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Metamizol potentiates morphine effects on visceral pain and evoked c-Fos immunoreactivity in spinal cord

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Abstract

In a model of visceral pain consisting of intraperitoneal injection of acetic acid (writhing test), simultaneous administration of subanalgesic doses of metamizol (150 mg/kg) and morphine (0.2 mg/kg) resulted in a potent analgesia (19 ± 1 vs. 2.3 ± 0.8 writhes; $P < 0.05$). While the analgesic effect of morphine (2 mg/kg) was antagonized by naloxone (1 mg/kg), the opioid antagonist did not reverse the analgesia induced by the combination of metamizol and morphine. Potentiation by metamizol was also observed as a bilateral decrease in stimulus-evoked c-Fos induction in superficial laminas (I–II) of the dorsal spinal cord after drug combination compared to single administration (66.5 ± 2.2 vs. 80.7 ± 4.2; $P < 0.05$). Conversely, the number of nuclei immunostained with an antibody that recognizes all proteins of the Fos family was not modified by the same dose combination compared to single treatment (21.1 ± 1.3 vs. 20.2 ± 1.2). Furthermore, in a model of somatic pain consisting of peripheral thermal stimulation of the paws, simultaneous administration of metamizol (100–250 mg/kg) and morphine (0.5 mg/kg) failed to modify flexor reflex latency.

Keywords: Metamizol; Dipyrone; Morphine; Fos; Synergism; Analgesia; Visceral pain

1. Introduction

The analgesic effect of nonsteroidal antiinflamatory drugs (NSAID) has been associated with decreased activation of peripheral nociceptors through local blockage of prostaglandin synthesis by cyclooxygenase activity inhibition (Ferreira and Vane, 1974; Lorenzetti and Ferreira, 1985), and with activation of nitric oxide synthetase in the injured tissue (Duarte et al., 1992; Granados-Soto et al., 1995). More recent evidence suggests that NSAID may have also direct central effects. Microinjection of dipyrone into the periaqueductal gray reduces the activity of spinal ascending axons following activation of peripheral C-fibres and increases tail flick latency (Carlsson et al., 1986, 1988; Carlsson and Jurna, 1987; Tortorici and Vanegas, 1994). Similarly, analgesia after microinjection of lysine acetylsalicylate into the periaqueductal gray (Tortorici and Vanegas, 1995) or by diclofenac applied directly into the periaqueductal gray, nucleus raphe magnus or thalamus (Jurna and Brune, 1990; Björkman et al., 1992) has been reported. Furthermore, the central effects of NSAID have been associated with endogenous opioid systems since the analgesic effect of diclofenac (Björkman et al., 1992) and dipyrone (Tortorici et al., 1996) were blocked by naloxone, and both drugs stimulate the release of pituitary and hypothalamic β-endorphin (Sacerdote et al., 1985; Vlaskovska et al., 1989). Moreover, coadministration of morphine and metamizol in a model of chronic somatic injury in rats is particularly effective at reducing hyperalgesia (Lopez-Muñoz et al., 1994), and administration of metamizol prior to abdominal surgery in man has been demonstrated to reduce the postoperative consumption of morphine (Rockemann et al., 1996; Tempel et al., 1996).

Inhibition of stimulus-evoked induction of Fos proteins at spinal and supraspinal levels has been observed after the administration of opioids (Presley et al., 1990; Gogas et al., 1991; Hammond et al., 1992; Abbadie and Besson, 1993; Tölle et al., 1994a), enkephalin-degrading enzyme inhibitors (Tölle et al., 1994b), NSAIDs (Abbadie and Besson, 1994; Buritova et al., 1995a,b; Honoré et al., 1991), electroacupunture (Lee and Beitz, 1992) and stimulation of diffuse noxious inhibitory controls (Morgan et al., 1994). Moreover, specific inhibition of c-Fos and FosB

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induction after thermal noxious stimulation has been reported after morphine administration (Tölle et al., 1994a). Functional correlates for this inhibition has been proposed (Presley et al., 1990; Abbadie et al., 1994; Munglani and Hunt, 1995), although not definitively established. After somatic pain, the inhibitory effect of morphine on the induction of Fos proteins shows laminar specificity, being greater in deeper laminas of the spinal cord (Presley et al., 1990; Gogas et al., 1991; Tölle et al., 1994a). However, this is not the case after visceral pain where a similar reduction was reported in superficial and inner laminas (Hammond et al., 1992). Furthermore, complete suppression of Fos induction is not necessary for the full analgesic effect by morphine (Presley et al., 1990; Gogas et al., 1991; Hammond et al., 1992; Tölle et al., 1994a), and beneficial effects of mild analgesics are sometimes independent on a decrease in Fos immunoreactive neurons (Abbadie et al., 1994).

The aim of the present study was, first, to investigate the dose-dependent inhibition of pain using metamizol and morphine in an established model of visceral pain, the writhing test in mice (Taber et al., 1969) and second, to identify the mechanism of a potentiating effect of this combination. In an attempt to correlate the effect of metamizol and morphine in mediating analgesia as measured in the visceral pain model, c-Fos as well as total Fos proteins were analyzed in the lower thoracic–upper lumbar spinal cord.

2. Materials and methods

2.1. Animal experiments and behavioural testing

Experiments were performed on male Balb/c mice weighing between 20–25 g, and maintained under a 12/12 h light/dark cycle with food and water freely available.

In a first series of experiments, we used the visceral pain model (Taber et al., 1969). A total of 170 mice were randomly selected for intraperitoneal (i.p.) pretreatment with either metamizol or/and morphine (see below) or with an equivalent volume of saline. An i.p. 0.3 ml bolus injection of 0.6% acetic acid was administered 30 min following the initial pretreatment injection. The animals were then separated and placed into individual observation chambers which facilitated the measurement of the number of writhes that each animal performed in response to the acetic acid injection. Counting was performed over a 10 min period beginning at 5 min following the acetic acid insult. We performed dose-response studies to identify low doses of metamizol and morphine which were relatively ineffective in producing analgesia alone but which would produce a significant effect when administered together. All drugs were administered as an i.p. 0.1 ml bolus volume. The doses of metamizol studied were between 50–250 mg/kg i.p. and morphine was tested at doses between 0.2–2 mg/kg i.p. Each experimental group consisted of 8 to 12 mice except for the control untreated group receiving only the acetic acid injection which included a total of 24 mice. Statistical analysis was made to compare the number of writhes using one way analysis of variance. For multiple comparisons, the Bonferroni's method was used.

In a second set of experiments we performed a limited analysis of the effect of metamizol and morphine in a model of somatic pain. This pain model was developed around the plantar test (Hargreaves et al., 1988) so that quantitative measures of hindlimb flexor reflex latency could be measured in response to application of radiant heat stimuli to the plantar pad of both hindfeet. The intensity of the radiant heat stimulus was set to evoke flexor reflex latencies of around 2 s. Measurements were performed once every 5 min to avoid heat sensitization. A total of 9 measurements were recorded over a 15 min control period before drug treatment and a further 15 min period 30 min after an i.p. injection of the drug or drug combination. A total of 30 mice were used in these experiments.

2.2. Tissue preparation and immunohistochemistry

To analyze the induction of total Fos and c-Fos immunoreactivity after visceral pain, 3 to 5 mice were taken from each experimental group after the writhing test and sacrificed by decapitation 90 min after the acetic acid insult. In addition, a group of 5 sham-paired mice pretreated with two injections of saline, 0.3 and 0.1 ml at the indicated times for acetic acid or drug injection, were included as control for the immunohistochemistry experiments. The spinal cord was removed by hydraulic pressure and placed on an ice-cold plate. The lower thoracic and upper lumbar segment was rapidly dissected. The tissue was fixed by immersion in 4% paraformaldehyde in phosphate buffered saline (PBS) for 24 h at 4°C, and cryoprotected in 30% sucrose in PBS for 2 days at 4°C. Cords were embedded in OCT–Tissue–Tek compound and kept at −80°C until cryostat sectioning. Coronal 40-μm sections corresponding to T11 to L2 levels were obtained from each cord, and processed for free-floating immunohistochemistry. All the subsequent procedures were carried out with continuous agitation at room temperature except when stated. After inactivation of endogenous peroxidase activity with 0.3% H_2O_2 in PBS for 20 min, sections were washed 3 times with phosphate buffered saline for 10 min each and incubated in a blocking solution of 20% normal goat serum in PBS containing 0.2% Triton X-100, for 30 min. Two different primary antibodies (Santa Cruz, CA), were used to localize Fos proteins: K-25 which recognize all proteins of the Fos family and 4 which recognizes only the c-Fos protein. Both antibodies were diluted 1:4000 in 0.1% bovine serum albumina and 0.1% Triton X-100 in PBS. Sections were incubated in either antibody overnight

at 4°C. After three washes of 10 min each in the same buffer, a 1:500 dilution of biotin-conjugated goat-antirabbit secondary antibody (Jackson ImmunoResearch) was applied to the sections for 1 h. Sections were washed again as above and incubated with peroxidase-conjugated streptavidin (Jackson ImmunoResearch), diluted 1:1000 for 45–60 min. After a final 3 times 10 min wash, the peroxidase activity was visualized by 0.5 mg/ml 3,3́diaminobenzidine and 0.003% H_2O_2 in PBS. Reaction was stopped by a final wash in PBS. Sections were dried onto gelatin-coated glass slides, dehydrated through serial alcohols and clarified in xylol, before mounting with DePeX and observed under a Zeiss-Axiophot microscope.

2.3. Quantification

Tissue sections were examined using darkfield microscopy to evaluate the spinal segmental level (Molander et al., 1984). A minimum of six sections from each mouse, from T11 to L2 were then examined with brightfield microscopy and Fos- and c-Fos-immunostained nuclei in superficial laminas I and II were counted. The immunohistochemistry was performed for each experimental group and was repeated three times. Statistical analysis was made to compare the number of labeled cells using the Student's *t*-test. Photomicrographs are taken from one representative experiment. The camara lucida drawings were made from the sections in the photomicrographs and include Fos- and c-Fos-immunostained nuclei across the different focal planes of the preparation.

3. Results

3.1. Effect of the combined administration of metamizol and morphine in a model of visceral pain

In order to analyze a potentiation between the NSAID metamizol and morphine we chose the writhing test in mice because it offers good sensitivity for all analgesics clinically analyzed and a good correlation between the analgesic potency in this test and in clinical trials (Taber et al., 1969). Injection of 0.3 ml of 0.6% acetic acid i.p. normally precipitated stereotypical chronic pain behaviour in mice starting between 3–5 min post-injection. From a pooled population of 24 mice treated with acetic acid a mean of 19.6 ± 1.7 stretches were observed over a 10 min period (control in Fig. 1). In contrast, injection of 0.3 ml of saline did not initiate this operationally-defined behaviour.

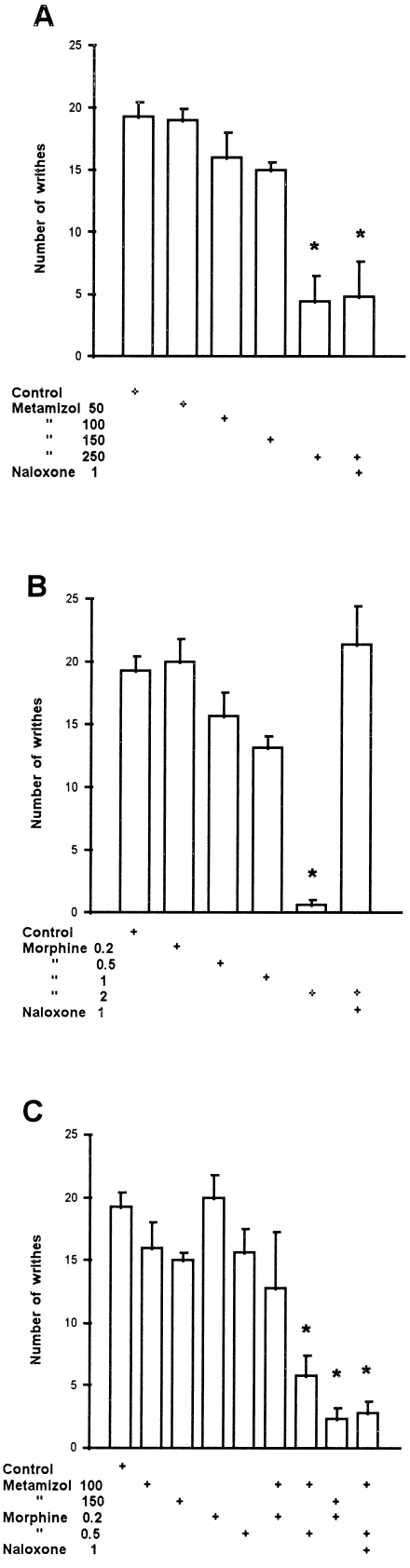


Fig. 1. Dose-dependent inhibition of the writhing behaviour in mice by metamizol (A), morphine (B) and combination of the two (C). Analgesics were injected at the indicated dose 30 min before the acetic acid insult. Naloxone, when used, was administered 10 min after the analgesic. Asterisks denote statistical significant ($P < 0.05$) difference compared to the control group.

Pretreatment 30 min before the acetic acid insult with a i.p. 0.1 ml bolus dose of metamizol at the concentrations studied was not associated with any obvious abnormalities in behaviour. The analgesic properties of metamizol in the visceral pain model was demonstrated by the dose-dependent inhibition of writhing behaviour induced by acetic acid so that, at 50 mg/kg i.p. no obvious analgesia was recorded while at 250 mg/kg the pain-induced behaviour was significantly reduced (Fig. 1A). Likewise, complete blockage of writhing behaviour was obtained with 2 mg/kg i.p. of morphine while no significant reduction was observed with doses of morphine between 0.2 and 1 mg/kg (Fig. 1B). The analgesic effect of morphine was totally blocked by 1 mg/kg naloxone even when administered 10 min after morphine. However, naloxone (1 mg/kg) did not modify the analgesia observed with metamizol (Fig. 1A,B).

To identify which low-dose combination would be particularly effective in mediating analgesia in this model, 100 or 150 mg/kg of metamizol was injected with either 0.2 or 0.5 mg/kg of morphine (Fig. 1C), doses which were ineffective in reducing visceral pain. Coadministration of 100 mg/kg metamizol with 0.2 mg/kg of morphine reduced writhing behaviour to 12.8 ± 4.5 counts, a reduction that was not significant when compared to the population control (Fig. 1C). However, application of 0.5 mg/kg morphine with 100 mg/kg of metamizol or 0.2 mg/kg morphine with 150 mg/kg of metamizol powerfully inhibited chronic visceral pain behaviour so that a mean of 5.7 ± 1.9 or 2.3 ± 0.8 writhes were recorded over the observation period, respectively (Fig. 1C, $P < 0.001$, compared to the response obtained with acetic acid). As described above, only small nonsignificant analgesic effects were observed when these doses were administered independently. The significant difference in number of writhes observed following coadministration compared to metamizol alone ($P < 0.001$) and morphine alone ($P < 0.05$) clearly indicates a potentiating effect of metamizol on morphine analgesia.

In order to understand the mechanism of this potentiation we applied 1 mg/kg naloxone 10 min after the simultaneous administration of 100 mg/kg metamizol and 0.5 mg/kg morphine. Under these experimental conditions, naloxone did not block the analgesia produced by the combination, suggesting that a nonopioid mechanism is responsible for the potentiation observed (Fig. 1C).

3.2. Lack of analgesic effect of metamizol in a model of somatic pain

To test the potentiating effect of metamizol on morphine analgesia in a model of pain that is neither chronic nor visceral, mice were trained so that the hindlimb flexor latency could be quantified following thermal test stimuli applied to both plantar pads. Five groups of mice were randomly assigned to receive one of the analgesics alone or to receive the combination of both. Control flexor latencies were obtained over the 15 min period before the administration of the assigned treatment and tested 30 min later. Administration of metamizol alone (100–250 mg/kg) or in combination with 0.5 mg/kg of morphine did not increase flexor reflex latency (Table 1).

Table 1
Effect of morphine and its combination with metamizol on hindlimb flexor reflex latencies in mice

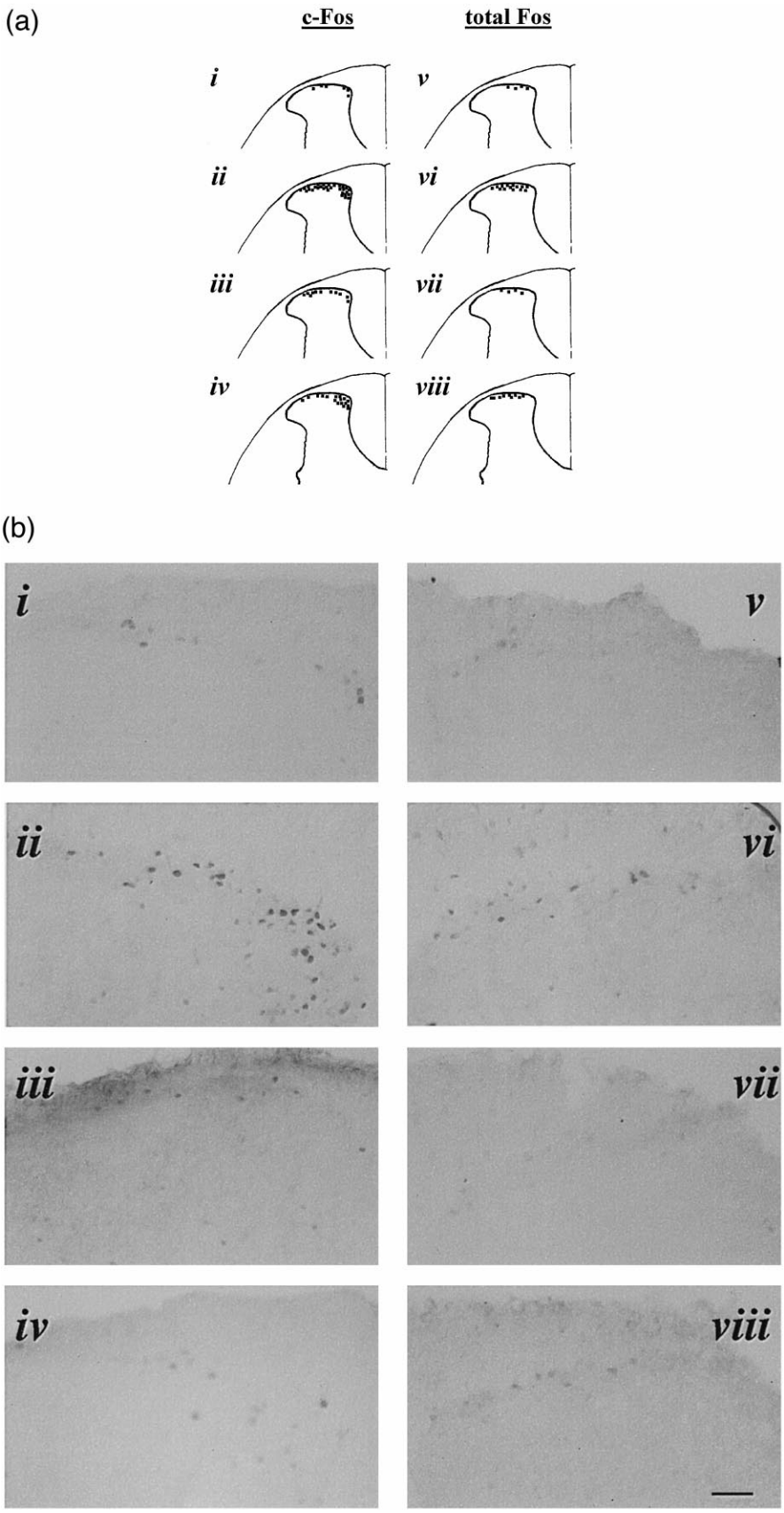
	Basal	30 min
Mo 0.5	2.2 ± 0.1	2.3 ± 0.1
Mo 0.5 + Me 100	1.9 ± 0.1	1.8 ± 0.1
Mo 0.5 + Me 250	2.3 ± 0.1	2.3 ± 0.1

Reflex latencies (seconds) were measured using the plantar test. Morphine (Mo) at an i.p. dose of 0.5 mg/kg was used alone and in combination with metamizol (Me) at i.p. doses of 100 or 250 mg/kg 30 min before measurements (mean ± S.D.; $n = 9$). No statistically significant difference was observed for any of the treatment groups compared to respective basal measurement (Student's *t*-test).

3.3. Fos immunohistochemistry in spinal cord

Peripheral noxious stimulation induces the expression of, among other immediate early genes, the different genes of the Fos family (Hunt et al., 1987; Herdegen et al., 1991). The physiological meaning of multiple induction of *fos* genes is presently not known and specific targets for secondary transactivation for each of them remains to be established (Munglani and Hunt, 1995). To analyze whether inhibition of stimulus-evoked *fos* induction accompanies the analgesic effect of metamizol on visceral pain and to test whether blockage of Fos induction after pain by analgesics may affect differentially c-Fos or other members of the Fos family, we used an antibody specific for the c-Fos protein (4) and an antibody (K-25) that recognizes total Fos proteins; c-Fos, FosB, Fra-1 and Fra-2. Injection of 0.6% acetic acid in mice induced widespread c-Fos and total Fos protein immunostaining in several segments including T11 to L2 as shown by the camara lucida drawings (Fig. 2A, ii and vi) and photomicrographs (Fig. 2B, ii and vi). The Fos–immunoreactive nuclei were distributed in the dorsal half of the spinal cord, preferentially in the superficial laminas (I and II), in agreement with previous reports (Menetrey et al., 1989; Hammond et al., 1992). Quantification of Fos labeling was therefore

Fig. 2. (A) Camera lucida drawings representing the induction of c-Fos (left column) and total Fos immunoreactivity (right column) in superficial laminas of the lower thoracic spinal cord. Dots represent immunostained nuclei, isolated or in groups. Samples were taken from the different experimental groups 90 min after saline (i,v) or acetic acid injection (ii–iv, vi–vii). Thirty minutes before the acetic acid injection mice were pretreated with saline (ii and vi), 2 mg/kg morphine (iii and vii) or the combination of 0.5 mg/kg morphine plus 100 mg/kg metamizol (iv and viii). (B) Photomicrographs of the spinal cord sections (i to viii) used for the camara lucida drawings. Labels (i to viii) are placed at the lateral part of the spinal cord section. Bar represents 50 μm.


(a)
c-Fos
total Fos
i
ii
iii
iv
v
vi
vii
viii
(b)
i
ii
iii
iv
v
vi
vii
viii

performed bilaterally and restricted to the superficial laminas (I–II) of the dorsal horn.

Analysis of c-Fos-labeled nuclei (specific antibody 4) revealed a robust induction of c-Fos in superficial dorsal laminas, 82.6 ± 7.3 labeled nuclei, 90 min after the acetic acid insult versus 20.1 ± 2.1 labeled nuclei in control mice injected with 0.3 ml of isotonic saline solution (panels i and ii in Fig. 2A,B and Fig. 3, $P < 0.001$). Pretreatment with metamizol did not significantly reduce c-Fos induction even with the highest analgesic dose of 250 mg/kg (Fig. 3). On the other hand, the analgesic dose of 2 mg/kg of morphine significantly reduced the number of c-Fos positive nuclei in superficial laminas of the dorsal horns (48.6% reduction, see panels iii in Fig. 2A,B and Fig. 3). Coadministration of metamizol (100 mg/kg) and morphine (0.5 mg/kg), only slightly but significantly reduced the number of c-Fos stained nuclei in the superficial laminas (18.5% reduction, see panels iv in Fig. 2A and BFig. 3).

Analysis of total Fos protein induction (antibody K-25) also revealed a significant induction 90 min following acetic acid insult. In the superficial laminas of the dorsal horn 27.8 ± 2.1 labeled nuclei were observed, in contrast to only 11.0 ± 1.5 nuclei following injection of 0.3 ml of isotonic saline solution (panels v and vi in Fig. 2A,B and Fig. 4, $P < 0.001$). Interestingly, metamizol at the subanalgesic dose of 100 mg/kg significantly reduced total Fos protein induction (32% reduction, Fig. 4) but no further reduction was obtained with the analgesic dose of 250 mg/kg (32.7% reduction, Fig. 4). Pretreatment with 0.5 mg/kg of morphine did not significantly modify the induction observed in the acetic acid insult group, while 2 mg/kg significantly reduced Fos induction in the superficial laminas (70.5% reduction, panels vii in Fig. 2A,B and Fig. 4). Interestingly, simultaneous administration of 100 mg/kg metamizol and 0.5 mg/kg morphine failed to reduce the induction of Fos immunolabeling below that seen with metamizol alone (panels viii in Fig. 2A,B and Fig. 4).

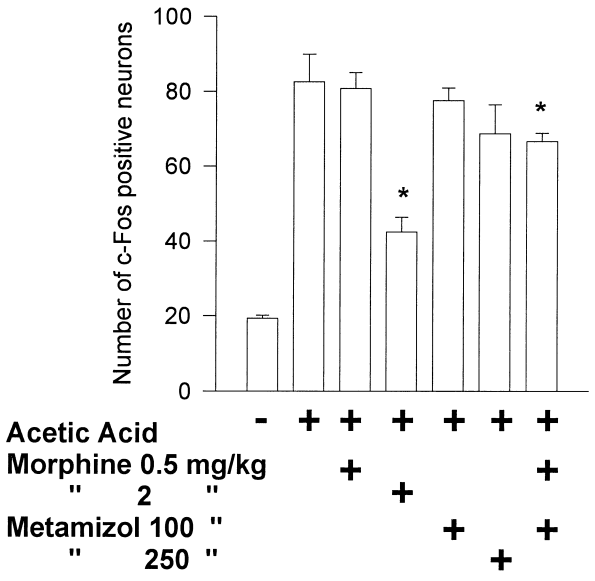


Fig. 3. Effect of metamizol, morphine and combinations of the two on the number of c-Fos positive nuclei in superficial laminas of the dorsal horn after acetic acid injection in mice. Asterisks denote statistical significant ($P < 0.05$) difference compared to the acetic acid group.

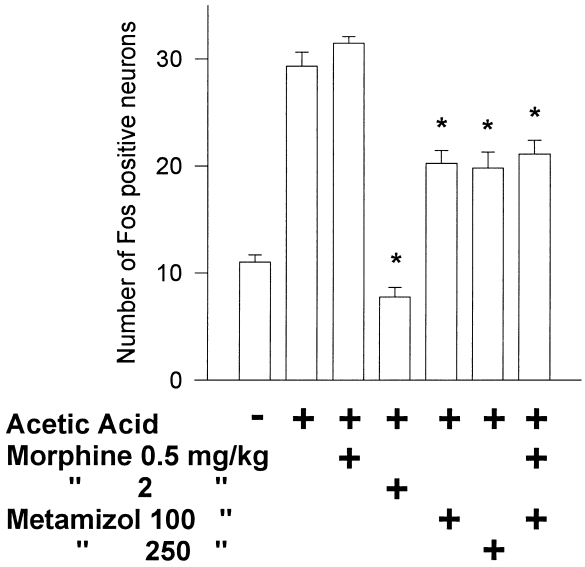


Fig. 4. Effect of metamizol, morphine and combination of the two on the number of total Fos positive nuclei in superficial laminas of the dorsal horn after acetic acid injection in mice. Asterisks denote statistical significant ($P < 0.05$) difference compared to the acetic acid group.

4. Discussion

We describe the potentiation by subanalgesic doses of metamizol on morphine-induced reduction of the behavioral symptoms of visceral pain and decrease in the extent of stimulus-evoked c-Fos induction after i.p. injection of acetic acid. Importantly, while morphine-induced analgesia is fully blocked by naloxone, the analgesia obtained with high doses of metamizol alone or with the combined administration of subanalgesic doses of metamizol and morphine was totally unaffected by 1 mg/kg naloxone.

In a previous study using a model of pain-induced functional impairment by intra-articular injection of uric acid in the knee joint (Lopez-Muñoz et al., 1994), combined administration of metamizol and morphine gave mainly additive effects. However, also potentiation was observed in 2 out of 24 dose combinations. In the absence of an exhaustive titration for synergistic combinations of metamizol and morphine in the writhing test, our results agree with the suggestion that the appearance of synergism may depend on the model of pain, since the same dose combination with which we observed potentiation in the visceral pain model, and even a combination of higher dose, failed completely to modify nociceptive thresholds in

a model of somatic pain consisting of thermal stimulation. Nevertheless, central administration of subanalgesic doses of indomethacin, acetylsalicylic acid or ketorolac markedly synergized with brain stimulation- and morphine-induced analgesia in models of somatic pain (Taiwo and Levine, 1988; Malmberg and Yaksh, 1993). Therefore, the molecular mechanisms underlying these interactions may differ for the different combinations of NSAID and opioids, the model of pain and the route of administration.

Blockage of prostaglandin synthesis at central and peripheral levels (Ferreira, 1972; Flower and Vane, 1972; Ferreira and Vane, 1974; Ferreira et al., 1978) have been associated with the antinociceptive effect of NSAIDs. Furthermore, a recent report by Vaughan et al. (1997) has shown that the sensitivity of periaqueductal grey slices to opioids is markedly increased when they are pretreated with inhibitors of the enzymes cycloxygenase and 5-lipoxygenase, and this potentiation is blocked by naloxone (Vaughan et al., 1997). Our results using an in vivo model of visceral pain, however, do not support a central opioid mechanism in metamizol-induced analgesia in visceral pain since naloxone was without effect on metamizol hypoalgesia. Remarkably, naloxone also failed to reduce the potentiation by metamizol of morphine activity, suggesting that the mechanism that reduced visceral hyperalgesia after this dose combination takes place peripherally and does not involve a direct opioid receptor activation centrally. In vitro versus in vivo experimental design is most likely responsible for the discrepancy between our results and those by Vaughan et al. (1997).

The noxious visceral stimulus applied in this study produced a highly significant increase in the bilateral induction of c-Fos and total Fos immunoreactivity in the superficial dorsal horn at the lower thoracic and upper lumbar segments (T11-L2), compared to the background activity observed following injection of saline solution. In keeping with previous studies (Menetrey et al., 1989; Hammond et al., 1992) bilateral total Fos labeling was observed mainly in superficial laminas (I–II), as was the main population of c-Fos positive nuclei. Analysis of the number of immunostained nuclei with the two antibodies clearly revealed their different sensitivity and precludes any direct comparison. Interestingly, the absolute number of total Fos immunostained nuclei are similar to those reported earlier by Hammond et al. (1992) using the same experimental model of pain and a different pan-Fos antibody. Furthermore, the absolute number of c-Fos nuclei in lower thoracic-upper lumbar segments 90 min after acetic acid injection reported here are of the same magnitude as that reported earlier in lumbar spinal cord 2 h after thermal noxious stimulation (Tölle et al., 1994a).

Pretreatment with an analgesic dose of morphine significantly reduced the number of c-Fos and total Fos-positive nuclei in the superficial laminas of the dorsal horn. However, the reduction in the c-Fos population was smaller (about half) than the reduction in the total Fos population (about two thirds). This can be related to the preferential effect of morphine on C-fibre evoked activity as compared with the effect on depolarizing inputs conducted by large myelinated fibres (Dickenson and Sullivan, 1986). However, the different time course for the induction of different Fos family members, i.e., the early and transient induction of c-Fos, and the delayed and sustained induction of Fra proteins (Herdegen et al., 1991; Tölle et al., 1994a) complicates the functional interpretation of this differential effect of morphine.

Contrary to the action of morphine, an analgesic dose of metamizol alone failed to reduce c-Fos induction. Instead, metamizol slightly but significantly reduced the number of total Fos positive nuclei. This reduction was observed already after the administration of subanalgesic doses and was not further increased neither with higher doses nor after coadministration with morphine. Furthermore, administration of an analgesic dose of acetylsalicylic acid (125 mg/kg) failed to reduce either c-Fos or total Fos induction after the acetic acid insult (unpublished observation). Taken together these results agree with the concept that changes in Fos induction is not a reliable parameter for the calibration of potency of mild non-opioid analgesics, while it offers a better correlation for morphine and its derivatives (Abbadie et al., 1994; Abbadie and Besson, 1994). On the other hand, the fact that metamizol failed to reduce c-Fos induction, but significantly reduced the number of total Fos nuclei suggests a preferential action on a Fos family member different from c-Fos. A specific target for this particular suppressive effect and the molecular basis for such a specificity is presently unknown.

In summary, the potentiation by metamizol of morphine analgesia in visceral pain provides a basis for further investigation into the possibility of combining low doses of NSAID's with morphine for the treatment of chronic pain to enhance the degree of analgesia with the further possibility of limiting unwanted side-effects that are normally associated with morphine administration.

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