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Subanalgesic doses of dexketoprofen and HCT-2037 (nitrodexketoprofen) enhance fentanyl antinociception in monoarthritic rats

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Abstract

Subanalgesic doses of the non-steroidal antiinflammatory drugs (NSAID) dexketoprofen trometamol and nitroparacetamol (NCX-701) enhance μ-opiate fentanyl effect in acute nociception. It is not known if a similar combination of drugs is effective in situations of spinal cord sensitization. The aim of this study was to assess if the enhancement of fentanyl antinociception can be observed in carrageenan-induced monoarthritis, when combined with dexketoprofen (DKT) or nitrodexketoprofen (HCT-2037). Withdrawal reflexes were recorded as single motor units in male Wistar rats anesthetized with α-chloralose. Fentanyl was studied alone and in the presence of 0.4, 0.8 μmol/kg of DKT or 0.3 μmol/kg of HCT-2037. In responses to noxious mechanical stimulation, the ID50 of fentanyl was enhanced twofold by 0.8 μmol/kg DKT and more than fourfold by HCT-2037 and no significant recovery was observed 45 min later. DKT 0.4 μmol/kg was, however, very little effective. The opioid antagonist naloxone did not reverse the effect. Enhancement of fentanyl effect on wind-up was only observed with HCT-2037 but not with DKT. We conclude that the combined administration of subanalgesic doses of dexketoprofen derivatives, specially its nitroderivative, and the μ-opiate fentanyl is an effective antinociceptive therapy in situations of articular inflammation involving a naloxone-independent mechanism of action.

Keywords: Pain; Analgesia; Spinal cord; Opioids; Non-steroidal antiinflammatory drugs; Nitric oxide; Wind-up

1. Introduction

The systemic administration of some antiinflammatory drugs (NSAIDs) like dexketoprofen trometamol or nitroparacetamol (NCX-701) induces a potent enhancement of the antinociceptive activity of the μ-opioid receptor agonist fentanyl in rat withdrawal reflexes in the acute nociceptive state (Gaitan et al., 2003). This effect is in agreement with other studies showing that the combination of some NSAIDs like aspirin or ketorolac and opiates like morphine induces an additive or supra-additive analgesia either on animals (Beaver, 1984; Malmberg and Yaksh, 1993; Maves et al., 1994; Taylor et al., 1998; Melis et al., 2000) or humans (Gillies et al., 1987; Laitinen and Nuutinen, 1992; Bosek and Miguel, 1994). The main difference with our work, however, was that we utilized subanalgesic doses of new-generation NSAIDs, with a high ability to penetrate the central nervous system and, as a consequence, a highly antinociceptive profile (Mazario et al., 1999, 2001; Romero-Sandoval et al., 2002; Herrero et al., 2003). In this case, we observed that subanalgesic doses of the NSAIDs studied were capable to enhance the effect of fentanyl and, in addition, to increase the duration of its effect. The use of such small doses of NSAIDs might improve the therapeutical profile of this type of combination of drugs, since it is not likely the appearance of secondary effects as in other protocols of treatment of pain, especially in the long-term treatment. Nevertheless, the combination of these drugs is intended for situations of sensitization and since our observation was made in normal animals, in the acute nociceptive state, it was important to assess if the enhancement of fentanyl antinociceptive activity by new generation NSAIDs also occurs in animals with spinal cord sensitization. We have therefore studied the antinociceptive activity

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of fentanyl, and the duration of its effect, in the absence and in the presence of subanalgesic doses of two different NSAIDs, dexketoprofen trometamol and a new nitroderivative of dexketoprofen, HCT-2037 (nitrodexketoprofen), that has been shown to be more potent than its parent compound (Gaitan et al., 2004) in animals with carrageenan-induced monoarthritis. The antinociceptive activity was studied in responses to noxious mechanical stimulation and to high-intensity repetitive electrical stimulation (wind-up) in order to discriminate a peripheral from a central action (Herrero et al., 2000). In addition, the effect of fentanyl in the presence of any of the NSAIDs was challenged with the opioid receptor antagonist naloxone, in order to check if the enhancement resulted from a direct action on opioid receptors. Preliminary results have been published in abstract form (Gaitan et al., 2004).

2. Methods

Details of methods have been described previously (Herrero and Headley, 1991; Solano and Herrero, 1997). Male Wistar rats (250–330 g) were anesthetized with halothane (5% for induction and 1.5–2.5% for maintenance in oxygen) and 50 μl of carrageenan λ (Sigma, 10 mg/ml, in distilled water) was administered into the right knee cavity 16 h before the experiment. Preparatory surgery was also made under halothane anesthesia (same regime) and included the cannulation of the trachea, carotid artery and two superficial branches of the jugular veins. Halothane was discontinued after surgery and the anesthesia was maintained with α-chloralose (50 mg/kg for induction and 30 mg/kg/h, by perfusion pump, for maintenance in a rate of 1 ml/h to assure a correct animal hydration). Core temperature was maintained at 37±0.5 °C by means of feedback controlled blanket. Blood pressure was monitored continuously and systolic levels were always above 100 mm Hg except for transient drops following i.v. injection of fentanyl. In all cases, the preparation was left to rest for at least 1 h after the surgery before any drug was tested. The degree of articular inflammation produced by carrageenan was assessed by comparing the knee perimeter before the induction of inflammation and after the experiment.

Single motor unit (SMU) activity was recorded from hind limb muscles by means of a bipolar Teflon-coated tungsten electrode (Solano and Herrero, 1997; Gaitan et al., 2003). Activity was elicited in 3-min cycles consisting of noxious mechanical stimulation (10 s, 200 mN above threshold over 14 mm^2) and one train of electrical stimuli (2-ms pulse width, 1 Hz and twice the threshold intensity for the recruitment of C-fibers) applied to the most sensitive area of the cutaneous receptive field of the unit. Mechanical stimulation was performed by a computer-controlled pincher device. The threshold force was considered as the minimum force required to obtain a sustained firing over the period of 10 s of stimulation. Only units with a steady firing rate were selected for experiments. Electrical stimulation was used to study the phenomenon of wind-up (see Herrero et al., 2000 for review). Data from electrical stimulation were analyzed by counting the responses evoked between 150 and 650 ms after each pulse, considered as C-fiber mediated inputs. At the end of the experiments, the animals were killed with an overdose of sodium pentobarbital (Euta-Lender, Normon). All experiments in this study were undertaken in accordance with European Union legislation (European Community Council Directive of 24 November 1986; 86/609/EEC) regarding the uses of animals for experimental protocols and all efforts were made to reduce the number of animals used.

The experimental protocol for the study of the antinociceptive activity of fentanyl has been described in detail elsewhere (Gaitan et al., 2003). The experiments were divided in four groups. In a control group (n=6), doses of i.v. fentanyl were administered every 6 min, starting with a dose of 1 μg/kg and ending with the dose that reduced responses to below 20% of control response, 32–64 μg/kg. One hour after the recovery, the protocol was repeated and responses were compared to those observed in the first test. In another three groups of experiments, the second fentanyl test was preceded by the administration of three cumulative doses (separated each by 21 min) of one of the following NSAIDs: dexketoprofen 0.1 to 0.4 μmol/kg (10 to 40 μg/kg, DKT 0.4, n=6); dexketoprofen 0.2 to 0.8μmol/kg (20 to 80 μg/kg, DKT 0.8, n=9); nitrodexketoprofen 0.1 to 0.3 μmol/kg (0.3 to 1 mg/kg, HCT-2037, n=6). In addition, the effect of fentanyl in the presence of DKT 0.8 (n=4) or HCT-2037 (n=3) was challenged with the opioid receptor antagonist naloxone (Sigma, 200 μg/kg) 45 min after the administration of fentanyl. Data are presented as percentage of control, control being the average of the three responses previous to the administration of the drug (mean±S.E.M.) and drug effect was assessed with the one-way analysis of variance (ANOVA) with post-hoc Dunnett's test. Intergroup comparisons were made using the Student's t-test (GraphPad-Prism and GraphPad-Instat for windows). Dexketoprofen and HCT-2037 were dissolved in DMSO (Sigma) and polyethylene glycol (1:1; Panreac) in a concentration of 50 mM, diluted in isotonic saline, and administered i.v. in a constant volume of 0.3 ml. All NSAIDs were kindly supplied by NicOx S.A.

3. Results

3.1. Effect of fentanyl in responses to noxious mechanical stimulation

The force used for mechanical stimulation was very similar in all groups, ranging from 0.75±0.1 N in the group of DKT 0.8 to 0.9±0.1 N in the group of HCT-2037. The μ-opioid receptor agonist fentanyl dose-dependently depressed SMU responses to noxious mechanical stimulation in all

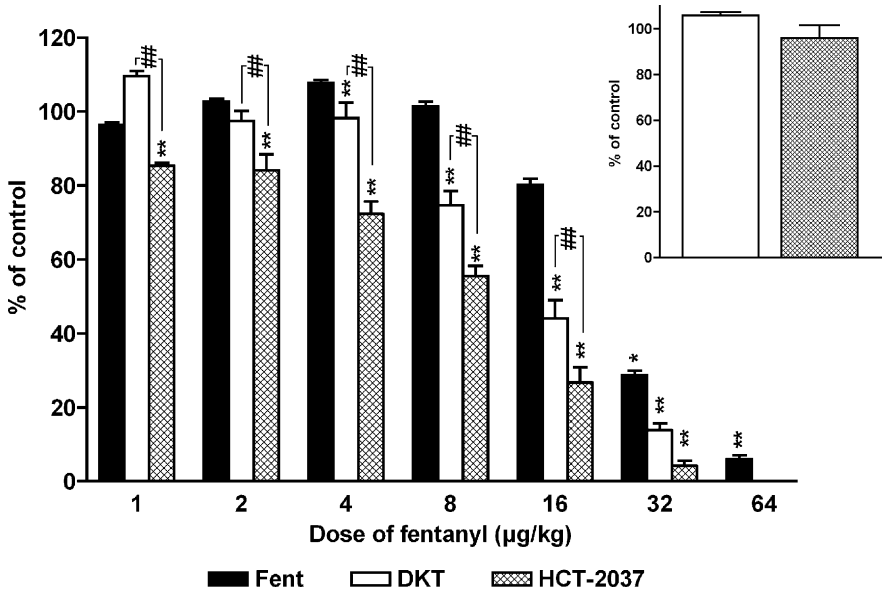

Fig. 1. Pooled data of the antinociceptive effects of fentanyl in responses to noxious mechanical stimulation. The effects were observed with the i.v. administration of the μ-opioid agonist fentanyl alone (Fent) and in the presence of 0.8 μmol/kg of dexketoprofen trometamol (DKT) or 0.3 μmol/kg of HCT-2037 in animals with monoarthritis. The potency of fentanyl was enhanced by twofold in the presence of DKT and by more than fourfold in the presence of HCT-2037. The administration of the NSAIDs by themselves (inset) did not modify the responses (*P<0.05, **P<0.01, comparison vs. control response with the one-way ANOVA, with the post-hoc Dunnett's test; ##P<0.01, comparison of the effect of fentanyl in the presence of HCT-2037 vs. DKT; Student's T-test).

experimental groups (Fig. 1). No differences were observed in the effect of fentanyl when given alone and so, data from the first dose–response curves in all groups were pooled together and compared to the effect of fentanyl in the presence of the NSAIDs. In the control group of experiments, depression of responses below 20% of control was achieved with 64 μg/kg, with an ID50 value of 29±2 μg/kg (Table 1).

The doses studied of dexketoprofen trometamol (0.4 μmol/kg; DKT 0.4, data not shown, and 0.8 μmol/kg, DKT 0.8) and HCT-2037 (0.3 μmol/kg) caused no change on responses to noxious mechanical stimulation on their own (Fig. 1, inset; comparison vs. control; control being the mean value of the three responses previous to the administration of the NSAID). In the presence of DKT 0.4, fentanyl was more potent than when given alone (ID50 value of 18±1 μg/kg, P<0.01, Table 1). The enhancement of the effect of fentanyl was, however, much greater when given in the presence of DKT 0.8 and HCT-2037 (Fig. 1 and Table 1). In these cases, depression below 30% control was achieved with 32 μg/kg and 16 μg/kg of fentanyl, respectively. The maximal depression of responses was observed with 32 μg/kg of fentanyl in the presence of HCT-2037: 4±3% (P<0.01). The ID50 of fentanyl was around twofold lower in the presence of DKT 0.8 than in its absence (15±1 vs. 29±2 μg/kg). The greater enhancement was, however, observed in the presence of HCT-2037 where fentanyl ID50 was over fourfold lower (ID50s: 7±1 vs. 29±2 μg/kg; Table 1). The inhibition of responses induced by fentanyl in the presence of HCT-2037 was significantly higher than that observed in the presence of its parent compound dexketoprofen for doses of 1 to 16 μg/kg of fentanyl (P<0.01 in all cases, Fig. 1).

The effect of fentanyl alone fully recovered within the following 30 min (Table 1, Fig. 2). The reduction of responses was still present 15 min later when coadminis-

Table 1
Effects of fentanyl alone and in the presence of dexketoprofen trometamol 0.4 and 0.8 μmol/kg (DKT 0.4 and 0.8) and HCT-2037 0.3 μmol/kg

Group	Mechanical stimulation					Wind-up		
	ID50 (μg/kg)	<30% (μg/kg)	Effect of 32 μg/kg (% control)	Recovery 15 min	Recovery 30 min	ID50 (μg/kg)	Effect of 32 μg/kg (% control)	Recovery 15 min (% control)
Fentanyl alone	29±2	64	29±6	75±8	111±8	26±2	48±9	110±6
DKT 0.4	18±1**	32	20±14	26±11**	82±17	56±2	74±8	95±10
DKT 0.8	15±1***	32	14±5**	19±8**	61±19**	19.7±2	35±14	76±8
HCT-2037	7±1***###	16	4±3**##	9±4**	57±12**	19.9±2	18±2**	92±10

The effect of fentanyl was twofold more potent in the presence of DKT 0.8, but not of DKT 0.4, and over fourfold more potent with HCT-2037. The greater effect in fentanyl effectiveness was seen with the dexketoprofen nitroderivative HCT-2037, with a reduction below 30% of control response with 16 μg/kg of fentanyl (<30%). Statistical analysis was made with the Student's T-test (**P<0.01; ***P<0.001 vs. fentanyl alone; ##P<0.01; ###P<0.001 vs. fentanyl in the presence of DKT 0.8).

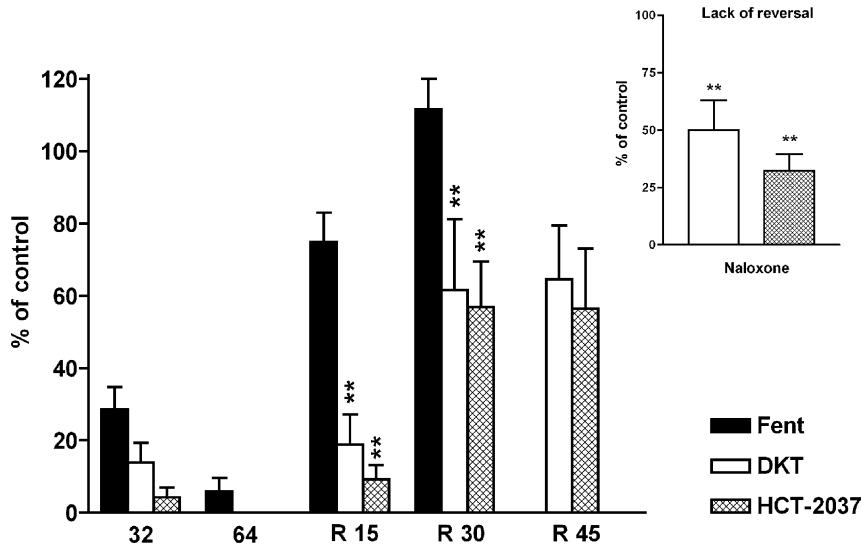

Fig. 2. Pooled data of the antinociceptive effect of fentanyl in responses to noxious mechanical stimulation after the administration of the highest cumulative doses (32 and 64 μg/kg) and recovery from the effect 15 (R15), 30 (R30) and 45 min later (R45). Recovery from fentanyl was fast when injected alone, but full recovery was not observed within 45 min of recording when administered in the presence of 0.8 μmol/kg of dexketoprofen trometamol (DKT) or 0.3 μmol/kg of HCT-2037. This effect was not reversed by the administration of naloxone (inset). Statistical significance and layout as for Fig. 1.

tered with DKT 0.4, but this effect recovered within the following few minutes (Table 1). In the presence of DKT 0.8 and HCT-2037, however, full recovery was never observed, and responses remained depressed at a level close to 60% of control response (Fig. 2, Table 1). When the effect of fentanyl was challenged with the opioid receptor antagonist naloxone, no significant reversal was observed in any of the tests performed (Fig. 2, inset; comparison vs. control; control being the mean value of the three responses previous to the administration of the NSAID).

3.2. Effect of fentanyl in wind-up

Repetitive high-intensity electrical stimulation produced a progressive increment in the number of spikes (wind-up) on all the units studied. Fentanyl alone dose-dependently reduced wind-up, observing a complete inhibition with the dose of 64 μg/kg. The calculated ID50 was 26±2 μg/kg (Table 1). The effect recovered within the following 15 min (Fig. 3). The administration of DKT 0.4 (data not shown in figures) and DKT 0.8 did not modify the effect of fentanyl,

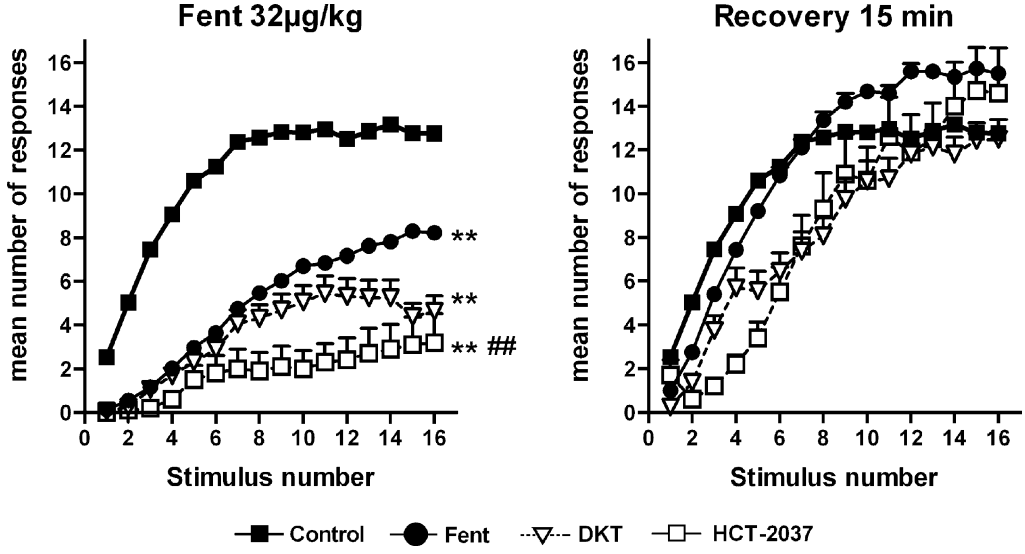

Fig. 3. Comparison of the effect of 32 μg/kg of fentanyl on single motor unit wind-up when injected alone (Fent) or in the presence of 0.8 μmol/kg of dexketoprofen trometamol (DKT) or 0.3 μmol/kg of HCT-2037. Complete inhibition of wind-up was observed with the dose of 32 μg/kg of fentanyl in the presence of HCT-2037 but not in the rest of the experimental groups. Full recovery of the responses was observed 15 min after the administration of fentanyl in all groups. Statistical comparison was made using the one-way analysis of variance, ANOVA, with the post-hoc Dunnett's test (**P<0.01 vs. control; ##P<0.01 vs. fentanyl alone).

and a slight and similar reduction of wind-up was observed with the dose of 32 μg/kg in the presence or in the absence of any of these NSAIDs (Fig. 3, Table 1). ID50s were, however, rather different: 56±2 and 19±7 μg/kg, respectively, as low doses of fentanyl in the presence of DKT 0.4 increased in a nonsignificantly way the level of wind-up. HCT-2037, however, induced a significant increment of the effect of fentanyl, in such a way that 32 μg/kg of fentanyl fully inhibited wind-up (Table 1, $P<0.01$). The ID50 was not significantly different of that observed with fentanyl alone: 19.9±2 μg/kg (Table 1). The duration of the effect was not modify by any of the NSAIDs tested and full recovery was always observed 15 min after the administration of fentanyl (Fig. 3, Table 1).

4. Discussion

The main observation in the present study is the significant enhancement of the antinociceptive potency of fentanyl by subanalgesic doses of new derivatives of some NSAIDs in a situation of central sensitization. In a previous study (Gaitan et al., 2003), we showed a similar effect produced by dexketoprofen trometamol and NCX-701 in the situation of acute nociception, and it was important to assess if the effect was also observable in arthritis, a situation more similar to what a combination of these drugs is intended for. In the present experiments, we confirm that the potency of fentanyl antinociception is also enhanced in the presence of subanalgesic doses of dexketoprofen and its nitroderivative HCT-2037 in monoarthritic animals. However, some differences were observed when compared to normal animals. In the latter (Gaitan et al., 2003), we observed an increment of above threefold on the potency of fentanyl when injected in the presence of 0.4 μmol/kg of DKT. In monoarthritic animals, the reduction of the ID50 of fentanyl was very low with this dose of DKT. Even when doubling the amount of DKT injected, the increment in the potency of fentanyl never rose as much as in normal animals. It is, therefore, possible to conclude that the enhancement of the effect of fentanyl by DKT requires higher doses of the NSAID in situations of hyperexcitability of the spinal cord.

In all these experiments, we studied the effect of dexketoprofen trometamol, which has been shown to be a very potent antinociceptive agent, more potent than dexketoprofen (Mazario et al., 1999 and references within), but we also had the chance to compare its effect with a new dexketoprofen derivative, HCT-2037. This new nitro derivative has also been shown to be more potent that its parent compound in SMU experiments (Gaitan et al., 2004) and in the present experiments had a better efficacy in the enhancement on fentanyl antinociception than dexketoprofen trometamol. DKT was effective in enhancing the antinociceptive activity of fentanyl in normal animals with a dose of 0.4 μmol/kg (Gaitan et al., 2003), but it was only effective at a dose of 0.8 μmol/kg when tested in monoarthritic rats. HCT-2037, however, was more effective, and a dose of 0.3 μmol/kg was able to enhance the effect of fentanyl with significantly more effectiveness than that of dexketoprofen trometamol. It is, therefore, important to point out that combination of fentanyl and NSAIDs is an effective therapy in normal animals and in animals with monoarthritis, but in the latter, there is some more resistance to this action, and it is very dependent of the type of molecule chosen. This also accounts for the effect of fentanyl (Herrero and Headley, 1991; Gaitan et al., 2003). The combination of nitroderivatives and fentanyl seems to be more advantageous than the combination of fentanyl with other cyclooxygenase-inhibitors. This implies a role of nitric oxide in the increment of the effect of the drugs which is supported by previous reports in which the antinociceptive activity of the NSAID ketorolac was shown to be mediated by the activation of nitric oxide synthase in the injured tissue (Duarte et al., 1992; Granados-Soto et al., 1995).

A longer duration of the effect of fentanyl was also observed in the presence of the NSAIDs, and no recovery at all was observed within 15 min after the opiate administration, whereas a very important recovery was observed when injected alone. This is important since the effect of fentanyl is very short lasting, with a half-life of 4 to 5 min in this preparation (Herrero and Headley, 1991 and references within). A reduction of 40% of nociceptive responses for a minimum of 45 min is something to be taken in consideration. However, although responses to noxious mechanical stimulation remained low during 45 min of observation, the effect was less strong than that observed in normal animals where the responses remained round about 30% of control response (Gaitan et al., 2003). Furthermore, fentanyl depressed the wind-up phenomenon and although this effect was twofold more potent in the presence of HCT-2037, no enhancement was observed in the presence of DKT, and in no case a more prolonged duration of the effect was seen. We and others have previously shown that the wind-up phenomenon is facilitated in situations of carrageenan-induced monoarthritis (Herrero et al., 2000), reflecting the hyperexcitability of spinal cord neurons proper of a state of sensitization as a consequence of a change in the spinal cord neuromodulation. It is therefore possible that the dosage required to maintain the reduction of wind-up is higher in hyperexcitability than that required to reduce responses to natural stimulation. On the other hand, wind-up is a centrally mediated phenomenon and its generation is dependent on the activity of NMDA and NK1 systems. The effect of fentanyl and other opiates on wind-up has been shown to be due to a reduction on the number of nociceptive inputs arriving the spinal cord, i.e., a peripheral unspecific action (Herrero et al., 2000 for review). The depression of wind-up by fentanyl was enhanced in the presence of the NSAIDs but to a lower extent than the increment observed in responses to noxious mechanical stimulation. This supports a peripheral action of the combination of the drugs. It is therefore also possible that a reduction of

peripheral inputs caused by fentanyl in combination with the NSAIDs was not enough to reduce the wind-up phenomenon in situations of carrageenan-induced hyperalgesia.

In any case, the enhancement of the effect of fentanyl was not reversed by naloxone. Further experiments are required to elucidate the mechanism of action of this observation, but the results suggest that the effect is independent of a direct activation of the opioid receptor (see Gaitan et al., 2003 for further discussion on this point).

In conclusion, the results of our experiments suggest that the combined administration of small doses of some NSAID derivatives might be useful in the treatment of pain derived of articular inflammation, with a possibility of reducing secondary effects associated with opiates and COX-inhibitors. Although the effect in normal animals is observed with different types of molecules, nitroderivatives of NSAIDs seem to be the most effective drugs in the enhancement of the antinociceptive effect of fentanyl in monoarthritis.

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References

Beaver WT. Combination analgesics. Am J Med 1984;77:38–53.

Bosek V, Miguel R. Comparison of morphine and ketorolac for intravenous patient-controlled analgesia in postoperative cancer patients. Clin J Pain 1994;10:314–8.

Duarte ID, dos Santos IR, Lorenzetti BB, Ferreira SH. Analgesia by direct antagonism of nociceptor sensitization involves the arginine–nitric oxide–cGMP pathway. Eur J Pharmacol 1992;217:225–7.

Gaitan G, Del Soldato P, Herrero JF. Subeffective doses of dexketoprofen trometamol or nitroparacetamol enhance the effectiveness of fentanyl in responses to noxious mechanical stimulation and wind-up. Eur J Pharmacol 2003;481:181–8.

Gaitan G, Ahuir FJ, Herrero JF. Comparison of the antinociceptive activity in rats of two new nitro-derivatives of the NSAID S-ketoprofen, HCT-2037 and HCT-2040. 4th Forum of European Neuroscience, Lisboa; 2004.

Gillies GW, Kenny GN, Bullingham RE, McArdle CS. The morphine sparing effect of ketorolac tromethamine A study of a new, parenteral non-steroidal anti-inflammatory agent after abdominal surgery. Anaesthesia 1987;42:727–31.

Granados-Soto V, Flores-Murrieta FJ, Castaneda-Hernandez G, Lopez-Munoz FJ. Evidence for the involvement of nitric oxide in the antinociceptive effect of ketorolac. Eur J Pharmacol 1995;277:281–4.

Herrero JF, Headley PM. The effects of sham and full spinalization on the systemic potency of μ- and k-opioids on spinal nociceptive reflexes in rats. Br J Pharmacol 1991;104:166–70.

Herrero JF, Laird JMA, Lopez-Garcia JA. Wind-up of spinal cord neurons and pain sensation: much ado about something? Prog Neurobiol 2000;61:169–203.

Herrero JF, Romero-Sandoval EA, Gaitan G, Mazario J. Antinociception and the new COX-inhibitors: research approaches and clinical perspectives. CNS Drug Rev 2003;9:227–52.

Laitinen J, Nuutinen L. Intravenous diclofenac coupled with PCA fentanyl for pain relief after total hip replacement. Anesthesiology 1992;76:194–8.

Malmberg AB, Yaksh TL. Pharmacology of the spinal action of ketorolac, morphine, ST-91, U50488H, and L-PIA on the formalin test and an isobolographic analysis of the NSAID interaction. Anesthesiology 1993;79:270–81.

Maves TJ, Pechman PS, Meller ST, Gebhart GF. Ketorolak potentiates morphine antinociception during visceral nociception in the rat. Anesthesiology 1994;80:1094–101.

Mazario J, Roza C, Herrero JF. The NSAID dexketoprofen trometamol is as potent as μ-opioids in the depression of wind-up and spinal cord nociceptive reflexes in normal rats. Brain Res 1999;816:512–7.

Mazario J, Gaitan G, Herrero JF. Cicloxygenase-1 versus cicloxygenase-2 inhibitors in the induction of antinociception in rodent withdrawal reflexes. Neuropharmacology 2001;40:937–45.

Melis M, Diana M, Gessa GL. Cyclo-oxygenase-inhibitors increase morphine effects on mesolimbic dopamine neurons. Eur J Pharmacol 2000;387:R1–3.

Romero-Sandoval EA, Mazario J, Howat D, Herrero JF. NCX-701 (nitroparacetamol) is an effective antinociceptive agent in rat withdrawal reflexes and wind-up. Br J Pharmacol 2002;135:1556–62.

Solano RE, Herrero JF. Cutaneous responsiveness of rat single motor units activated by natural stimulation. J Neurosci Methods 1997;73:135–40.

Taylor J, Mellstrom B, Fernaud I, Naranjo JR. Metamizol potentiates morphine effects on visceral pain and evoked c-*Fos* immunoreactivity in spinal cord. Eur J Pharmacol 1998;351:39–47.