ± s.e. of 5–6 animals. *$P<0.05$ versus morphine alone; **$P<0.01$ versus DPDPE alone.

Effect of ovine β-endorphin$_{1-27}$ on responses to i.t. morphine and DPDPE

In subsequent experiments, the action of ovine β-endorphin$_{1-27}$ on the antinociceptive action of morphine and DPDPE in the two tests was examined. Before evaluating these interactions, the dose-response curves for the spinal antinociceptive effects of morphine and DPDPE were derived with a view to selecting a submaximally effective dose of these agonists for testing in the combination experiments. Figure 4 shows the comparative effects of morphine (7.5, 14.9, 22.4, 44.8, 59.8 nmol, i.t.) and DPDPE (7.7, 15.5, 38.7, 77.4 nmol, i.t.), as well as the effects of β-endorphin (1.44, 2.88, 5.8, 11.5, 23.1 nmol, i.t.) in the two tests. The rank order of apparent agonist potency of the three agents in both tests was: β-endorphin > morphine > DPDPE. The efficacy of morphine and DPDPE in the tail-flick test was comparable to that of β-endorphin in this test; however, DPDPE was considerably less efficacious than morphine or β-endorphin in the paw-pressure test. The maximal response produced by DPDPE (77.4 nmol) in the paw-pressure tests approximated only 50% of that observed in the tail-flick test. Higher doses of DPDPE were not tested owing to their tendency to interfere with motor function.

Figure 5 shows the results of experiments in which ovine β-endorphin$_{1-27}$ (1.44 nmol) was administered in combination with a submaximal dose of morphine (14.9 nmol) or DPDPE (38.7 nmol). In these experiments, β-endorphin$_{1-27}$ attenuated the antinociceptive effect of morphine in both tests and that of DPDPE in the tail-flick test. In the tail-flick and paw-pressure tests, the effect of morphine was significantly reduced by the peptide by 20% and 30%, respectively. In the tail-flick test, the same dose of β-endorphin$_{1-27}$ produced a 72% decrease in the DPDPE-induced response, but failed to modify significantly this response in the paw-pressure test. Thus, a dose of ovine β-endorphin$_{1-27}$ which attenuated the effect of β-endorphin in preceding experiments also inhibited the antinociceptive effect of morphine in both tests and of DPDPE in the tail-flick test.

Discussion

In this study, the intrathecal administration of two shorter carboxy terminal endorphin fragments, ovine and human β-endorphin$_{1-27}$, produced much weaker antinociceptive effects than those of β-endorphin in the tail-flick and paw-pressure tests in the rat. The ovine peptide also effectively attenuated the antinociceptive action produced by intrathecal β-endorphin. The choice of ovine β-endorphin$_{1-27}$ for use in combination experiments was influenced by the observation that it had a very low intrinsic activity, a property which would minimally interfere with the observance of its potential antagonist action. The inhibition of the β-endorphin-induced effect by intrathecal β-endorphin$_{1-27}$ seen here has been reported in earlier antinociception studies which evaluated the action of i.c.v. human β-endorphin$_{1-27}$ on β-endorphin-induced effects in mice (Hammonds *et al.*, 1984; Nicolas & Li, 1985; Suh *et al.*, 1988). However, in contrast to the results of those i.c.v. studies showing that administration of human β-endorphin$_{1-27}$ was ineffective against the antinociceptive effects of morphine or DPDPE, in the present study intrathecal injection of ovine β-endorphin$_{1-27}$ reduced the effect of spinal morphine in both antinociceptive tests and DPDPE in the tail-flick test. This discrepancy between the results obtained with the i.c.v. and those with the i.t. route of administration may be related to differences in the doses and forms of β-endorphin$_{1-27}$, or in the species of animals used in these experiments. However, the discrepancy could also reflect potential differences in the mechanism underlying the actions of β-endorphin, morphine and DPDPE in the brain and spinal cord.

Tseng and coworkers explained the differential action of

β-endorphin$_{1-27}$ against i.c.v. β-endorphin and morphine on the basis that these two agonists produce their antinociceptive effects by activating distinct receptors linked to different descending spinal pathways (Tseng & Fujimoto, 1985). According to their proposal, i.c.v. β-endorphin activates a descending bulbospinal pathway resulting in the release of spinal methionine-enkephalin, an action which is blocked by i.c.v. β-endorphin$_{1-27}$ (Tseng & Li, 1986). On the other hand, i.c.v. morphine activates a similar non-opioid, possibly 5-hydroxytryptaminergic or noradrenergic, pathway to produce antinociceptive effects (Tseng *et al.*, 1985, 1986). Since morphine did not induce a release of spinal methionine-enkephalin, it was suggested that β-endorphin mediates its action through the activation of a brainstem ε-receptor which is not sensitive to morphine (Tseng *et al.*, 1985). Thus, the ability of β-endorphin$_{1-27}$ to antagonize the action of β-endorphin has been attributed to a blockade of the ε-receptor (Suh & Tseng, 1988; Suh *et al.*, 1988). The failure of β-endorphin$_{1-27}$ to influence the action of morphine, a μ-receptor selective agonist, has suggested that this peptide does not interact with the μ-receptor. However, this differential action of β-endorphin$_{1-27}$ is surprising in view of the observation that this shorter peptide fragment does not show selectivity for a specific opioid receptor type in receptor binding studies (Hammonds & Li, 1985). Furthermore, the notion that β-endorphin-induced antinociception involves ε-receptor activation is at variance with the finding that this response is inhibited by antagonists selective for μ- and δ-receptors (Shook *et al.*, 1988). This finding suggests that both μ- and δ-receptors may have a role in β-endorphin-induced effects.

Since evidence for the existence of ε-receptors in the spinal cord is lacking, it is difficult to attribute the antagonist action of intrathecal β-endorphin$_{1-27}$ seen here to a blockade of these receptors. Since β-endorphin is recognized to activate both μ- and δ-receptors (McKnight *et al.*, 1983), its potent spinal antinociceptive effects most probably involve a synergistic activation of these two receptor types. Thus, intrathecal β-endorphin$_{1-27}$ could antagonize the effect of β-endorphin by blocking spinal μ- and/or δ-receptors and thus preventing this synergistic activation. This explanation is supported by the observation that in the present study, β-endorphin$_{1-27}$ attenuated the antinociceptive effects of morphine and DPDPE, agonists acting at μ- and δ-receptors, respectively. Additional support is provided by a previous observation that in certain isolated tissue preparations β-endorphin$_{1-27}$ interacts with μ- and δ-receptors (McKnight *et al.*, 1983). However, in such preparations an inhibition of the β-endorphin effect by the shorter peptide fragment has not been demonstrated (Wuster *et al.*, 1979). In behavioural experiments, i.c.v. human β-endorphin$_{1-27}$ has been found to inhibit motivational effects of β-endorphin, DPDPE, and DAMGO (Bals-Kubik *et al.*, 1988). It is not clear at present, why i.c.v. injections of human β-endorphin$_{1-27}$ influence the motivational but not antinociceptive effects of centrally injected DPDPE and DAMGO.

Although intrathecal β-endorphin$_{1-27}$ significantly reduced the effect of DPDPE in the tail-flick test, it surprisingly failed to influence the antinociceptive effect of this agonist in the paw-pressure test. The effect of DPDPE in the latter test was considerably weaker than that in the tail-flick test, suggesting spinal δ-receptors may be less important in the modulation of mechanically-induced nociception. Alternatively, considering the potential heterogeneity of the δ-receptor (Porreca *et al.*, 1987), the subtypes mediating antinociception in the tail-flick and paw-pressure tests may be different, with β-endorphin$_{1-27}$ preferentially interfering with that involved in thermal antinociception. Additional experiments are required to evaluate the role of different δ-receptors in various antinociception tests.

The presence of β-endorphin$_{1-27}$ in spinal regions (Gianoulakis & Angelogianni, 1989) and the finding that this peptide influences antinociceptive actions of opioids at the spinal level suggests that certain endorphin fragments may have a role in modulating the transmission of nociceptive signals. Its ability to inhibit spinal antinociception mediated via μ- and δ-receptors suggests that this peptide may behave as an inhibitory modulator of endogenous opioids. However, the full significance of β-endorphin$_{1-27}$ action cannot be ascertained until its effects on other opioid receptor-mediated responses including the development of tolerance and dependence have been evaluated.

This work was supported by the Medical Research Council of Canada. The authors acknowledge the assistance of Mrs J. LeSarge in the preparation of the manuscript.

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(Received September 18, 1992
Revised December 16, 1992
Accepted December 21, 1992)