ELSEVIER

European Journal of Pharmacology 370 (1999) 287–296

ejp

Effects of U-69,593, a κ-opioid receptor agonist, on carrageenin-induced peripheral oedema and Fos expression in the rat spinal cord

Gwénaëlle Catheline *, Stéphanie Le Guen, Jean-Marie Besson

Unité de Recherche de Physiopharmacologie du Système Nerveux, I.N.S.E.R.M. U 161 and E.P.H.E. 2 rue d'Alésia, 75014 Paris, France

Received 26 November 1998; received in revised form 24 February 1999; accepted 2 March 1999

Abstract

In an attempt to study the anti-inflammatory and the antinociceptive effects of a κ_1-opioid receptor agonist (U-69,593: *trans*-3,4-dichloro-*N*-methyl-*N*-[7-(1-pyrrolidinyl)cycloexil]benzene acetamide methanesulfonate), we used a combination of the measurement of peripheral oedema (with a calliper) and Fos immunodetection in the carrageenin model of inflammation. The intraplantar injection of carrageenin-induced the development of a peripheral oedema, associated with an increase in Fos-like immunoreactivity at the level of the dorsal horn of the spinal cord. U-69,593 administered intravenously (i.v.) 10 min before carrageenin administration over the dose range 0.75, 1.5 and 3 mg/kg, reduced both paw and ankle oedema in a non dose-dependent manner. The maximal decrease was observed at the highest dose and did not exceed 21% and 20% for the paw and the ankle respectively. These effects were κ-opioid receptor specific since the anti-inflammatory effect of 1.5 mg/kg i.v. of U-69,593 was antagonised by a specific κ-opioid receptor antagonist nor-binaltorphimine. Pre-treatment with U-69,593 strongly decreased the number of Fos-like Immunoreactive neurones of the spinal cord in a dose-dependent, antagonist reversible manner; maximal effect was 65%. The disparate results between the anti-inflammatory effects and the depressive effects on Fos expression suggest that anti-inflammatory effects of κ-opioid receptor agonist are of minor importance for the antinociceptive effects of this compound.

Keywords: c-Fos; Spinal cord; κ-Opioid receptor agonist; U-69,593; Carrageenin-induced inflammation

1. Introduction

It is admitted that κ-opioid receptor agonists produce antinociceptive effects (Millan, 1990; Dhawan et al., 1996; Barber and Gottschlich, 1997). Despite controversial results, the antinociceptive effects of these compounds could result in part from a spinal and/or supraspinal sites of action. In addition, numerous investigations clearly described a peripheral analgesic effects of κ-opioid receptor agonists (Russell et al., 1987; Hargreaves et al., 1988; Abbott, 1988; Stein et al., 1989; Haley et al., 1990; Taiwo and Levine, 1991; Andreev et al., 1994; Kolesnikov et al., 1996; Bilsky et al., 1996; Nagasaka et al., 1996; Beyer et al., 1997; Catheline et al., 1998; Machelska et al., 1998; Inoue and Ueda, 1998). This peripheral site of action is of special interest, since κ-opioid receptor agonists produce dysphoria, resulting from their action at the Central Nervous System (CNS) level (Pfeiffer et al., 1986; Dykstra et al., 1987). Thus, the development of κ-opioid receptor agonists, which do not cross the blood brain barrier, could present several advantages for the treatment of pain from peripheral origin (for review see Barber and Gottschlich, 1997; Stein et al., 1997).

The peripheral effects of opioid receptor agonists are especially observed in inflammatory conditions (Stein et al., 1997). Moreover, it is well established that opioids contribute to the regulation of inflammatory response by modulation of inflammatory cell infiltrate (see Ref. in Stefano et al., 1996). Some studies have in fact demonstrated that opioid receptor agonists could have anti-inflammatory effects (Gyires et al., 1985; Joris et al., 1990; Wheeler-Aceto and Cowan, 1991; Planas et al., 1994; Earl et al., 1996; Sacerdote et al., 1996; Wilson et al., 1996; Binder and Walker, 1998). Unfortunately, only few studies evaluated quantitatively the effects of κ-opioid receptor agonists on the clinical signs of inflammation (see Ref. in

* Corresponding author. Tel.: +33-1-40789350; Fax: +33-1-45881304; E-mail: cathel@broca.inserm.fr

0014-2999/99/$ - see front matter
PII: S0014-2999(99)00153-3

Walker et al., 1997). Recently, using an adjuvant model of arthritis it has been shown that the κ-opioid receptor agonist U-50,488H (*trans*-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]-benzenacetamide) markedly attenuates adjuvant arthritis as judged by oedema, radiography and histological changes (Wilson et al., 1996). Moreover, U-50,488H was more potent after local compared to distant subcutaneous (s.c.) injections, and when the drug was administered at the disease onset. The same group have confirmed and extended these results with asimadoline, a peripherally selective κ-opioid receptor agonist (Binder and Walker, 1998).

We have previously demonstrated that the combination of the measure of peripheral oedema with the evaluation of spinal Fos expression represent a suitable and appropriate approach to judge the effectiveness of drugs in the model of inflammatory nociception. Indeed, we have observed that different non-steroidal anti-inflammatory drugs (NSAIDs) dose-dependently reduce the development of the carrageenin-induced oedema, as well as the number of neurones expressing Fos protein at the spinal cord level. A direct correlation between the size of the oedema and Fos expression was clearly established (Buritova and Besson, 1998; Buritova et al., 1998).

The objectives of the present study were to evaluate 1) the eventual anti-inflammatory effects of i.v. administration of U-69,593 (*trans*-3,4-dichloro-*N*-methyl-*N*-[7-(1-pyrrolidinyl)cycloexil]benzene acetamide methanesulfonate)), a κ_1-opioid receptor agonist, on acute inflammation induced by the intraplantar (i.pl.) administration of carrageenin 2) its antinociceptive effects judged by Fos expression induced in the spinal cord in the same animals.

2. Materials and methods

2.1. Experimental animals

Adult male albino Sprague–Dawley rats (Charles River, France), weighing 175–200 g on arrival, were housed 6 per cage in a room with controlled temperature (22 ± 1°C) and a 12 h alternating light–dark cycle for a week before experiments. Food and water were made available ad libitum. Guidelines on ethical standards for investigations of experimental pain in conscious animals were followed (Zimmermann, 1983).

Forty four rats were divided into 7 groups. A control group ($n = 10$) of rats received intravenous (i.v.) saline 10 min before intraplantar (i.pl.) injection of carrageenin 6 mg/150 μl of saline (λ-carrageenin, Sigma, St. Quentin Fallavier, France). The effects of three intravenous (i.v.) doses (0.75, 1.5 and 3 mg/kg) of U-69,593 (Sigma) a κ_1-opioid receptor agonist were evaluated on carrageenin-induced Fos expression ($n = 4$, $n = 10$, $n = 4$ respectively). The receptor specificity of the effect of U-69,593 (1.5 mg/kg) was tested in two groups of rats receiving either 2 ($n = 5$) or 4 mg/kg i.v. ($n = 6$) of a specific κ-opioid receptor antagonist, nor-binaltorphimine (RBI, Research Biochemical, Natick, MA, USA). In addition, we examined the effect of nor-binaltorphimine administered alone (4 mg/kg i.v.) on carrageenin-induced spinal Fos expression ($n = 5$). Dosing with the agonist and/or the antagonist (0.2 ml) were successively made, 10 min before i.pl. injection of carrageenin.

In this study, animals which did not receive carrageenin were not included since we have previously shown that in these animals, there was almost no Fos-like Immunoreactive neurones at the level of the spinal cord (less than 5 Fos-like Immunoreactive neurones per section; Chapman et al., 1995).

We have previously demonstrated that the maximum depressive effect of another opioid receptor agonist (i.v. morphine) on spinal Fos expression was induced 1.5–2 h after i.pl. carrageenin injection (Honoré et al., 1995), and we have previously observed in behavioural experiments that U-69,593 effects last at least 80 min (Desmeules et al., 1993; Catheline et al., 1996, 1998), thus the rats were sacrificed 2 h after i.pl. carrageenin.

2.2. Evaluation of inflammation

In order to assess the development of inflammation, we considered the peripheral oedema at the time the animals were killed. The ankle and paw diameters, both ipsilateral and contralateral to the stimulation were measured using a calliper. Both the assessment of inflammatory oedema and Fos immunohistochemistry were performed in the same rats.

2.3. Immunohistochemistry for Fos-like immunoreactivity

Two hours after carrageenin injection, the animals were deeply anaesthetised with 55 mg/kg intraperitoneal pentobarbital (Sanofi, Libourne, France) and underwent intracardiac perfusion with 200 ml phosphate-buffered saline 0.1 M (PBS) followed by 500 ml of 4% paraformaldehyde in 0.1 M phosphate buffer (PB). The spinal cord was then removed and post fixed for 4 h in the same fixative, and cryoprotected overnight in 30% sucrose in PB. Frontal frozen sections of the lumbar spinal cord, L4–L5 segment, 40 μm thick, were cut and collected in PB to be processed immunohistochemically as free floating sections. The serial sections from the lumbar segment were immunostained for Fos-like protein according to the avidin–biotin–peroxidase method (Hsu et al., 1981). The tissue sections were incubated for 30 min at room temperature in a blocking solution of 3% normal goat serum in PBST (0.1 M PBS and 0.3% Triton X100) and were then incubated overnight at 4°C in the primary antiserum directed against

the Fos protein. The c-Fos antibody (Tebu, sc-52, 0.1 mg/ml diluted at 1:30,000) is a rabbit polyclonal antibody raised against a peptide corresponding to amino acids 3–16, mapping at the amino-terminus of human c-Fos p62. The incubated sections were washed in two changes of normal goat serum (1% in PBST) and incubated in biotinylated goat anti-rabbit immune globulin G for 1 h at room temperature, then washed twice in 1% normal goat serum and incubated for 1 h in avidin–biotin–peroxidase complex (Vectastain, Vector Laboratories). Finally, the sections were washed two times in PB and developed in 1-naphtol ammonium carbonate solution (89.5 ml 0.1 M PB, 10 ml ammonium carbonate −1% in distilled water- and 0.5 ml 1-naphtol-N199 2 Aldrich, 10% in absolute alcohol-). The incubation was done at room temperature for 5 min with 0.1 ml hydrogen peroxide 30% (w/w solution). Sections were then washed three times in PB to stop the staining reaction. The 1-naphtol staining is a fine grey–violet precipitate, which must be intensified and made alcohol resistant to be easily identified. Thus, sections were mounted on gelatine-coated slides, air dried, and dye-enhanced for 3 min in 0.025% crystal violet (solution in PB, Aldrich). After 2 short distilled water rinses to remove the excess staining, sections were sequentially differentiated in 70% and absolute alcohol with the time of differentiation being evaluated under a microscope. At that time, staining appears as an intense blue–violet colour. Sections were finally air dried, xylene treated, and cover-slipped with Eukitt. To minimise between day variability and for valid comparisons, immunohistochemistry was carried out on the same day for each individual experiment.

2.4. Counting of Fos labelled neurones

Tissue sections were first examined using dark field microscopy to determine the segmental level according to Molander et al. (1984) as well as the grey matter landmarks. The sections were then examined under light field microscopy at ×125 magnification to localise Fos positive cells. Labelled nuclei were counted using a camera lucida attachment. The number of Fos-like Immunoreactive neurones present in the spinal grey matter was evaluated in 10 sections of L4–L5 segment. All Fos-like Immunoreactive neurones were analysed without considering the intensity of the staining. The investigator responsible for plotting and counting the Fos-like Immunoreactive neurones was unaware of the treatment regimen.

2.5. Statistical analysis

Statistical analysis was performed to compare the number of spinal Fos-like Immunoreactive neurones in the whole 40 μm thick section and in the different laminae of the spinal cord, using one-way analysis of variance for the different groups of animals. One-way analysis of variance was used to compare the oedema data, between the different groups of animals. Post-hoc multiple comparisons were carried out using Fisher's PLSD (Protected Least Significant Difference) test. The dose–response data was examined using a simple regression calculation.

3. Results

3.1. Carrageenin evoked spinal Fos expression

As previously described, after intraplantar carrageenin, we observed the development of a unilateral peripheral oedema; both the paw and ankle diameters of the injected hind-paw were increased (111 ± 5% and 16 ± 4% of increase respectively when compared to the contralateral side). Two hours after i.pl. carrageenin injection, Fos-like Immunoreactive neurones, which were stained to a variable degree, were located in the ipsilateral dorsal horn of the spinal cord (80 ± 4 neurones/section for L4-L5 segments). The number of Fos-like Immunoreactive neurones in the contralateral dorsal horn was not significantly different from the extremely low number of spinal Fos-like Immunoreactive neurones in non-stimulated rats (< 5 neu-

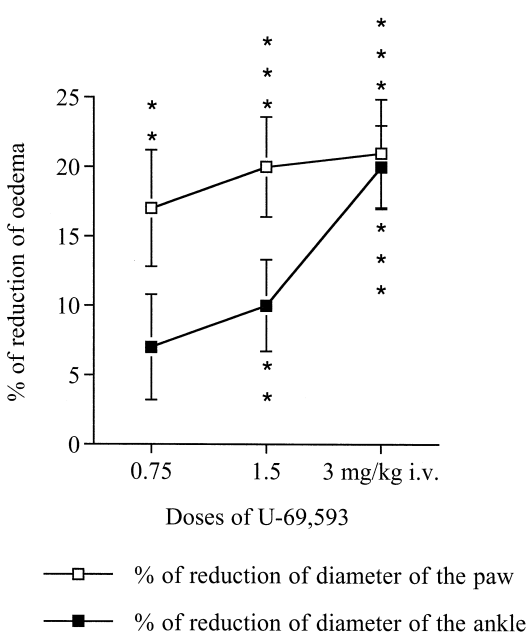

Fig. 1. Effect of i.v. pre-administration of U-69,593 (0.75, 1.5 and 3 mg/kg) on the carrageenin-induced oedema. Results are expressed as percentage of reduction (±SEM) of the control carrageenin-induced oedema for both paw (□) and ankle (■). Significance as compared with the control value was performed using ANOVA and Fisher's PLSD test (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

rones Fos-like Immunoreactive/40 μm section, Abbadie and Besson, 1992; Chapman et al., 1995). The Fos labelling was mainly located in the superficial dorsal horn (I–II) and to a lesser extent in the deep dorsal horn (V–VI). In untreated animals, 2 h after carrageenin, the number of Fos-like Immunoreactive neurones in these laminae, represent $57 \pm 4\%$ and $30 \pm 2\%$ of the total number of neurones/section, respectively. In contrast, very few Fos-like Immunoreactive neurones were observed in laminae III–IV and in laminae VII–X (Figs. 2B and 3A).

3.2. Effects of U-69,593 on the peripheral oedema

All the rats receiving an i.v. injection of U-69,593 had a reduced inflammatory oedema when compared to the oedema observed in the control group of rats. The diameter of the ipsilateral paw was significantly decreased for the three doses ($17 \pm 6\%$, $20 \pm 4\%$ and $21 \pm 5\%$ of decrease for 0.75, 1.5 and 3 mg/kg respectively—$P < 0.01$, $P < 0.001$ and $P < 0.001$, Fisher's PLSD test). Compared to control animals, the diameter of the ipsilateral ankle was decreased between 7–20% over the dose range 0.75 to 3 mg/kg respectively. The decrease of the diameter of the ankle was statistically significant only for the two highest doses of U-69,593 ($P < 0.01$ and $P < 0.001$, Fisher's PLSD test) (Fig. 1).

The anti-inflammatory effects of 1.5 mg/kg i.v. of U-69,593 were blocked by the concomitant administration of nor-binaltorphimine (2 or 4 mg/kg i.v.). Indeed, no

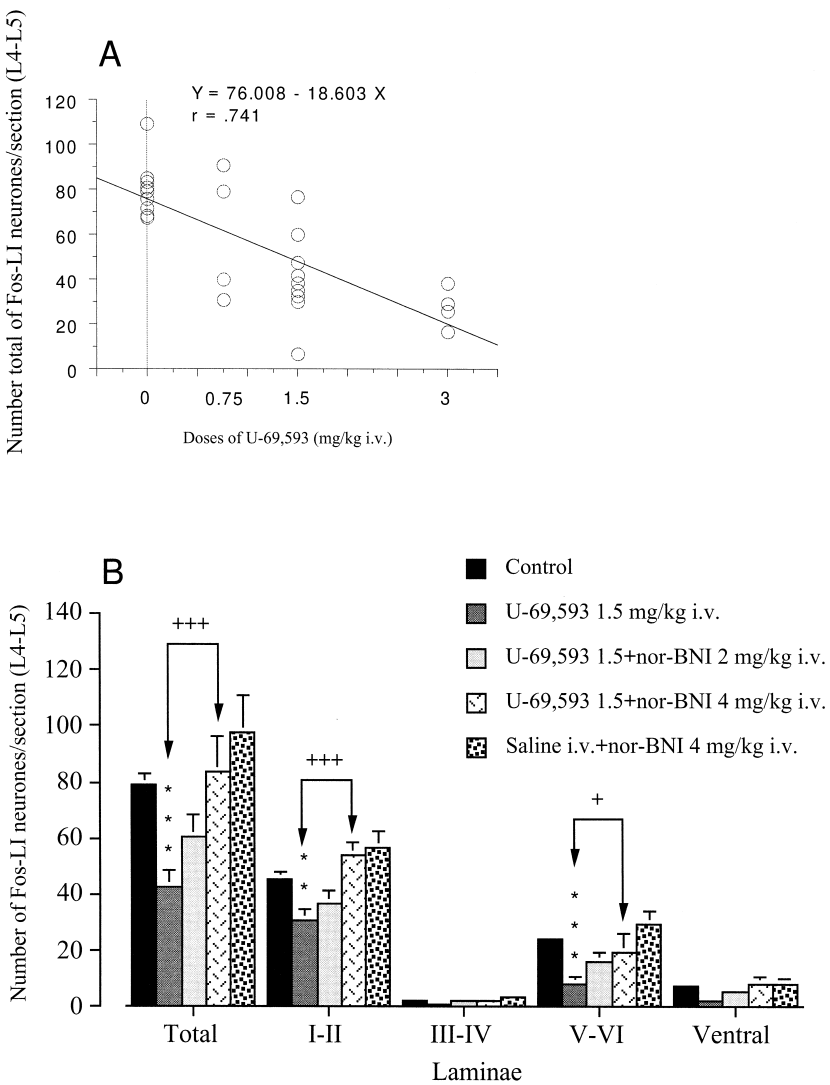

Fig. 2. (A) Simple regression curves illustrating the dose-dependent effects of U-69,593 (0.75, 1.5 and 3 mg/kg i.v.) on the total number of Fos-like Immunoreactive neurones/section of L4–L5. (B) Effect of i.v. pre-administration of 1.5 mg/kg of U-69,593 alone, and in combination with nor-binaltorphimine (2 or 4 mg/kg i.v.) on the number of Fos-like Immunoreactive neurones in the L4–L5 segments of the rat spinal cord, 2 h after i.pl. injection of carrageenin. Results are expressed as mean number of Fos-like Immunoreactive neurones (±SEM), per L4–L5 segments (Total), and per laminar region (laminae I–II, III–IV, V–VI, ventral). Statistical comparison with control (***$P < 0.001$) and between groups (†††$P < 0.001$) was performed using ANOVA and Fisher's PLSD test.

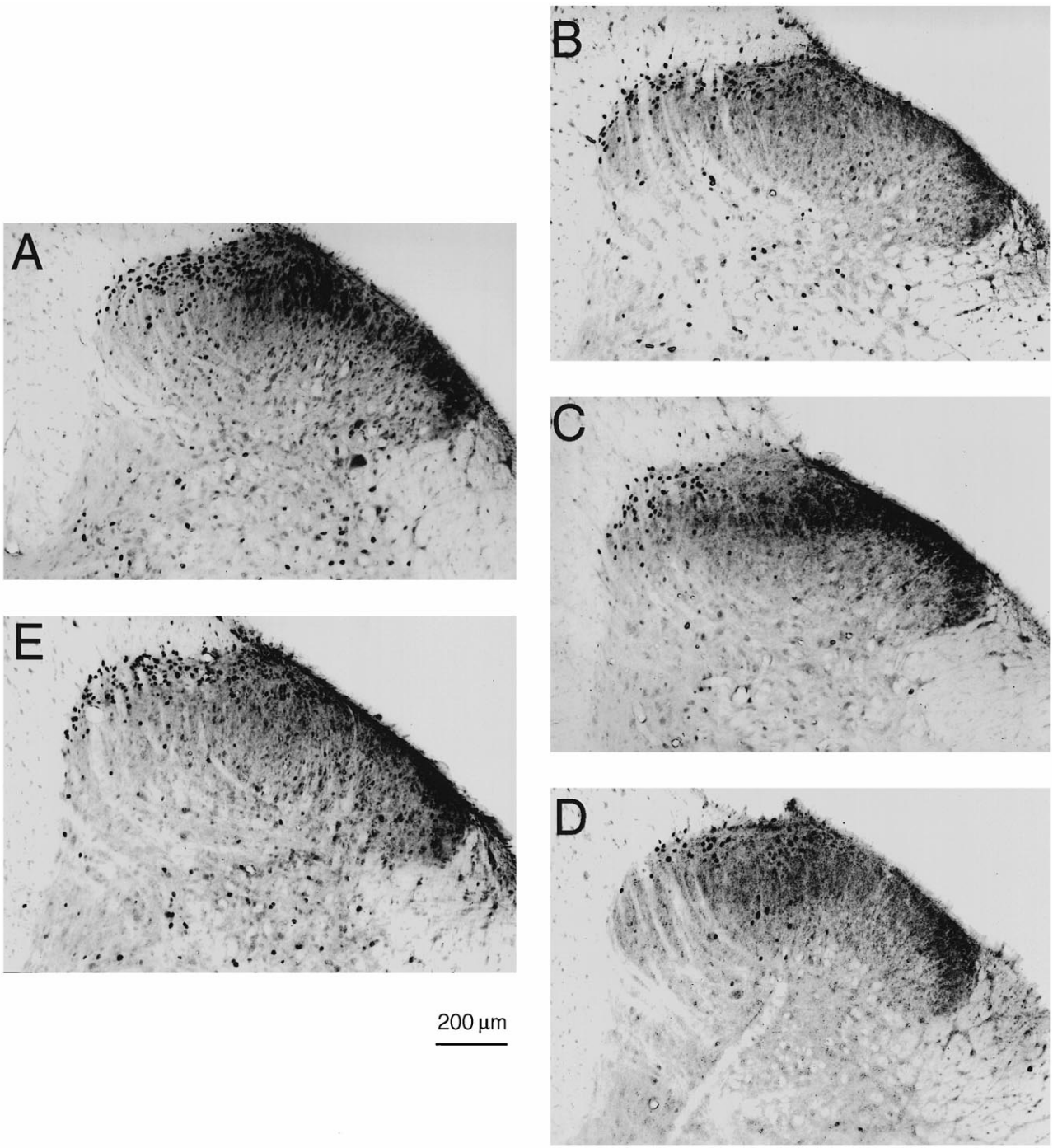

Fig. 3. Photomicrographs of individual representative examples of 40 µm sections in segment L4–L5 of rat spinal cord, illustrating the effects of i.v. U-69,593 on carrageenin-induced Fos immunoreactivity. Five experimental situation are represented: (A) control (B) 0.75, (C) 1.5 and (D) 3 mg/kg i.v. of U-69,593 and (E) blockade of the depressive effect of 1.5 mg/kg i.v. of U-69,593 by simultaneous i.v. injection of nor-binaltorphimine (4 mg/kg).

significant decrease of the oedema was observed in both groups when compared to control group (N.S., Fisher's PLSD test).

3.3. Effects of systemic U-69,593 on carrageenin-induced Fos expression

The i.v. injection of 0.75 mg/kg of U-69,593 reduced the number of carrageenin-induced Fos-like Immunoreactive neurones (25% of reduction), but this decrease is not statistically significant (N.S., Fisher's PLSD test, Figs. 2A and 3B). In contrast, 1.5 and 3 mg/kg i.v. of this substance significantly reduced the mean number of Fos-like Immunoreactive neurones (46% and 65% of reduction respectively—Fisher's PLSD test, $P < 0.001$). After the injection of these two highest doses, the total number of Fos-like Immunoreactive neurones observed was 43 ± 6

and 28 ± 4 of neurones/section respectively for 1.5 and 3 mg/kg i.v. of U-69,593 (Figs. 2A, 3C and D). The effects of U-69,593 were dose-related ($r = 0.741$, $P < 0.0001$) (Fig. 2A).

3.4. Blockade of the depressive effects of systemic U-69,593 on Fos expression by the systemic injection of nor-binaltorphimine

The effects of 1.5 mg/kg U-69,593 were partially blocked by the injection of 2 mg/kg i.v. of nor-binaltorphimine. In this latter group, the total number of Fos-like Immunoreactive neurones/section was 60 ± 15, which was not significantly different (N.S., Fisher's PLSD test) from the control group (80 ± 4 neurones/section), but neither from the group receiving 1.5 mg/kg U-69,593 (43 ± 6 neurones/section). Nor-BNI 4 mg/kg i.v.was able to completely block the effects of the κ-opioid receptor agonist in laminae I–II and laminae V–VI. Indeed the total number of Fos-like Immunoreactive neurones in this group was 84 ± 12, which was not different from the control group (N.S., PLSD fisher's test), but significantly different from the group receiving 1.5 mg/kg U-69,593 alone ($P <$ 0.001, Fisher's PLSD test) (Figs. 2B and 3E).

3.5. Effects of systemic nor-binaltorphimine on carrageenin-induced Fos expression

As shown in Fig. 2B, following the injection of the κ-opioid receptor antagonist nor-binaltorphimine alone (4 mg/kg i.v.), we observed a non-significant trend for an increase in the total number of neurones (98 ± 13 neurones/section versus 80 ± 4 neurones/section; N.S., Fisher's PLSD test).

4. Discussion

In this study, we have observed that an i.v. administration of a κ_1-opioid receptor agonist, the U-69,593, has weak but not dose-dependent anti-inflammatory effects on the development of carrageenin-induced oedema. In contrast, this compound dose-dependently and strongly decreased the number of carrageenin-induced Fos-like Immunoreactive neurones, at the spinal cord level. Both effects (on oedema and on Fos expression) were κ-receptor specific since they were blocked by specific κ-opioid receptor antagonist, nor-binaltorphimine.

In our study, U-69,593 exhibits weak but well reproducible anti-inflammatory effects, as judged by a decrease in peripheral oedema in all rats. At the injection site (paw diameter), the anti-inflammatory effect is almost maximum with 0.75 mg/kg i.v. of U-69,593 (reduction of 17 ± 6% of the paw oedema), while a very small additional decrease was observed for higher doses (20 ± 4% and 21 ± 5% for 1.5 and 3 mg/kg i.v. respectively). This effect was not dose-dependent. At distance of the injection site (ankle diameter), significant decrease was only observed for 1.5 mg/kg i.v. and further decrease was obtained with 3 mg/kg i.v. Here again, this effect was not dose-dependent. We did not use higher doses here because doses above 3 mg/kg produces catatonic effects on some rats (Desmeules et al., 1993).

The results in the present study also concurs with other studies who have described a decrease in oedema of various origins by exogenously administered opioids (Gyires et al., 1985; Joris et al., 1990; Wheeler-Aceto and Cowan, 1991; Earl et al., 1996; Wilson et al., 1996; Binder and Walker, 1998 however see also Earl et al., 1994; Hall et al., 1996). Further they support the results of others who have demonstrated that opioids inhibit plasma extravasation either by local or systemic administration (Bartho and Szolcsanyi, 1981; Joris et al., 1990; Barber, 1993; Hong and Abbott, 1995). However, the weak anti-inflammatory effects we observed on the development of acute carrageenin inflammation contrast with the potent anti-inflammatory effects obtained with others κ-opioid receptor agonists on the development of chronic arthritis induced by the administration of Freund adjuvant (Wilson et al., 1996; Binder and Walker, 1998). Indeed, combining the comprehensive criteria of oedema, radiography and histological changes, these authors described a potent inhibition of arthritis which is > 60% following a 3 days administration of U-50,488H, or > 75% following the administration of asimadoline, a peripherally selective κ-opioid receptor agonist. Walker et al. (1997) suggest that these effects could result from an effect on primary afferent nerve fibers which possess κ-opioid receptors (Coggeshall and Carlton, 1997), and from an effect on various immune cells like macrophages that release pro-inflammatory neuropeptides, the cytokines Tumor Necrosis Factor-α and Interleukine1 (see also Schäfer et al., 1994; Belkowski et al., 1995). Chronic arthritis in the rat however, is a very severe auto-immune disease making comparison with carrageenin-induced inflammation used here difficult.

It is interesting to compare the anti-inflammatory effects observed here with the anti-inflammatory effects of NSAIDs, which were evaluated under the same experimental paradigm. It appears that the effects of U-69,593 which did not exceed 20% of reduction of oedema with the highest dose (3 mg/kg i.v.) are extremely weak when compared to the marked effects of various NSAIDs (50–70% of reduction Buritova and Besson, 1998 and see Ref. therein). For example, with 1 mg/kg i.v. of Lornoxicam, the decrease of the ankle oedema was of 66 ± 8%. Without excluding a central effect (i.e., the spinal cord, Malmberg and Yaksh, 1992), we attributed their depressive effects on spinal Fos expression to their anti-inflammatory effects, because there is a positive correlation between the oedema and the Fos expression. Interestingly, if we roughly com-

pare the effects of U-69,593 and Lornoxicam on carrageenin-induced Fos expression, their depressive effects on spinal Fos expression are of the same order (65% of reduction for 3 mg/kg i.v. of U-69,593 and 61% of reduction for 1 mg/kg i.v. of Lornoxicam). Taken together, these data suggest that in contrast to NSAIDs, the effects of U-69,593 observed on Fos expression are weakly related to a decrease of inflammation but could be the result of a direct effect at the level of primary afferent fibers and/or at the CNS levels.

Several studies have demonstrated a peripheral site of action of κ-opioid receptor agonists. Initial findings have demonstrated that U-50,488H inhibits the discharges of fine afferent units from inflamed knee joint of the cat when the drug was injected intra-arterially close to the joint (Russell et al., 1987). Further, using an in vitro preparation of the saphenous nerve hindpaw skin from adult rats it has been demonstrated that U-69,593 suppressed the spontaneous activity of polymodal nociceptors induced by ultraviolet irradiation (Andreev et al., 1994). In addition, it has been demonstrated that prior administration of U-50,488H directly into the site of formalin injection results in a dose-dependent decrease in the response of nociceptive dorsal horn neurones (Haley et al., 1990). These electrophysiological findings are in good agreement with several behavioural experiments showing the antinociceptive potency of κ-opioid receptor agonists injected locally in normalgesic (Kolesnikov et al., 1996), inflammatory (Stein et al., 1989; Barber et al., 1994) or neuropathic conditions (Keïta et al., 1995). Furthermore, some data suggest that when opioids are administered systemically, concentration sufficient to produce peripheral antinociception are achieved (Stein et al., 1988; Catheline et al., 1998). Indeed, the antinociceptive effects of κ-opioid receptor agonists administered systematically are partially antagonised by the i.pl. injection of naloxone in monoarthritic rats (Stein et al., 1988) and by i.pl. injection of nor-binaltorphimine in a model of mononeuropathic rats (Catheline et al., 1998). These studies that suggest a peripheral site of action of κ-opioid receptor agonists, support autoradiographic, immunohistochemical and in situ hybridisation findings that indicate the presence of κ-opioid receptors at the level of the dorsal root ganglion and of the peripheral sensory axons and tissues (see Ref. in Coggeshall and Carlton, 1997).

In addition to the peripheral sites of action of κ-opioid receptor agonists, there are also strong evidences in favour of their direct action at the spinal cord level. Our data demonstrating potent depressive effects on Fos-like Immunoreactivity following i.v. administration of U-69,593 is in good agreements with those of Hammond et al. (1992). In their study, the U-50,488 injected s.c. (30 mg/kg) reduced the number of Fos-like Immunoreactive neurones by 54% in the acetic acid induced visceral stimulation. The potency of κ-opioid receptor agonists is surprising since it is well known from both behavioural (see Ref. in Yaksh, 1997) and electrophysiological (see Ref. in Dickenson, 1994) studies, that they have lower antinociceptive effects than the μ-opioid receptor agonists. Moreover, even if there is not necessarily a correlation between a pharmacological effect of a drug and the density of its receptors, it must be emphasized that the density of κ-opioid receptors in the dorsal horn of the spinal cord is very weak by comparison with those of μ receptors (Besse et al., 1990). The potency of κ-opioid receptor agonists supports numerous electrophysiological studies showing that the activity of U-50,488H administered by systemic routes strongly depressed the activity of dorsal horn neurones (Parsons and Headley, 1989; Parsons et al., 1989; Thorn et al., 1994) and tend to suggest a direct spinal site of action. A direct spinal site of action of κ-opioid receptor agonists has been clearly established by electrophysiological studies using either i.t. application or microiontophoretic technique (Willcockson et al., 1986; Knox and Dickenson, 1987; Fleetwood-Walker et al., 1988; Hope et al., 1990; Hylden et al., 1991; Stanfa et al., 1992; Randic et al., 1995). With such techniques, both excitatory and inhibitory effects have been reported on spinal dorsal horn neurones. Interestingly, the excitatory effect observed with low doses in normalgesic conditions was not observed in inflammatory conditions (Stanfa et al., 1992). In addition, in this study, Stanfa et al. (1992) have demonstrated that U-69,593 had a more pronounced effect on the activity of spinal dorsal horn neurones in inflammatory pain state induced by i.pl. injection of carrageenin than in normal pain state. Taken together, these electrophysiological investigations and our present study using Fos-like Immunoreactivity are in good agreement with numerous behavioural studies showing the antinociceptive effects of i.t. administration of κ-opioid receptor agonists (Millan et al., 1989; Ossipov et al., 1990; Machelska et al., 1997 see however Schmauss and Yaksh, 1984; Leighton et al., 1988; Przewlocka et al., 1992; Ho et al., 1997).

We used an i.v. administration therefore we cannot exclude a supraspinal site of action of U-69,593 in the decrease of Fos-like Immunoreactivity we observed in the present study. Indeed, intracerebroventricular (i.c.v.) administration of CI-977 ((5R)-(5a,7a,8b)-*N*-methyl-*N*-[7-(1-pyrrolindinyl)-oxaspiro[4,5]dec-8-yl]-4benzofurnacetamide monohydrocloride), another κ-opioid receptor agonist, decreases the number of Fos-like Immunoreactive neurones of the spinal dorsal horn induced by i.pl. formalin (Gogas et al., 1996). This study suggest that a descending component which did not travel via the dorso-lateral funiculus is involved in the effect of the agonist. This involvement is in agreement with the fact that spinalization reduced the potency of CI-977 for depression of hindlimb flexor muscle (Herrero and Headley, 1993).

Finally, in this study, as in others studies based on carrageenin-induced Fos expression (Honoré et al., 1996, 1997; Catheline et al., in press), the administration of the antagonist alone did not significantly modify the Fos la-

belling. In other terms, we were unable to reveal a tonic activity of κ-endogenous opioid systems at the dorsal horn level, under our experimental conditions.

In conclusion, further experiments should be done so as to confirm the anti-inflammatory potency of κ-opioid receptor agonists. It could be of particular interest to evaluate in our technical paradigm 1) the anti-inflammatory effects of the U-69,593 administered directly into the paw 2) the anti-inflammatory effects of a κ-opioid receptor agonist which does not cross the blood brain barrier.

Acknowledgements

The authors are grateful to Dr. Judith-S. Walker for English revisions. S. Le Guen was supported by a fellowship from the Ministère de l'Enseignement Supérieur, de la Recherche et de la Technologie. G. Catheline was supported by European BIOMED 2 Program no. BMH4-CT95-0172.

References

Abbadie, C., Besson, J.M., 1992. C-*fos* expression in rat lumbar spinal cord during the development of adjuvant-induced arthritis. Neuroscience 48, 985–993.

Abbott, F.V., 1988. Peripheral and central antinociceptive actions of ethylketocyclazocine in the formalin test. Eur. J. Pharmacol. 152, 93–100.

Andreev, N., Urban, L., Dray, A., 1994. Opioids suppress spontaneous activity of polymodal nociceptors in rat paw skin induced by ultraviolet irradiation. Neuroscience 58, 793–798.

Barber, A., 1993. Mu and kappa-opioid receptor agonists produce peripheral inhibition of neurogenic plasma extravasation in rat skin. Eur. J. Pharmacol. 12, 113–120.

Barber, A., Gottschlich, R., 1997. Novel developments with selective, non-peptidic kappa-opioid receptor agonists. Expert Opin. Invest. Drugs 6, 1351–1368.

Barber, A., Bartoszyk, G.D., Greiner, H.E., Mauler, F., Murray, R.D., Seyfried, C.A., Simon, M., Gottschlich, R., Harting, J., Lues, I., 1994. Central and peripheral actions of the novel κ-opioid receptor agonist, EMD 60400. Br. J. Pharmacol. 111, 843–851.

Bartho, L., Szolcsanyi, J., 1981. Opiate agonists inhibit neurogenic plasma extravasation in the rat. Eur. J. Pharmacol. 73, 101–104.

Belkowski, S.M., Alicea, C., Eisenstein, T.K., Adler, M.W., Rogers, T.J., 1995. Inhibition of interleukin-1 and tumor necrosis factor-α synthesis following treatment of macrophages with the kappa opioid agonist U-50,488H. J. Pharmacol. Exp. Ther. 273, 1491–1496.

Besse, D., Lombard, M.-C., Zajac, J.-M., Roques, B.P., Besson, J.-M., 1990. Pre- and postsynaptic distribution of μ, δ and κ opioid receptors in the superficial layers of the cervical dorsal horn of the rat spinal cord. Brain Res. 521, 15–22.

Beyer, A., Schäfer, M., Stein, C., 1997. Antinociceptive effects of dynorphin peptides in a model of inflammatory pain. Pain 70, 141–147.

Bilsky, E.J., Wang, T., Lai, J., Porreca, F., 1996. Selective blockade of peripheral delta opioid agonist induced antinociception by inthratecal administration of delta receptor antisense oligodeoxynucleotide. Neurosci. Lett. 220, 155–158.

Binder, W., Walker, J.S., 1998. Effect of the peripherally selective κ-opioid agonist, asimadoline, on adjuvant arthritis. Br. J. Pharmacol. 124, 641–654.

Buritova, J., Besson, J.-M., 1998. Dose-related anti-inflammatory/analgesic effects of lornoxicam: a spinal c-Fos protein study in the rat. Inflammation Res. 47, 18–25.

Buritova, J., Besson, J.-M., Bernard, J.-F., 1998. Involvement of the spinoparabrachial pathway in inflammatory nociceptive processes: a c-Fos protein study in the awake rat. J. Comp. Neurol. 397, 10–28.

Catheline, G., Kayser, V., Idänpään-Heikkilä, J.J., Guilbaud, G., 1996. The antinociceptive activity of κ- but not δ-opioid receptor agonists is maintained in morphine-tolerant neuropathic rats. Eur. J. Pharmacol. 318, 273–281.

Catheline, G., Guilbaud, G., Kayser, V., 1998. Peripheral component in the enhanced antinociceptive effect of systemic U-69,593, a κ-opioid receptor agonist in mononeuropathic rats. Eur. J. Pharmacol. 357, 171–178.

Catheline, G., Le Guen, S., Besson, J.-M., 1999. Effects of opioid receptor antagonists on the effects of i.v. morphine on carrageenin evoked c-Fos expression in the superficial dorsal horn of the rat spinal cord. Brain Res., in press.

Chapman, V., Honoré, P., Buritova, J., Besson, J.-M., 1995. Cholecystokinin B receptor antagonism enhances the ability of a low dose of morphine to reduce c-Fos expression in the spinal cord of the rat. Neuroscience 67 (3), 731–739.

Coggeshall, R.E., Carlton, S.M., 1997. Receptor localization in the mammalian dorsal horn and primary afferent neurons. Brain Res. Rev. 24, 28–66.

Desmeules, J.A., Kayser, V., Guilbaud, G., 1993. Selective opioid receptor agonists modulate mechanical allodynia in an animal model of neuropathic pain. Pain 53, 277–285.

Dhawan, B.N., Cesselin, F., Rhaghubir, R., Reisine, T., Bradley, P.B., Porthogese, P.S., Hamon, M., 1996. International union of pharmacology: XII. Classification of opioid receptors. Pharmacol. Rev. 48, 567–592.

Dickenson, A.H., 1994. Where and how do opioids act? In: Gebhart, G.F., Hammond, D.L., Jensen, T.S. (Eds.), Proceedings of the 7th World Congress on Pain, Progress in Pain Research and Management. IASP Press, Seattle, pp. 525–552.

Dykstra, L.A., Gmerek, D.E., Winger, G., Woods, J.H., 1987. Kappa opioids in rhesus monkeys: I. Diurisis, sedation, analgesia and discriminative stimulus effects. J. Pharmacol. Exp. Ther. 242, 413–420.

Earl, J.R., Claxson, A.W., Blake, D.R., Morris, C.J., 1994. Proinflammatory effects of morphine in the rat adjuvant arthritis model. Int. J. Tissue React. XVI, 163–170.

Earl, J.R., Grootveld, M.C., Blake, D.R., Morris, C.J., 1996. Effect of mu, delta and kappa opioid receptor agonists on a reactive oxygen species mediated model of skin inflammation. Skin Pharmacol. 9, 250–258.

Fleetwood-Walker, S.M., Hope, P.J., Mitchell, R., El-Yassir, N., Molony, V., 1988. The influence of opioid receptor subtypes on the processing of nociceptive inputs in the spinal dorsal horn of the cat. Brain Res. 451, 213–226.

Gogas, K.R., Levine, J.D., Basbaum, A.I., 1996. Differential contribution of descending controls to the antinociceptive actions of kappa and mu opioids: an analysis of formalin-evoked c-Fos expression. J. Pharmacol. Exp. Ther. 276, 801–809.

Gyires, K., Budavari, I., Fürst, S., Molnar, I., 1985. Morphine inhibits the carrageenan-induced oedema and the chemoluminiscence of leucocytes stimulated by zymosan. J. Pharm. Pharmacol. 37, 100–104.

Haley, J., Ketchum, S., Dickenson, A., 1990. Peripheral κ-opioid modulation of the formalin response: an electrophysiological study in the rat. Eur. J. Pharmacol. 191, 437–446.

Hall, T.J., Jagher, B., Schaueblin, M., Wiesenberg, I., 1996. The analgesic drug buprenorphine inhibits osteoclastic bone resorption in vitro, but is proinflammatory in rat adjuvant arthritis. Inflammation Res. 45, 299–302.

Hammond, D.L., Presley, R.W., Gogas, K.R., Basbaum, A.I., 1992. Morphine or U-50,488 suppresses Fos protein-like immunoreactivity in the spinal cord and nucleus tractus solitarii evoked by a noxious visceral stimulus in the rat. J. Comp. Neurol. 315, 244–253.

Hargreaves, K.M., Dubner, R., Joris, J.L., 1988. Peripheral actions of opiates in the blockade of carrageenan-induced inflammation. In: Dubner, R., Gebhart, G.F., Bond, M.R. (Eds.), Proceedings of the Vth World Congress on Pain. Elsevier, pp. 55–60.

Herrero, J.F., Headley, P.M., 1993. Functional evidence for multiple receptor activation by kappa-ligands in the inhibition of spinal nociceptive reflexes in the rat. Br. J. Pharmacol. 110, 303–309.

Ho, J., Mannes, A., Dubner, R., Caudle, R.M., 1997. Putative kappa-2 opioid antagonists are antihyperalgesic in a rat model of inflammation. J. Pharmacol. Exp. Ther. 281, 136–141.

Hong, Y., Abbott, F.V., 1995. Peripheral opioid modulation of pain and inflammation in the formalin test. Eur. J. Pharmacol. 277, 21–28.

Honoré, P., Chapman, V., Buritova, J., Besson, J.-M., 1995. When is the maximal effect of pre-administered systemic morphine on carrageenin evoked spinal c-Fos expression in the rat?. Brain Res. 705, 91–96.

Honoré, P., Chapman, V., Buritova, J., Besson, J.-M., 1996. Concomitant administration of morphine and an *N*-methyl-D-aspartate receptor antagonist profoundly reduces inflammatory evoked spinal c-Fos expression. Anesthesiology 85 (1), 150–160.

Honoré, P., Buritova, J., Fournié-Zaluski, M.C., Roques, B.P., Besson, J.M., 1997. Antinociceptive effect of RB-101, a complete inhibitor of enkephalin-catabolizing enzymes, are enhanced by cholecystokinin type B receptor antagonist, as revealed by noxiously evoked spinal c-Fos expression in rats. J. Pharmacol. Exp. Ther. 281, 208–217.

Hope, P.J., Fleetwood-Walker, S.M., Mitchell, R., 1990. Distinct antinociceptive actions mediated by different opioid receptors in the region of laminae I and laminae III–V of the dorsal horn of the rat. Br. J. Pharmacol. 101, 477–483.

Hsu, S., Raine, L., Fanger, H., 1981. Use of avidin–biotin–peroxydase complex (ABC) in immunoperoxydase techniques: a comparison between ABC and unlabelled antibody (PAP) procedures. J. Histochem. Cytochem. 29, 577–580.

Hylden, J.L.K., Nahin, R.L., Traub, R.J., Dubner, R., 1991. Effects of spinal kappa-opioid receptor agonists on the responsiveness of nociceptive superficial dorsal horn. Pain 44, 187–193.

Inoue, M., Ueda, I., 1998. In vivo signalling of peripheral opioid analgesia. International Narcotic Research Conference (Abstract)

Joris, J.L., Costello, A., Dubner, R., Hargreaves, K.M., 1990. Opiates suppress carrageenan-induced edema and hyperthermia at doses that inhibit hyperalgesia. Pain 43, 95–103.

Keïta, H., Kayser, V., Guilbaud, G., 1995. Antinociceptive effect of a κ-opioid receptor agonist that minimally crosses the blood–brain barrier (ICI 204448) in a rat model of mononeuropathy. Eur. J. Pharmacol. 277, 275–280.

Knox, R.J., Dickenson, A.H., 1987. Effects of selective and non-selective κ-opioid receptor agonists on cutaneous C-fibre-evoked responses of rats dorsal horn neurones. Brain Res. 415, 21–29.

Kolesnikov, Y., Jain, S., Wilson, R., Pasternak, G.W., 1996. Peripheral κ_1-opioid receptor-mediated analgesia in mice. Eur. J. Pharmacol. 310 (2–3), 141–143.

Leighton, G.E., Rodriguez, R.E., Hill, R.G., Hughes, J., 1988. κ-opioid agonists produce antinociception after i.v., i.c.v. but not intrathecal administration in the rat. Br. J. Pharmacol. 93, 533–560.

Machelska, H., Kabuz, D., Przewlocki, R., Przewlocka, B., 1997. Inhibition of nitric oxide synthetase enhances antinociception mediated by Mu, Delta and Kappa opioid receptors in acute and prolonged pain in the rat spinal cord. J. Pharmacol. Exp. Ther. 282, 977–984.

Machelska, H., Maycock, A.L., Stein, C., 1998. Peripheral effects of the kappa agonist EMD 61753 on nociception and inflammation. International Narcotic Research Conference (Abstract).

Malmberg, A.B., Yaksh, T.L., 1992. Antinociceptive actions of spinal nonsteroidal anti-inflammatory agents on the formalin test in the rat. J. Pharmacol. Exp. Ther. 263, 136–146.

Millan, M.J., 1990. κ-Opioid receptors analgesia. Trends Pharmacol. Sci. 11, 70–76.

Millan, M.J., Czlonkowski, A., Lipkowski, A., Herz, A., 1989. Kappa-opioid receptor-mediated antinociception in the rat: II. Supraspinal in addition to spinal sites of action. J. Pharmacol. Exp. Ther. 251, 342–350.

Molander, C., Xu, Q., Grant, G., 1984. The cytoarchitectonic organization of the spinal cord in the rat: I. The lower thoracic and lumbosacral cord. J. Comp. Neurol. 230, 133–141.

Nagasaka, H., Awad, H., Yaksh, T.L., 1996. Peripheral and spinal actions of opioids blockade of the autonomic response evoked by compression of the inflamed knee joint. Anesthesiology 85, 808–816.

Ossipov, M.H., Lozito, R., Messineo, E., Green, J., Harris, S., Lloyd, P., 1990. Spinal antinociceptive synergy between clonidine and morphine, U69593, and DPDPE: isobolographic analysis. Life Sci. 47, 71–76.

Parsons, C.G., Headley, P.M., 1989. Spinal antinociceptive actions of μ- and κ-opioids: the importance of stimulus intensity in determining 'selectivity' between reflexes to different modalities of noxious stimulus. Br. J. Pharmacol. 98, 523–532.

Parsons, C.G., West, D.C., Headley, P.M., 1989. Spinal antinociceptive actions and naloxone reversibility of intravenous μ- and κ-opioids in spinalized rats: potency mismatch with values reported for spinal administration. Br. J. Pharmacol. 98, 533–543.

Pfeiffer, A., Branti, V., Herz, A., Emrich, H.M., 1986. Psychomimesis mediated by kappa-opioid receptors. Science 233, 774–776.

Planas, M.E., Rodriguez, L., Sanchez, S., Pol, O., Puig, M.M., 1994. Pharmacological evidence for the involvement of the endogenous opioid system in the response to local inflammation in the rat paw. Pain 60, 67–71.

Przewlocka, B., Dziedzicka, M., Lason, W., Przewlocki, R., 1992. Differential effects of opioid receptor agonists on nociception and cAMP level in the spinal cord of monoarthritic rats. Life Sci. 50, 45–54.

Randic, M., Cheng, G., Kojic, L., 1995. κ-Opioid receptor agonists modulate excitatory transmission in substantia gelatinosa neurons of the rat spinal cord. J. Neurosci. 15, 6809–6826.

Russell, N.J.W., Schaible, H.-G., Schmidt, R.F., 1987. Opiates inhibit the discharges of fine afferents units from inflamed knee joint of the cat. Neurosci. Lett. 76, 107–112.

Sacerdote, P., Bianchi, M., Panerai, A.E., 1996. Involvement of β-endorphin in the modulation of paw inflammatory edema in the rat. Regulatory Peptides 63, 79–83.

Schäfer, M., Carter, L., Stein, C., 1994. Interleukin 1β and corticotropin-releasing factor inhibit pain by releasing opioids from immune cells in inflamed tissue. Proc. Natl. Acad. Sci. USA 91, 4219–4223.

Schmauss, C., Yaksh, T.L., 1984. In vivo studies on spinal opiate receptor systems mediating antinociception: II. Pharmacological profiles suggesting a differential association of mu, delta and kappa receptors with visceral chemical and cutaneous thermal stimuli in the rat. J. Pharmacol. Exp. Ther. 228, 1–12.

Stanfa, L.C., Sullivan, A.F., Dickenson, A.H., 1992. Alterations in neuronal excitability and the potency of spinal mu, delta and kappa opioids after carrageenan-induced inflammation. Pain 50, 345–354.

Stefano, G.B., Scharrer, B., Smith, E.M., Hughes, T.K., Magazine, H.I., Bilfinger, T.V., Hartman, A.R., Fricchione, G.L., Liu, Y., Makman, M.H., 1996. Opioid and opiate immunoregulatory processes. Critical Reviews in Immunology 16 (2), 109–144.

Stein, C., Millan, M.J., Yassouridis, A., Herz, A., 1988. Antinociceptive effects of μ- and κ-agonists in inflammation are enhanced due to a peripheral opioid-specific mechanism. Eur. J. Pharmacol. 155, 255–264.

Stein, C., Millan, M.J., Shippenberg, T.S., Peter, K., Herz, A., 1989. Peripheral opioid receptors mediating antinociception in inflammation. Evidence for involvement of mu, delta and kappa receptors. J. Pharmacol. Exp. Ther. 248, 1269–1275.

Stein, C., Schäfer, M., Cabot, P.J., Zhang, Q., Zhou, L., Carter, L., 1997. Opioids and inflammation. In: Borsook, D. (Ed.), Molecular Neurobi-

ology of Pain, Progress in Pain Research and Management. IASP Press, Seattle, pp. 25–43.

Taiwo, Y.O., Levine, J.D., 1991. κ- and δ-opioids block sympathetically dependent hyperalgesia. J. Neurosci. 11, 928–932.

Thorn, S.A., Herrero, J.F., Headley, P.M., 1994. Stimulus intensity and the comparative efficacy of mu- and kappa-opioid agonists on nociceptive spinal reflexes in the rat. Brain Res. 663, 352–356.

Walker, J.S., Wilson, J.L., Binder, W., Scott, C., Carmody, J.J., 1997. The anti-inflammatory effects of opioids: their possible relevance to the pathophysiology and treatment of rheumatoid arthritis. Rheumatoid Arthritis ID research alert 1, 291–299.

Wheeler-Aceto, H., Cowan, A., 1991. Standardization of the rat paw formalin test for the evaluation of analgesics. Psychopharmacology 104, 35–44.

Willcockson, W.S., Kim, J., Shin, H.K., Chung, J.M., Willis, W.D., 1986. Actions of opioids on primate spinothalamic tract neurons. J. Neurosci. 6, 2509–2520.

Wilson, J.L., Nayanar, V., Walker, J.S., 1996. The site of anti-arthritic action of the κ-opioid, U-50,488H, in adjuvant arthritis: importance of local administration. Br. J. Pharmacol. 118, 1754–1760.

Yaksh, T.L., 1997. Pharmacology and mechanisms of opioid analgesic activity. Acta Anaesthesiol. Scand. 41, 94–111.

Zimmermann, M., 1983. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 16, 109–110.