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European Journal of Pharmacology 481 (2003) 181–188
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Low doses of nitroparacetamol or dexketoprofen trometamol enhance fentanyl antinociceptive activity

Gema Gaitán[a], Piero Del Soldato[b], Juan F. Herrero[a,*]

[a] *Departamento de Fisiología, Facultad de Medicina, Campus Universitario, Universidad de Alcalá, Alcalá de Henares, 28871 Madrid, Spain*
[b] *NicOx S.A., Sophia-Antipolis, France*

Received 5 June 2003; received in revised form 5 September 2003; accepted 10 September 2003

Abstract

We have reported that subeffective doses of the nonsteroidal anti-inflammatory drug (NSAID) dexketoprofen trometamol enhances μ-opioid receptor agonist fentanyl antinociception. The aim of this study was to assess if this effect can also be observed with other new cyclooxygenase-inhibitors such as nitroparacetamol, and in responses to high intensity electrical stimulation (wind-up). Single motor units were recorded in male Wistar rats under α-chloralose anaesthesia. The antinociceptive effect of fentanyl was studied alone and in the presence of subeffective doses of dexketoprofen trometamol or nitroparacetamol. In responses to noxious mechanical stimulation, the potency of fentanyl was enhanced by more than threefold in the presence of the NSAIDs and no significant recovery was observed after 45 min. The opioid antagonist naloxone and the α_2-adrenoceptor antagonist atipamezol did not reverse the effect. The enhancement of the effect of fentanyl in wind-up was lower though significant. We conclude that the co-administration of subeffective doses of new cyclooxygenase-inhibitors and the μ-opioid receptor agonist fentanyl should be considered as a potential pain therapy.

Keywords: Pain; Analgesia; Spinal cord; Opioid; Nonsteroidal anti-inflammatory drug; Wind-up

1. Introduction

We have observed that the systemic administration of the nonsteroidal anti-inflammatory drug (NSAID) dexketoprofen trometamol induces a potent enhancement of the antinociceptive activity of the μ-opioid receptor agonist fentanyl in rat withdrawal reflexes recorded as single motor unit activity (Gaitan and Herrero, 2002). The effect was observed in responses to noxious mechanical stimulation and was in agreement with previous studies that had shown that the combination of some NSAIDs like aspirin or ketorolac and opiates like morphine induces an additive or supra-additive analgesia (Beaver, 1984; Malmberg and Yaksh, 1993; Maves et al., 1994; Melis et al., 2000). This effect has been proposed to be mediated by a periaqueductal grey circuitry (Vaughan et al., 1997). In our study, however, the effect was accompanied by a potent increment of the duration of the effect of fentanyl. This might be advantageous in the use of fentanyl since this opiate is metabolized quickly and, though it is around 1000 times more potent than morphine as an antinociceptive drug, its half-life is very short, only around 4 to 5 min (Herrero and Headley, 1991 and references within). On the other hand, we observed that the doses of dexketoprofen required to induce the enhancement of the fentanyl effect were not effective in the reduction of nociceptive responses and, therefore, the presence of unwanted side effects might be lower than those expected with the use of higher doses of NSAIDs. This potent interaction might be due to the higher antinociceptive effectiveness of dexketoprofen trometamol, when compared to other NSAIDs (Mazario et al., 1999, 2001), or might be due to a preferred central versus peripheral action of this compound (Herrero et al., 2000; Mazario et al., 2001), in favor of a mechanism of action located at central sites of the nervous system. In order to check this possibility, we have performed some more experiments following the same protocol as that followed in our previous study (Gaitan and Herrero, 2002). On this occasion, however, we have studied the effectiveness of fentanyl when given alone and when given in the presence of subeffective doses (i.e. doses that do not cause an observable change in single motor unit responses to

* Corresponding author. Tel.: +34-91-885-45-16; fax: +34-91-885-45-90.
E-mail address: juanf.herrero@uah.es (J.F. Herrero).

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doi:10.1016/j.ejphar.2003.09.024

noxious stimulation) of dexketoprofen trometamol in responses to noxious mechanical stimulation and in responses to high-intensity repetitive electrical stimulation. The latter induces the phenomenon of wind-up, which has been shown to be a spinal cord-mediated phenomenon that involves the activation of NMDA and tachykinin NK1 receptors (see Herrero et al., 2000 for review) and that, if reduced, should indicate a central mechanism of action.

On the other hand, we do not know whether the enhancement of the fentanyl activity was exclusively due to dexketoprofen trometamol or whether it may be observed with subeffective doses of other new cyclooxygenase-inhibitors such as nitroderivatives like nitroparacetamol. This compound combines an inhibitory action over the cyclooxygenase enzymes with a release of low amounts of nitric oxide (NO; Del Soldato et al., 1999) that improves the antinociceptive activity of its parent compound, paracetamol (Al-Swayeh et al., 2000; Romero-Sandoval et al., 2002), and reduces its unwanted side effects (Futter et al., 2001; Fiorucci et al., 2002). Also, nitroparacetamol, as well as dexketoprofen trometamol, has been shown to have central actions and to reduce the phenomenon of wind-up (Romero-Sandoval et al., 2002). We have therefore checked if the combination of subeffective doses of nitroparacetamol also induces an enhancement of the fentanyl effectiveness and the duration of its effect in responses to noxious mechanical stimulation and wind-up.

Finally, it is not known whether the enhancement of fentanyl activity was the result of an actual interaction to opioid receptors, since the administration of naloxone did not reverse the inhibition of responses in our previous study. Since we have observed in the same experimental model that the effect of fentanyl may be modulated by the activity of the α_2-adrenoceptor systems (Herrero and Solano, 1999), we have studied the possible reversal of the effect with naloxone and with the α_2-adrenoceptor antagonist atipamezol.

2. Materials and methods

2.1. Animals and experimental groups

The experiments were made on adult male Wistar rats weighing 270–320 g divided in four groups (Table 1): (A) control group ($n=9$) in which two dose–response curves of fentanyl were studied in the same unit (1 to 32 μg/kg) separated by a gap of 1 h; (B) group of dexketoprofen ($n=10$), in which a first dose–response curve of fentanyl (1 to 32 μg/kg) was followed 1 h later by a second dose–response curve of fentanyl in the presence of 40 μg/kg of dexketoprofen trometamol; (C) group of nitroparacetamol ($n=7$) following a protocol similar to group B, but studying fentanyl in the presence of 17 mg/kg of nitroparacetamol instead; (D) group of vehicle ($n=3$), in which the effect of fentanyl was studied in the presence and in the absence of equivalent doses of the vehicle used to dissolve nitroparacetamol. In groups B ($n=5$) and C ($n=4$), the effect of fentanyl was challenged at the end of the experiment with a single dose of 200 μg/kg of naloxone (Sigma, Table 1). Also, the antinociceptive effect of fentanyl in the presence of paracetamol (group C) was challenged in three occasions with a further dose of 100 μg/kg of the α_2-adrenoceptor antagonist atipamezol (antisedan, SB). An extra group of experiments was carried out to check the effectiveness of naloxone (200 μg/kg) prior to the administration of a single dose of 32 μg/kg of fentanyl ($n=3$).

2.2. Recording of single motor units

Withdrawal reflexes were recorded as single motor unit activity following the technique previously described in detail (Herrero and Headley, 1991; Solano and Herrero, 1997). Briefly, the preparatory surgery consisted in the cannulation of the trachea, two superficial branches of the jugular veins for the administration of drugs and anesthesia, respectively, and one carotid artery to monitor the blood pressure. Core temperature was maintained at 37 ± 0.5 °C by means of a homoeothermic blanket. The surgery was performed under halothane anesthesia (5% for induction and 2.5% for maintenance in oxygen) and, once it was finished, the animal was transferred to a recording frame and the right hind limb was fixed in an inframaximal extension in a perspex block using plaster. Halothane was then stopped and the anesthesia continued with α-chloralose (Sigma, 50 mg/kg for induction and 20 mg/kg/h for maintenance, by a perfusion pump, diluted in saline). The preparation was left to rest for at least 1 h before the experiment was started. Blood pressure was continuously monitored throughout the experiment.

Table 1
Experimental groups and protocol of administration of drugs

Group	*n*	Fent 1 (μg/kg)	Time (min)	Dose	Fent 2 (μg/kg)	Time (min)	Naloxone (μg/kg)	Atipamezol (μg/kg)
(A) Control	9	1–32	60	–	1–32	–	–	–
(B) DKT	10	1–32	60	40 μg/kg	1–32	45	200 ($n=5$)	–
(C) NOP	7	1–32	60	17 mg/kg	1–32	45	200 ($n=4$)	100 ($n=3$)
(D) Vehicle	3	1–32	60	Eq. dose	1–32	45	–	–

Fentanyl was tested prior to and in the presence of subeffective doses of dexketoprofen trometamol (DKT) or nitroparacetamol (NOP) with a 60-min gap between tests. The effect of fentanyl was challenged by naloxone and atipamezol, 45 min after the second test. Control experiments were made by studying the effect of fentanyl twice in the same unit and with the vehicle used.

Bipolar tungsten electrodes inserted percutaneously into muscles of the right hind limb (usually peroneous longus and extensor digitorum longus; Schouenborg and Weng, 1994; Solano and Herrero, 1997) were used to record single motor units activated by mechanical (natural) and electrical (artificial) stimulation (wind-up). Isolation of motor units was performed by moving the electrode with a micromanipulator while a mild pressure was applied to the paw. Only the units with a steady firing rate were selected for experiments. The nociceptive activity was evoked on 3-min cycles consisting of 10 s of mechanical stimulation and 16 electrical stimuli applied to the most sensitive area of the cutaneous receptive field of the unit. Examples of recordings and cycles of stimulation are shown in Fig. 1. Electrical stimulation was used to study the phenomenon of wind-up (see Herrero et al., 2000 for review) and was applied in pulses of 2-ms duration, 1 Hz and twice the threshold intensity for the recruitment of long latency responses or C-fibers (Herrero and Cervero, 1996). Mechanical stimulation was performed by a computer-controlled pinch device, using a force of 0.2 N above the threshold over an area of 14 mm^2. The threshold force was considered as the minimum force needed to obtain a sustained firing over the period of 10 s of stimulation. 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 regarding the uses of animals for experimental protocols and all efforts were made to reduce the number of animals used.

2.3. Drugs

Fentanyl (Sigma) was dissolved in saline and was administered i.v. in log2 cumulative doses every two cycles of stimulation (6 min), starting with 1 μg/kg until the responses fell below 20% of control response (maximal dose used of 32 μg/kg). Dexketoprofen trometamol (Menarini) was dissolved in saline and administered in a volume of 0.3 ml, in doses of 10, 20 and 40 μg/kg. Nitroparacetamol (NicOx) was dissolved in dimethyl sulfoxide (Sigma, 50%) and polyethylene glycol (Panreac, 50%) in a concentration of 50 μmol/ml and diluted in saline to a final volume of 0.3 ml for each dose. The doses injected were 4.25, 8.5 and 17 mg/kg. Equivalent doses of the vehicle were studied following the same protocol of administration. The NSAIDs and vehicle were freshly prepared a few minutes before their administration, and they were administered intravenously in log2 cumulative doses every seven cycles of stimulation (21 min). The doses used were chosen based on results observed in previous experiments (Mazario et al., 2001; Romero-Sandoval et al., 2002; Gaitan and Herrero, 2002). Vehicle, dexketoprofen and nitroparacetamol were administered 1 h after the end of the first dose–response curve made with fentanyl, and 21 min prior to the second dose–response curve of fentanyl (Table 1). Naloxone (200 μg/kg) was injected 45 min after the second dose–response curve of fentanyl in five of the experiments performed with dexketoprofen and four of the experiments performed with nitroparacetamol, in order to challenge the opioid activity of fentanyl. In the group of experiments performed with nitro-

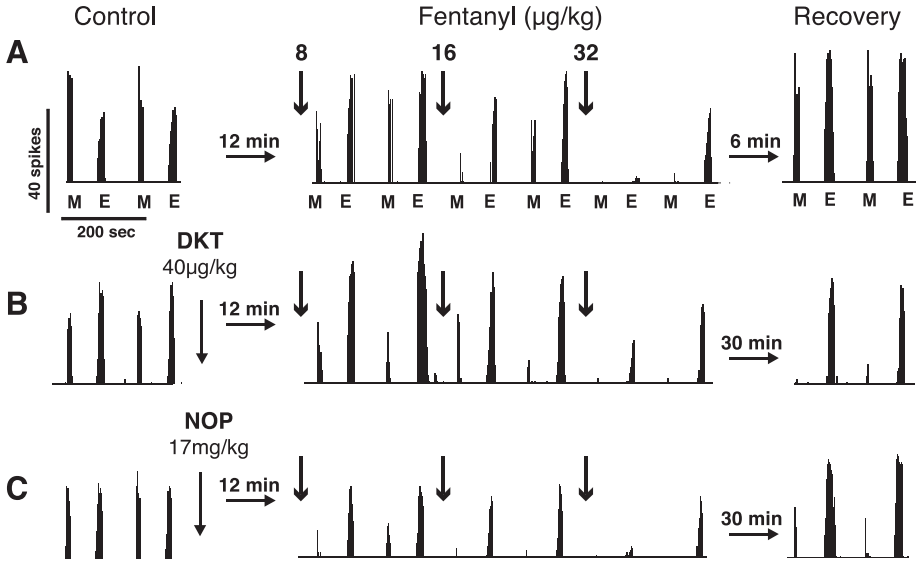

Fig. 1. Original recordings of single motor unit activity evoked by 3-min cycles of noxious mechanical (M) and electrical (E) stimulation in the absence (A) and in the presence of dexketoprofen trometamol (B) or nitroparacetamol (C). Panel A shows two control responses prior to the administration of cumulative doses of fentanyl (initial dose of 1 μg/kg), the effect observed after the administration of fentanyl and the full recovery of responses after the administration of the last dose. Panels B and C show the responses observed with different units prior to and after the administration of a total cumulative dose of 40 μg/kg of dexketoprofen trometamol (DKT) or 17 mg/kg of nitroparacetamol (NOP). The doses of dexketoprofen or nitroparacetamol used did not cause any significant change in the nociceptive activity. In the presence of dexketoprofen or nitroparacetamol, the administration of fentanyl induced a more potent reduction of nociceptive responses (especially in responses to noxious mechanical stimulation) and the effect did not recover 30 min later.

paracetamol, a further dose of 100 μg/kg of the α_2-adrenoceptor antagonist atipamezol was injected in three experiments, 6 min after naloxone, in an attempt to induce a reversion of the inhibition of responses (Table 1).

2.4. Collection of data and statistical analysis

Data of each group of experiments were joint and analyzed together as raw data and expressed as mean ± S.E.M. The responses observed after the administration of each drug were compared to the control response, control being the mean of the responses obtained in the three cycles of stimulation prior to the administration of the first dose of each drug. Data used for comparison were the averaged responses of the two cycles after each dose of fentanyl and mean responses in the last four cycles after each dose of dexketoprofen, nitroparacetamol or vehicle. Statistical significance of the effect observed by the administration of each dose was studied with the one-way analysis of variance (ANOVA) with post-hoc Dunnett test. Comparison of responses of fentanyl between groups was performed using the nonparametric Mann–Whitney *U*-test. The comparisons of ED_{50} were made by the Student's *t* test. (GraphPad-Prism and GraphPad-Instat for windows).

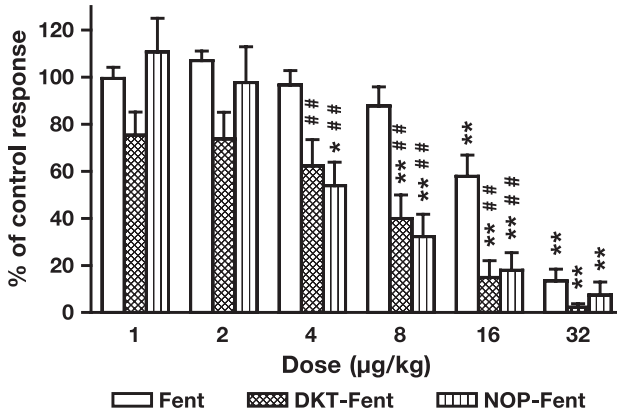

Fig. 3. Pooled data of the antinociceptive effects obtained with the cumulative i.v. administration of the μ-opioid receptor agonist fentanyl prior to (Fent) and after the administration of 40 μg/kg of the NSAID dexketoprofen trometamol (DKT-Fent) or 17 mg/kg of nitoparacetamol (NOP-Fent). The diagram represents the percentage of reduction of single motor unit responses to noxious mechanical stimulation. The potency of fentanyl was enhanced by more than threefold in the presence of either cyclooxygenase-inhibitors. The administration of the same doses of either dexketoprofen or nitroparacetamol on their own did not induce any significant reduction of responses. $^{*}P<0.05$; $^{**}P<0.01$, comparison vs. control response using the one-way ANOVA, with the post-hoc Dunnett test; $^{\#}P<0.05$; $^{\#\#}P<0.01$, comparison between DKT-Fent and NOP-Fent vs. Fent using the nonparametric Mann–Whitney *U*-test.

3. Results

3.1. Effects of fentanyl in responses to noxious mechanical stimulation

Fentanyl was effective in the reduction of responses to noxious mechanical stimulation (Fig. 1). Fig. 2 shows pooled data of these results. The effect was dose-dependent and no differences were observed between the first and the second dose–response curves. The ED_{50S} were respectively 16.1 ± 0.1 and 14.1 ± 0.1, the minimum effective dose (MED) was 16 μg/kg in either case and the maximum effect observed was very similar in the two curves: 89.8 ± 8.3% in the first test and 94.3 ± 2.1 in the second test. Full recovery of the responses was observed between 15 and 20 min after the administration of the highest dose in the two dose–response curves. These data were similar to those observed in a previous study (Gaitan and Herrero, 2002).

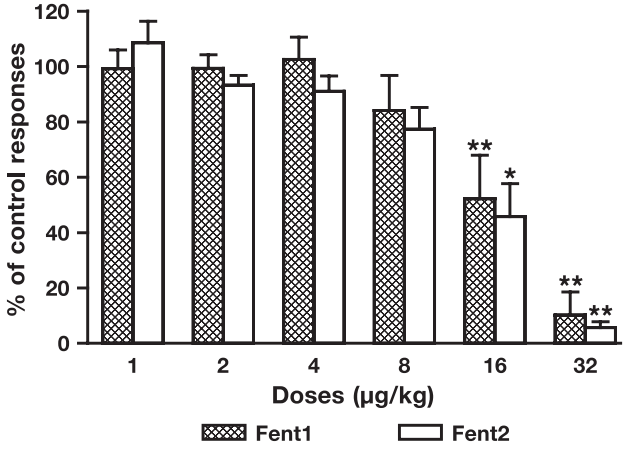

Fig. 2. Pooled data of the antinociceptive effects of fentanyl in responses to noxious mechanical stimulation when tested twice with an interval of 1 h. No significant differences were observed in this case between the two tests performed. Statistical comparison with the control response was performed using the one-way analysis of variance, ANOVA, with the post-hoc Dunnett test, $^{*}P<0.05$, $^{**}P<0.01$.

3.2. Effects of fentanyl in responses to noxious mechanical stimulation in the presence of dexketoprofen and nitroparacetamol

Fentanyl was tested in groups B and C in the absence and in the presence of subeffective doses of dexketoprofen or nitroparacetamol, following the same protocol as for group A. The effect of fentanyl in the absence of the NSAIDs was very similar in the two groups, and very similar to that observed in the control group of experiments (group A). Data from the first dose–response curves were therefore pooled and are represented in Fig. 3 and Table 2. The ED_{50} was 17 ± 0.1 μg/kg, the MED was 16 μg/kg and the maximum effect observed was 86 ± 13% of control response.

In the presence of 40 μg/kg of dexketoprofen, however, the MED was 8 μg/kg, and the ED_{50} was 4.7 ± 1 μg/kg, above threefold more potent than in the absence of dexketoprofen ($P<0.001$, Figs. 1 and 3 and Table 2). In the presence of 17 mg/kg of nitroparacetamol, the MED was 4 μg/kg, and the ED_{50} was 5.1 ± 1 μg/kg, again more than a threefold

Table 2
Comparison of data observed in responses to noxious mechanical stimulation and wind-up after the administration of fentanyl alone or in the presence of dexketoprofen trometamol, nitroparacetamol or vehicle

Group	Mechanical stimulation					Wind-up	
	ED_{50} (μg/kg)[a]	MED (μg/kg)	16 μg/kg (% control)[a]	Recovery, 30 min[a]	MED (μg/kg)	16 μg/kg (% control)[a]	Recovery, 15 min[a]
Fentanyl alone	17 ± 0.1	16	58 ± 8	102 ± 9	32	85 ± 2	123 ± 3
Fentanyl and DKT	4.7 ± 1[b]	8	14 ± 7[c]	30 ± 10[c]	16	63 ± 3[c]	73 ± 3[c]
Fentanyl and NOP	5.1 ± 1[b]	4	18 ± 7[c]	29 ± 8[c]	16	65 ± 3[c]	54 ± 3[c]
Fentanyl and vehicle	16 ± 0.7	32	95 ± 19	137 ± 22	32	75 ± 33	174 ± 11

The effect of fentanyl was more than threefold more potent in the presence of subeffective doses of dexketoprofen trometamol or nitroparacetamol. In these cases, no recovery of the responses was observed 30 min after the administration.
Student's *t* test for comparison of ED_{50s} and Mann–Whitney *U*-test for comparison of responses of fentanyl between groups.
[a] Data are expressed as mean ± S.E.M.
[b] $P < 0.001$.
[c] $P < 0.01$.

difference and more potent than in the absence of nitroparacetamol ($P < 0.001$, Table 2). The effect induced by the administration of fentanyl in the presence of either dexketoprofen or nitroparacetamol was significantly different to that observed with fentanyl alone with doses of 4, 8 and 16 μg/kg ($P < 0.01$ in all cases, Figs. 1 and 3 and Table 2). The effect observed with a dose of 16 μg/kg in the presence of dexketoprofen or nitroparacetamol (14 ± 7% and 18 ± 7%, respectively) was not significantly different to that observed with 32 μg/kg of fentanyl when studied alone (13 ± 5%, Fig. 3).

In the presence of the equivalent doses of the vehicle used, no enhancement of the potency or effectiveness of fentanyl was observed. In this case, the MED was 32 μg/kg and the ED_{50} was 16 ± 0.7 μg/kg.

The administration of 40 μg/kg of dexketoprofen, 17 mg/kg of nitroparacetamol or equivalent doses of the vehicle did not reduce significantly the responses to noxious mechanical stimulation on their own. The responses observed after the administration of dexketoprofen were of a maximum of 93 ± 7% of control. The administration of nitroparacetamol increased slightly the responses, with a maximum effect of 116 ± 10% of control, similar to the effect observed by the administration of vehicle: 114 ± 7% of control response, and similar to the effect observed in previous studies with similar doses (Mazario et al., 2001; Gaitan and Herrero, 2002; Romero-Sandoval et al., 2002). These effects were not significant.

3.3. Duration of the effect and no reversal action of naloxone and atipamezol

Fig. 4 shows pooled data of the recovery of responses to noxious mechanical stimulation after the administration of fentanyl in the absence and in the presence of the NSAIDs studied. When fentanyl was studied alone, the inhibitory effect disappeared very quickly, observing a 75.5 ± 10% of control response 15 min later and 102 ± 9% of control response 30 min after the completion of the dose–response curve (Fig. 4). In the presence of either dexketoprofen or nitroparacetamol, no significant recovery was observed up to 45 min after the administration of the highest dose of fentanyl, with responses at this time of 24 ± 10% and 32 ± 12%, respectively (Fig. 4). Surprisingly, the administration of either naloxone or atipamezol did not induce any significant reversal of the inhibition of responses (Fig. 4).

In order to test the effectiveness of the opioid receptor antagonist, 200 μg/kg of naloxone was injected in a different set of experiments 6 min prior to the administration of a single dose of 32 μg/kg of fentanyl. In this case, the administration of fentanyl did not cause any effect (114 ± 12% of control response).

3.4. Effect of fentanyl on wind-up in the absence and in the presence of dexketoprofen and nitroparacetamol

As in responses to noxious mechanical stimulation, the effect of fentanyl was very similar when tested twice in the

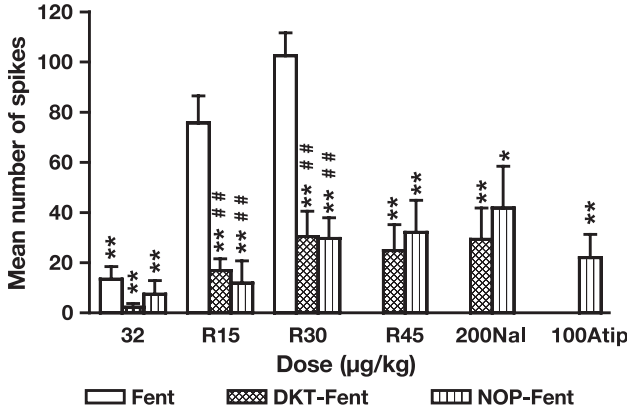

Fig. 4. Pooled data of the antinociceptive effects of fentanyl in responses to noxious mechanical stimulation after the administration of the highest cumulative dose of 32 μg/kg (32) and recovery of the responses observed 15 (R15), 30 (R30) and 45 min later (R45). The figure shows the quick recovery of the responses observed when fentanyl was injected alone (Fent) and the lack of recovery when administered in the presence of dexketoprofen (DKT-Fent) or nitroparacetamol (NOP-Fent). This effect was not reversed by the administration of 200 μg/kg of naloxone (200Nal) or 100 mg/kg of atipamezol (100Atip). Statistical significance and layout as for Fig. 2.

same unit, and when tested alone prior to the administration of the NSAIDs. Pooled data of the effect of fentanyl in the absence of any other drug are represented in Fig. 5A. The MED was 32 μg/kg in all cases and the maximum effect observed was 35 ± 2% of control response ($P < 0.01$). As Fig. 5A shows, a full disappearance of the effect was observed 15 min after the administration of fentanyl. The maximum firing rate observed was similar in all the experiments, with a maximum of 13 ± 1 spikes. This firing rate was lower to that observed in dexketoprofen experiments, 21 ± 1 spikes (Fig. 5B), and similar to that observed in the experiments performed with fentanyl in the presence of nitroparacetamol, 14 ± 2 spikes (Fig. 5C). In the presence of 40 μg/kg of dexketoprofen, the maximum reduction of wind-up was 33 ± 3% of control response ($P < 0.01$), with a dose of 32 μg/kg of fentanyl, similar to that observed when fentanyl was tested alone. The MED was, however, 16 μg/kg, which induced a reduction of 63 ± 3% of control ($P < 0.01$, Fig. 5B). A similar observation was made when fentanyl was studied in the presence of nitroparacetamol. In this case, the MED was also 16 μg/kg with an effect of 65 ± 3% of control ($P < 0.01$, Fig. 5C). The maximum effect observed was 23 ± 4% of control, with a dose of 32 μg/kg of fentanyl. The recovery of the effect of fentanyl on wind-up was slower when studied in the presence of dexketoprofen or nitroparacetamol in comparison to that of fentanyl alone. In the presence of dexketoprofen, only a recovery of 73 ± 3% was observed 15 min after the administration of fentanyl (Fig. 5B) and this effect was still significantly different to the control response ($P < 0.01$). Full recovery of the wind-up response was observed after 30 min. In the presence of nitroparacetamol, the recovery was similar to that observed in the presence of dexketoprofen, with a maximum of 54 ± 3% of control response ($P < 0.01$) 15 min after the administration of fentanyl (Fig. 5C) and full recovery observed 30 min after the administration of fentanyl.

3.5. Effect of fentanyl on blood pressure in the absence and in the presence of dexketoprofen and nitroparacetamol

The administration of fentanyl was always made very slowly in order to diminish the effect on blood pressure. Nevertheless, a dose-dependent drop in blood pressure was always observed in all cases. This effect was not modified by the presence of dexketoprofen or nitroparacetamol in any case. Prior to the administration of fentanyl the mean arterial pressure observed was 131 ± 5 mm Hg in group A of the experiments, when fentanyl was studied alone. This pres-

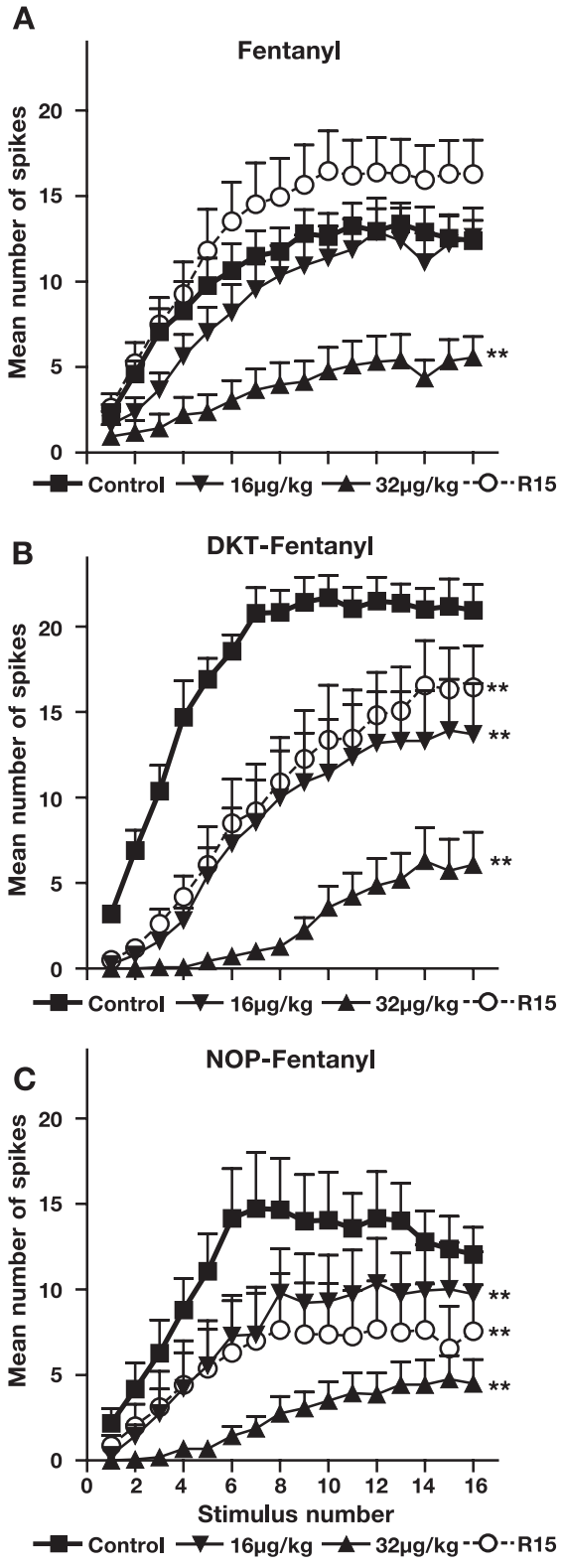

Fig. 5. Single motor unit wind-up observed after the administration of cumulative doses of fentanyl alone (A), in the presence of 40 μg/kg of dexketoprofen (DKT-Fentanyl, B) or in the presence of 17 mg/kg of nitoparacetamol (NOP-Fentanyl, C). The minimum effective dose of fentanyl was 32 μg/kg when given alone and 16 μg/kg when given in the presence of dexketoprofen or nitroparacetamol. Full recovery of the responses was observed 15 min (R15) after the administration of fentanyl alone. The recovery of the responses when fentanyl was injected in the presence of the cyclooxygenase-inhibitors was only partial, and full recovery was only observed 30 min after the administration. Only some of the doses tested are represented to illustrate the effect. Statistical comparison between responses observed with each dose and the control response was made using the one-way analysis of variance, ANOVA, with the post-hoc Dunnett test, $^{**}P < 0.01$.

sure was very similar to that observed in groups B: 127 ± 5 mm Hg and C: 130 ± 7 mm Hg. The mean arterial pressure values 6 min after the administration of a cumulative dose of 32 μg/kg of fentanyl alone were 81 ± 2 mm Hg, 78 ± 6 mm Hg in the presence of dexketoprofen and 76 ± 5 mm Hg in the presence of nitroparacetamol. The effect on blood pressure fully recovered 30 min after the administration of fentanyl in all cases.

4. Discussion

The results observed in this study confirm the enhancement of the antinociceptive activity of fentanyl by subeffective doses of dexketoprofen trometamol as previously observed (Gaitan and Herrero, 2002). The potency, effectiveness and duration of the effect of fentanyl were significantly enhanced, in withdrawal reflexes recorded as single motor unit activity, by doses of dexketoprofen trometamol that did not cause any apparent effect on their own. The effect was very potent on responses to noxious mechanical stimulation, in which an enhancement of more than threefold of the potency of fentanyl and twofold of its effectiveness was accompanied by an important increment of the duration of the antinociceptive effect. A clear conclusion of the present study is that the effect is not exclusive of dexketoprofen since a similar, and even more potent action, was observed with the administration of subeffective doses of nitroparacetamol. In this case, the enhancement of the potency and duration of the effect of fentanyl on responses to noxious mechanical stimulation was similar to that observed with dexketoprofen. The effectiveness of fentanyl was, however, increased by nitroparacetamol by fourfold, indicating that the combination of nitroderivatives and fentanyl might be even more advantageous than the combination of fentanyl with other cyclooxygenase-inhibitors. Dexketoprofen and nitroparacetamol have a good ability to cross the blood–brain barrier (see Mazario et al., 1999; Romero-Sandoval et al., 2002 for further details on this issue) and this might be crucial to explain the enhancement of opioid-mediated analgesia by cyclooxygenase-inhibitors. In fact, the mechanism of action throughout neurons in the periaqueductal grey area, proposed by Vaughan et al., (1997), or a mechanism of action involving serotonergic systems (Tjølsen et al., 1991; Sandrini et al., 1998) requires a central action of the cyclooxygenase-inhibitors. Also, the fact that low doses of other more classic NSAIDs do not increase that much the potency of opioid-mediated analgesia (Mas Nieto et al., 2001) might be due to a lower penetration of the central nervous system by the drugs used. Therefore, the combined administration of low doses of cyclooxygenase-inhibitors, with a high ability to cross the blood–brain barrier, with the μ-opioid receptor agonist fentanyl should be taken in consideration as a potential therapy in nociception.

The potent effect of nitroparacetamol and fentanyl might be due to a better interaction between the two compounds rather than to a different mechanism of action to that of dexketoprofen and fentanyl, since the effect on the wind-up phenomenon was very similar. The effect on responses to repetitive electrical stimulation was smaller, though significant, when compared to the effect on responses to noxious mechanical stimulation, with a clear increase in the effectiveness of fentanyl by twofold when combined with either dexketoprofen or nitroparacetamol. The enhancement of the duration of the effect was in this case more modest since it was only enlarged by 15 min. This effect, though modest, should be taken into consideration in a drug-like fentanyl whose half-life is so short. Also, this effect supports a central action but, since it is of a low intensity, shows a little interaction with the mechanisms involved in the generation of the wind-up phenomenon, i.e. NMDA receptors (Davies and Lodge, 1987; Dickenson and Sullivan, 1987) and tachykinin NK1 receptors (De Felipe et al., 1998).

The mechanism by which the enhancement of the effect is produced remains, however, unknown. As stated in the previous paragraph, an interaction with NMDA or NK1 systems does not seem likely. Also, the effect of fentanyl in the presence of dexketoprofen or nitroparacetamol was not reversed by naloxone. The dose of naloxone used has been previously shown to be enough to reverse the antinociceptive effect of similar doses of fentanyl or even the effect of kappa receptor agonists (Herrero and Headley, 1991). In control experiments, however, the dose used of naloxone was effective in the prevention of any effect of a dose of 32 μg/kg of fentanyl. This indicates that naloxone was effective when fentanyl was administered alone, and, therefore, that the mechanism of enhancement of the effect of fentanyl is not directly related to an action on opioid receptors, or that, at least, this is not the solely mechanism of action. A similar lack of full naloxone antagonism was observed when morphine was applied in combination with acetylsalicylic acid (Sandrini et al., 1998). This, however, was only observed in the second phase of the formalin test, which is considered as a result of spinal cord neuronal activity (Tjølsen et al., 1991), i.e. as a central process and, therefore, it will be in agreement with our hypothesis of a centrally mediated effect, as mentioned above. An interaction with serotonergic systems has been proposed to explain some of the central actions of paracetamol (Tjølsen et al., 1991) and even to explain the enhancement of the effect of morphine by acetylsalicylic acid (Sandrini et al., 1998). This is a possibility to be explored in future experiments, though in the above studies the effect was observed in the formalin test and never in the non-inflamed situation. Since previous studies have shown an interaction between μ-opioid systems and α_2-adrenoceptor systems (Herrero and Solano, 1999 and references within), we studied this possibility by injecting a high dose of the α_2-adrenoceptor antagonist atipamezol. The effect of fentanyl was, however, not modified by the

administration of atipamezol, indicating a lack of interaction with this system. A third possibility is that the lack of recovery of responses to noxious mechanical stimulation was not a pharmacological action, but a habituation of the units or an influence of the first dose–response curve of fentanyl, studied prior to the administration of the cyclo-oxygenase-inhibitor. These possibilities do not seem likely since neither a different effect nor a lack of recovery was ever observed in the control group of experiments.

In conclusion, the inhibitory effect of fentanyl and its duration on nociceptive responses evoked either by noxious mechanical or electrical stimulation are enhanced by the prior administration of low doses of dexketoprofen trometamol or nitroparacetamol. The effect is especially important in responses to noxious mechanical stimulation, though it is also present in wind-up. The mechanism of action remains unclear and it does not seem to be fully mediated by a direct action on opioid systems, α_2-adrenergic systems, NMDA or tachykinin NK1 receptors.

Acknowledgements

This work has been supported by NicOx and by a grant from the Comunidad de Madrid (grant 08.5/0036.1/2003). Gema Gaitan is a fellow of the Spanish Ministry of Science and Technology. We are grateful to Lawrence JC Baron for a critical review of the manuscript and for his help with the English revision.

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