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Subeffective doses of nitroparacetamol (NCX-701) enhance the antinociceptive activity of the α_2-adrenoceptor agonist medetomidine

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ABSTRACT

The α_2-adrenergic system is involved in pain processing and inflammation-induced sensitization. α_2-adrenoceptor agonists induce analgesia, and this effect is greater when administered in combination with other analgesics. In the present study, we assessed a possible enhancement of antinociception combining the α_2-adrenoceptor agonist medetomidine with subeffective doses of NCX701 (nitroparacetamol). The effects of the drugs were studied in spinal cord neuronal responses from adult male Wistar rats with carrageenan-induced inflammation, using the recording of single motor unit technique. The experiments showed that the i.v. administration of medetomidine and NCX701 induced a more potent and effective antinociceptive effect than medetomidine when given alone (ID_{50}: 0.47 ± 0.1 vs. 1.1 ± 0.1 μg/kg) or in the presence of paracetamol, in naturally-evoked nociceptive responses. In addition, the duration of antinociception was significantly longer ($P<0.001$, 100 min after administration). The use of low doses of NCX701 and α_2-adrenoceptor agonists might open new perspectives in the treatment of inflammatory pain.

1. Introduction

Numerous studies have shown a direct involvement of the α_2-adrenergic system in the processing of nociceptive information, especially in situations of inflammation (Millan, 1992). It is well stablished, for example, that α_2-adrenoceptor agonists induce analgesia (see for example Takano et al., 1993; Eisenach et al., 1996). However, the mechanism of action by which adrenoceptor agonists induce analgesia includes not only the activation of α_2-adrenoceptors at spinal and/or supraspinal sites (Peng et al., 1996; Asano et al., 2000), but also other mechanisms of action as, for example, the activation of cholinergic receptors (Duflo et al., 2003), GABAergic receptors (Nguyen et al., 1997), opioid receptors (Ossipov et al., 1990), the release of nitric oxide (NO; Aronov et al., 2005a,b) and the activation of spinal cord descending inhibitory pathways (Cui et al., 1999), among others. The involvement of different mechanisms of action has lead to the search for an enhancement of antinociception by combining α_2-adrenoceptor agonists with other compounds with an antinociceptive activity located preferably on any of those systems. As a result, enhancement of analgesia has been observed after the combination of α_2-adrenoceptor agonists and opiates (Meert and De Kock, 1994), serotonergic agonists (Dukat and Wesolowska, 2005) or GABAergic agonists (Przesmycki et al., 1998).

On the other hand, the α_2-adrenergic system is involved in the generation and maintenance of inflammation-induced sensitization. It is well established, for example, that joint-inflammation is associated with an enhancement of the expression of α_2-adrenoceptors in the spinal cord (Brandt and Livingston, 1990). In addition, the intensity of the antinociception induced by medetomidine, a selective α_2-adrenoceptor agonist, depends on the time course of soft-tissue inflammation (Molina and Herrero, 2006). Furthermore, α_2-adrenoceptor activation in inflammatory neuritis increases local apoptosis and anti-inflammatory products early after treatment (Romero-Sandoval and Eisenach, 2007). It is, therefore, not surprising that the combined administration of α_2-adrenoceptor agonists with cyclooxygenase (COX) inhibitors induced an enhancement of analgesia in situations of inflammation (Malmberg and Yaksh, 1993, Miranda and Pinardi, 2004). The benefits of the combination of α_2-adrenoceptor agonists and COX-inhibitors include, not only an enhancement of antinociceptive and anti-inflammatory activities, but also a reduction of some of the unwanted side-effects observed after the administration of high doses of these drugs, such as sedation (Sinclair, 2003) or hepatotoxicity (Moling et al., 2006).

Nitro-NSAIDs, like nitroparacetamol (NCX701), have shown an intense antinociceptive activity in different preparations, either in inflammatory or non-inflammatory conditions (Romero-Sandoval et al., 2002, 2003; see Curros-Criado and Herrero, 2009 for references). In addition, NCX701 enhances the antinociceptive activity induced by the opioid-receptor agonist fentanyl (Gaitan et al., 2003) and by gabapentin (Curros-Criado and Herrero 2009), and has shown a lower side-effect profile as well as a higher antinociceptive effectiveness than its parent compound, paracetamol (Romero-

Abbreviations: NO, nitric oxide; COX, cyclooxygenase; SMU, single motor unit; PAR, paracetamol; NCX701, nitroparacetamol.

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Sandoval et al., 2007; Curros-Criado and Herrero, 2009). However, there are no published studies assessing a possible enhancement by nitro-NSAIDs of the antinociceptive activity induced by α_2-adrenergic agonists, despite the antecedents described above. Therefore, the purpose of the present study was to investigate a possible interaction of subeffective doses of i.v. NCX701 and paracetamol (PAR; acetaminophen) on the intensity and duration of the antinociceptive action of the α_2-adrenoceptor agonist medetomidine in animals with soft-tissue carrageenan-induced inflammation.

2. Methods

2.1. Animals and preparation

Electrophysiological experiments were carried out on male Wistar rats weighing 240–302 g using the single motor unit (SMU) technique. The preparation has been described previously in detail (Herrero and Headley, 1991, 1996; Herrero and Cervero, 1996a; Solano and Herrero, 1997). Briefly, a small preparatory surgery was performed under halothane anesthesia (5% in oxygen for induction and 2% for maintenance) and consisted of the cannulation of the trachea, two superficial branches of the jugular veins (for the administration of the maintenance anesthetic and drugs, respectively), and one carotid artery to register the blood pressure. After the surgery, halothane was ceased and anesthesia continued with α-chloralose (Sigma; 50 mg/kg for induction and 30 mg/kg/h by a perfusion pump for maintenance at a rate of 1 ml/h). This rate allowed also for the correct hydration of the animal. Once transferred to an appropriate frame, the right hind limb was fixed into a Perspex block in inframaximal extension using plaster. Core body temperature was maintained at 37 ± 0.5 °C by means of a homeothermic blanket connected to a thermal rectal probe via an automatic feedback control unit throughout the surgery and the experiment. The preparation was left to rest for at least 1 h before the experiment started. Blood pressure was monitored constantly, so the animals with a systolic blood pressure below 100 mm Hg before the administration of the drugs were considered unhealthy and discarded.

The induction of soft-tissue inflammation was achieved by the intraplantar injection of 100 μl de carrageenan λ (10 mg/ml in distilled water, Sigma) in the right hind paw. The procedure was followed under brief halothane anesthesia (same protocol as for the surgery) 20 h before the experiment (Molina and Herrero, 2006). The level of inflammation was evaluated by measuring the volume of the paw by plethysmometry (Letica plethysmometer) before the administration of carrageenan and after each of the drug treatments.

The recording of withdrawal reflexes as SMUs has been used to study the phenomenon of wind-up and to test the analgesic activity of different drugs, and has been used and described in detail several times (Herrero and Headley, 1991; Romero-Sandoval et al., 2003; Ramos-Zepeda et al., 2004; Molina and Herrero, 2006; Curros-Criado and Herrero, 2007). Very briefly, nociceptive withdrawal reflexes were recorded as SMUs by means of a bipolar tungsten electrode inserted percutaneously into muscles of the right hind limb. The units were activated by mechanical (natural) and electrical (win-up) stimulation in cycles of three minute duration, each cycle consisting of 10 s of noxious mechanical stimulation (0.2 N above the threshold over an area of 14 mm^2) and 16 electrical stimuli (2 ms width, 1 Hz, twice the threshold intensity for the recruitment of C-fiber responses; Herrero and Cervero, 1996b). The protocol of stimulation and recording examples are illustrated in Fig. 1. Isolation of motor units was performed by moving the electrode with a micromanipulator while a mild pressure was applied to the paw. Electrical stimulation was applied through two 0.2 mm needles inserted percutaneously in the most sensitive area of the cutaneous receptive field. Mechanical stimulation was performed by a computer-controlled pincher (Cibertec), which was also used to determine the threshold force required to trigger the withdrawal response using a pressure-ramp stimulation.

The experiments were carried out in three experimental groups: the antinociceptive effect of medetomidine was studied in animals with carrageenan-induced inflammation either alone (n = 7) or in the presence of subeffective doses (see below) of NCX701 (n = 7) or PAR (n = 7). The effect of medetomidine was challenged by the i.v. administration of the α_2-adrenergic antagonist atipamezole in half of the experiments in each group, in order to confirm an action of medetomidine on α_2-adrenoceptors. The time-course recovery of the antinociceptive effect of medetomidine was studied in the rest of the experiments during 100 min, time enough to observe full recovery of the effect of the drug according to previous experiments (Molina and Herrero, 2006) and to the pharmacokinetics of the drug (Scheinin et al., 1992).

2.2. Drugs and analysis of data

Medetomidine (Domtor, Pfizer) was studied at doses of 0.3 to 4.8 μg/kg and administered in a cumulative $\log_2$ regime every two cycles of stimulation (6 min). It was dissolved in saline (1–10 μg/ml) and injected i.v. in a constant volume of 0.3 ml. The effect of medetomidine was challenged with a single dose of 100 μg/kg of

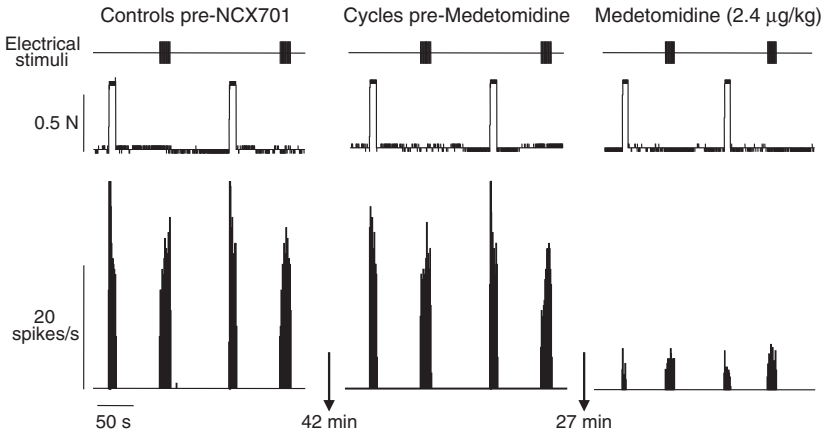

Fig. 1. Original recordings of single motor unit activity. Nociceptive responses were elicited by mechanical and electrical stimulation in animals with carrageenan-induced inflammation. The figure shows two control responses previous to the administration of NCX701 (30 μmol/kg), two cycles of stimulation after NCX701 and previous to the administration of cumulative doses of medetomidine (initial dose of 0.3 μg/kg), and the two first cycles of stimulation after the dose of 2.4 μg/kg of medetomidine. Upper and middle panels show the stimuli applied in cycles of three minute duration. Lower panel shows the SMU responses recorded with each stimulus represented as 1 s bar histograms.

atipamezole (Antisedan, SB; 500 μg/ml in saline) injected i.v. in a volume of 0.3 ml. PAR (Nicox) or NCX701 (Nicox) were dissolved in dimethyl sulfoxide (DMSO; Sigma; 50%) and polyethylene glycol 300 (PEG 300; Panreac; 50%; final concentration of 50 mM), and administered i.v. in two doses of 15 μmol/kg with a gap of seven cycles of stimulation (21 min) between doses. The two drugs were diluted in saline and injected i.v. in a constant volume of 0.5 ml (proportion of 1 μl DMSO/PEG: 4 μl saline). The cumulative amount of PAR or NCX701 administered was 30 μmol/kg. This dose did not reduce, in our experiments, the responses to noxious stimulation below 20% of control (subeffective dose). All doses used were calculated in prelimar experiments as well as according to previous studies made in similar experimental conditions (Gaitan et al., 2003; Gaitan et al., 2005; Romero-Sandoval et al., 2002, 2003; Molina and Herrero, 2006). Control experiments were carried out with the solvents used. However, the effect of PAR, as the parent compound, was considered as the most appropriate "vehicle" control group to compare the effects of NCX701. Therefore, statistical comparisons were made between the effects observed after the administration of medetomidine in the presence of NCX701 vs. medetomidine alone, but, in addition, vs. medetomidine in the presence of PAR. In addition, a group of control experiments ($n=4$) was carried out to test the possible effect of the NO donor, NOC-18 (DETA NONOate, Alexis), with similar NO release rate to that of NCX-701 (Romero-Sandoval et al., 2002).

Data are expressed as mean ± S.E.M. of percentage of control, control being the mean of the three responses previous to the injection of the drugs (Fig. 1). Responses to mechanical and electrical stimulation were counted and analyzed separately. The quantitative analysis was based on counts of spikes evoked during each of the two cycles of stimulation between each dose, in the case of medetomidine, or the last three cycles for PAR and NCX701. The data from the electrical stimulation were analyzed by counting the number of spikes evoked between 150 and 650 ms after each stimulus (C-fiber responses; Herrero and Cervero, 1996b).

The protocol for stimulation and the collection of data were performed by computer using commercial software (CED, U.K.; Spike 2 for Windows). Statistical comparisons were made using commercial software (GraphPad Prism and GraphPad-Instat for Windows). The responses were compared as raw data, using the one-way analysis of variance (ANOVA) for repeated measures with the Dunnett's post-test.

Animals were used for one procedure only and were humanely euthanized on completion of experiment by an overdose of sodium pentobarbital (Euta-Lender, Normon). All efforts were made to reduce the number of animals used. All experiments in this study were undertaken in accordance with Spanish and European Union legislation (European Communities Council Directive of 24 November 1986; 86/609/EEC) regarding the uses of animals for experimental protocols. In addition, the methods used in the present study were approved by the Committee of Ethics in Research of the University of Alcala.

3. Results

3.1. Noxious mechanical stimulation

The intraplantar injection of carrageenan induced a significant paw swelling in the three experimental groups. The mean volume of the paw before the injection of carrageenan was 1.53 ± 0.1 ml in animals treated with medetomidine, 1.58 ± 0.1 ml in animals treated with medetomidine and PAR and 1.60 ± 0.1 ml in animals treated with medetomidine and NCX701. Immediately before the experiments, and 20 h after the injection of carrageenan, the paw volumes were: 2.32 ± 0.2 ml in the group of animals treated with medetomidine; 2.4 ± 0.1 ml in the group of medetomidine and PAR and 2.55 ± 0.1 ml in animals treated with medetomidine and NCX701. After the experiments, the volume of the paw did not vary significantly: 2.4 ± 0.1, 2.42 ± 0.1 and 2.57 ± 0.1 ml, respectively. No significant differences were observed between groups.

The effects observed after the administration of medetomidine on responses to noxious mechanical stimulation are illustrated in Fig. 2. The i.v. administration of medetomidine induced a full inhibition of the responses elicited by noxious mechanical stimulation. This effect was dose-dependent, and a complete inhibition of the nociceptive activity was observed with the dose of 4.8 μg/kg (8 ± 3% of control response, $P<0.001$; Fig. 2). The minimum effective dose was 1.2 μg/kg ($P<0.01$) and the ID_{50} was 1.1 ± 0.1 μg/kg.

In a second group of animals, a total cumulative dose of 30 μmol/kg of PAR was injected 21 min previous to the administration of medetomidine, following the same protocol as in the previous experimental group. The administration of PAR did not modify significantly the responses to noxious mechanical stimulation: 91 ± 4% of control response (Fig. 2, inset). The effect of medetomidine in the presence of PAR was very similar to that seen when administered alone (Fig. 2). In this case, the minimum effective dose was 1.2 μg/kg ($P<0.05$), the ID_{50} was 1.5 ± 0.1 μg/kg and the maximal inhibitory effect observed was of 7 ± 4.5% of control response ($P<0.001$), with the cumulative dose of 4.8 μg/kg (Fig. 2).

The i.v. injection of small doses of NCX701 (final cumulative dose of 30 μmol/kg) did not modify significantly the level of mechanically-evoked nociceptive responses (83 ± 2% of control; Fig. 2, inset). However, the antinociceptive effect observed after the administration of medetomidine in the presence of NCX701 was more potent (ID_{50}: 0.47 ± 0.1 μg/kg, $P<0.001$, Fig. 2) than in the absence of NCX701 (ID_{50}: 1.1 ± 0.1 μg/kg) or in the presence of PAR (1.5 ± 0.1 μg/kg). In addition, the effect observed after the administration of medetomidine in the presence of NCX701 was significantly more intense than that observed when medetomidine was injected alone or in the presence of PAR, with the doses of 0.3 and 0.6 μg/kg ($P<0.001$ and $P<0.05$, Fig. 2). The minimum effective dose (0.3 vs. 1.2 μg/kg) and the dose that induced a reduction of responses below 20% of control (full inhibition) were also lower when the two drugs were co-administered than when medetomidine was injected alone or in the presence of PAR (2.4 vs. 4.8 μg/kg; Fig. 2). To assess whether the antinociceptive effects observed with NCX-701 were only due to the release of NO, four experiments were

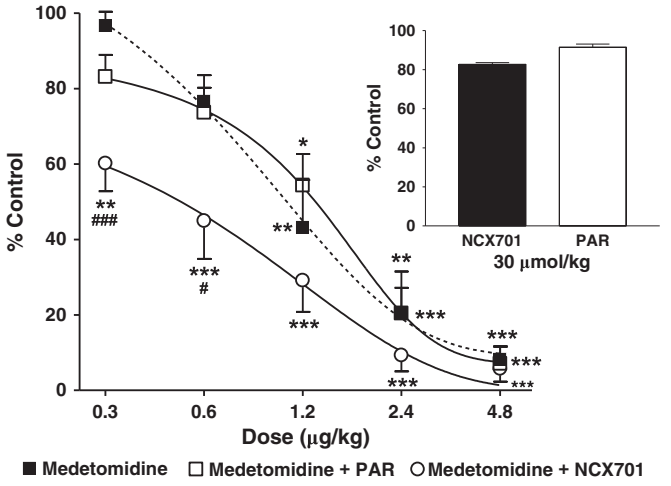

Fig. 2. Effect of medetomidine in responses to noxious mechanical stimulation. Medetomidine induced a reduction of nociceptive responses very similar when studied alone (medetomidine) and in the presence of PAR (medetomidine + PAR). However, the effect of medetomidine was significantly more potent in the presence of subeffective doses of NCX701 (medetomidine + NCX701). Inset shows the lack of effect on nociceptive responses induced by the i.v. administration of low doses of PAR and NCX701 (statistical comparison using the one-way ANOVA with the post hoc Dunnett test, *$p<0.05$, **$p<0.01$, ***$p<0.001$ comparison vs. control responses; #$p<0.05$, ###$p<0.001$, comparison vs. animals treated with medetomidine or medetomidine and PAR).

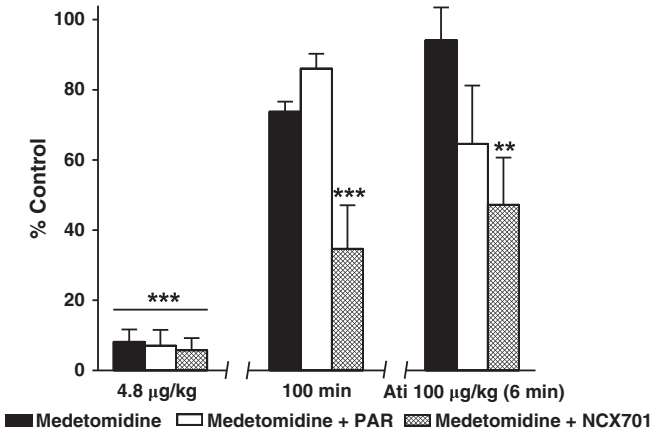

Fig. 3. Recovery of the effect of medetomidine on SMU responses to noxious mechanical stimulation. Left columns show the effect observed after the administration of the highest dose of medetomidine studied. The inhibition of responses fully recovered within 100 min after the administration of medetomidine alone or in the presence of PAR. However, a lack of recovery was observed when medetomidine was administered in the presence of NCX701. In this case, the antinociceptive effect of medetomidine was not reversed by the administration of 100 μg/kg of the α_2-adrenoceptor antagonist atipamezole (statistical comparison and layout as for Fig. 2).

performed using equivalent amounts of the NO donor NOC-18. The experiments showed a lack of enhancement of the activity of medetomidine (minimum effective dose: 1.2 μg/kg; ID_{50} was 1.7 ± 0.9 μg/kg).

In order to study the duration of the antinociceptive effect of medetomidine, nociceptive responses were recorded for a minimum of 100 min after the administration of the highest dose of medetomidine, following the same protocol of stimulation as that described in the Methods section. Fig. 3 illustrates these data. The effect of medetomidine when injected alone fully recovered within the period of time studied, and always within 60 min after the administration of the latest dose. The administration of low doses of PAR previous to the injection of medetomidine did not induce any change in the duration of the antinociception (Fig. 3). However, the duration of the antinociception was significantly longer when medetomidine was studied in the presence of subeffective doses of NCX701. In this case, the responses to noxious mechanical stimulation remained significantly lower than control responses (35 ± 12%, $P<0.001$) 100 min after the administration of the highest dose of medetomidine.

In a different group of experiments, the antinociceptive effect induced by the injection of medetomidine was challenged with a single dose of 100 μg/kg of the selective α_2-adrenoceptor antagonist atipamezole, administered i.v. 6 min after the highest cumulative dose of medetomidine (Fig. 3). The effect observed by the injection of medetomidine alone or in the presence of PAR recovered immediately after the administration of the antagonist. However, the antinociceptive responses observed after the administration of medetomidine in the presence of NCX701 remained significantly low: 47 ± 13% of control response ($P<0.01$, Fig. 3).

3.2. Noxious electrical stimulation: wind-up

Wind-up consists of a progressive increase of the number of spikes produced by repetitive and high intensity electrical stimulation (Herrero et al., 2000). All the units studied showed the phenomenon of wind-up, which is represented in Fig. 4 as pooled data, as well as the mean number of spikes per stimulus (inset).

The administration of cumulative doses of medetomidine dose-dependently reduced the level of wind-up to a complete inhibition, observed with the dose of 4.8 μg/kg (4 ± 3.5% of control response, $P<0.01$). The minimum effective dose was 1.2 μg/kg ($P<0.01$, Fig. 4) and the ID_{50} was 1.16 ± 0.2 μg/kg. As in responses to noxious mechanical stimulation, the combination of medetomidine and PAR did not cause any significant change in the antinociceptive activity (ID_{50}: 1.5 ± 0.2 μg/kg; minimum effective dose: 1.2 μg/kg, $P<0.01$, Fig. 4), with a maximum inhibitory effect of 17 ± 8% of control ($P<0.01$). The administration of medetomidine, in the presence of NCX701, induced a similar effect than that observed in previous experiments, with a minimum effective dose of 1.2 μg/kg ($P<0.01$), an ID_{50} of 1.13 ± 0.2 μg/kg and the maximum reduction of 11 ± 11% ($P<0.01$, Fig. 4). Finally, as in previous experiments, the administration of equivalent doses of NOC-18 did not modify the effect observed by the administration of medetomidine (data not shown).

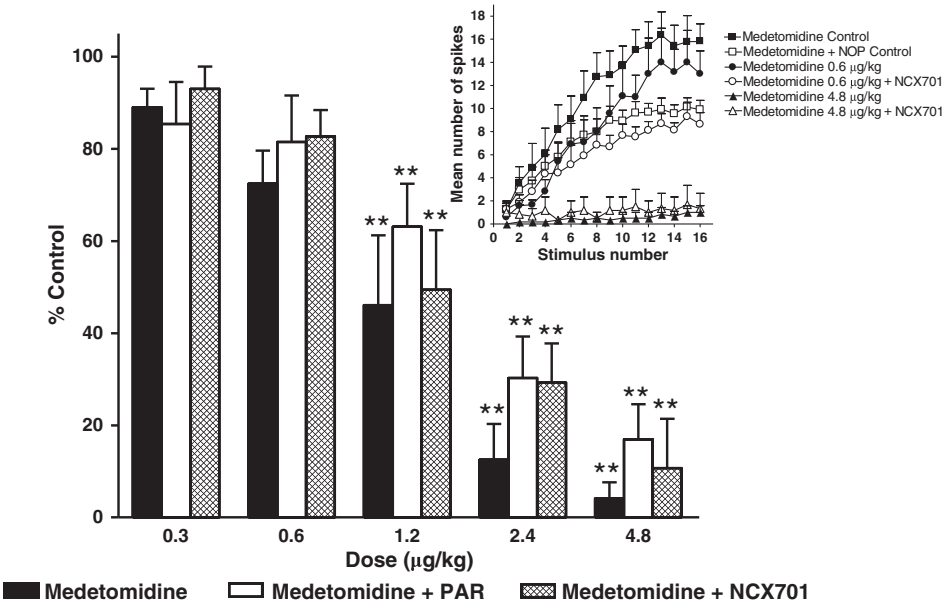

Fig. 4. Effect of medetomidine on single motor unit wind-up when injected alone or in the presence of PAR or NCX701. A dose-dependent reduction of wind-up was observed in all the experimental groups and no significant differences were observed between them. Inset shows the effect observed with some doses of medetomidine alone and with NCX701 using the actual mean number of spikes per stimulus (statistical significance as for Fig. 2).

4. Discussion

The main observation made in the present study is the significant enhancement of the antinociceptive activity of medetomidine when given in the presence of subeffective doses of the NO-releasing COX-inhibitor NCX701, in animals with soft-tissue carrageenan-induced inflammation. Previous studies have shown an increase of antinociception resulting from the combination of the α_2-adrenoceptor agonist clonidine or tizanidine with some COX-inhibitors, as for example naproxen or meloxicam, using the writhing test as a model of acute visceral pain (Jain et al., 2002). Similar results were observed when clonidine was studied in combination with piroxicam or PAR (Miranda and Pinardi, 2004), using the same algesiometric test. However, to our knowledge, this is the first study which shows an enhancement of the antinociceptive activity of the selective α_2-adrenoceptor agonist medetomidine, and its duration, induced by the administration of subeffective doses of a NO-releasing derivative of PAR.

Previous studies carried out in our and other labs have shown NCX701 as a potent and efficacious antinociceptive agent showing, in addition, a lower profile of unwanted side-effects than its parent compound (see Romero-Sandoval et al., 2007 for review). Further, an enhancement of antinociception has been also described, following the same protocol and technique as in the present study, after the combination of low doses of NCX701 and the opiate fentanyl (Gaitan et al., 2003, 2005). The enhancement of the antinociceptive activity of medetomidine by very low doses of this novel analgesic, confirms that NCX701 might be a compound with a potential interest in the treatment of inflammatory pain, either when given alone or, better, when combined at very low doses with medetomidine. In this case, the dose of medetomidine needed to induce a 50% reduction of nociceptive activity lowered by almost 2.5 fold, and the dose of NCX701 required to observe the effect was so low that no significant effect was observed in the nociceptive responses studied. This low dosage might also suggest a safety profile of the drugs when given in combination.

The reduction of nociceptive responses seemed to be a direct antinociceptive action rather than a consequence derived of a depression of the level of inflammation, since no reduction in the level paw swelling was observed in any of the experiments. In addition, it does not seem likely that NO, derived from NCX701, induced an antinociceptive effect on its own since the experiments carried out with NOC-18 showed no variation on the effect of medetomidine.

The experiments carried out in the present study showed an increased duration of the antinociception when medetomidine was studied in the presence of NCX701. Whereas the antinociception induced by the administration of medetomidine alone or in the presence of PAR lasted for a maximum of 1 h in all the experiments, the antinociceptive effect observed after the administration of NCX701 and medetomidine did not fully recover within the following 100 min, and the nociceptive responses were still significantly lower than control response. This observation is in agreement with a similar lack of recovery of the antinociception observed when similar doses of NCX701 were co-administered with the opiate fentanyl (Gaitan et al., 2005). The enhancement of the duration of the effect suggests a specific interaction between NCX701 and the α_2-adrenergic system, since the effect was not observed when medetomidine was studied in combination with its parent compound, PAR. In addition, the injection of the α_2-adrenoceptor antagonist atipamezole quickly reversed the antinociception induced by medetomidine alone or in the presence of PAR. This indicates that the antinociceptive effect involved an activation of α_2-adrenoceptors. However, the administration of atipamezole only modified partially the antinociception observed after the administration of medetomidine in the presence of NCX701. Further experiments are needed to clarify the specific mechanism involved in this lack of full reversal effect, although, it seems to indicate an action in which the α_2-adrenoceptors might be involved in the initiation of the antinociceptive effect but only partially in its maintenance. The lack of full effect of atipamezole can be therefore interpreted as the result of the involvement of a mechanism of action independent of the activation of α_2-adrenoceptors, which might involve a medetomidine-mediated enhancement of the COX-inhibition induced by NCX701. This might explain not only the increment of the antinociceptive activity observed in the experiments, but also its longer duration, which matches better with the pharmacokinetics of a COX-inhibitor than of a α_2-adrenoceptor agonist. Though NCX701 has a clear action as a COX-inhibitor (Marshall et al., 2006), a mechanism of action independent of COX inhibition is also a possibility to take in consideration, as suggested in previous studies with some nitro-derivatives, including NCX701 (Del Soldato et al., 1999; Fiorucci, 2001; Kiss and Vizi, 2001).

A possible action by NO, on its own, resulting on an enhancement of the antinociceptive activity of medetomidine is also possible. In fact, some studies have shown an interaction between clonidine-mediated analgesia and NO (Aronov et al., 2005a,b; Ge et al., 2006). However, this does not seem the case in the present study since previous experiments carried out in our lab have shown that the antinociceptive activity of NCX701, using the single motor unit technique, is not due to the sole action of a NO-donor, but to the joint activity of PAR and NO (Romero-Sandoval et al., 2002).

Finally, the combined administration of medetomidine and NCX701 in the present experiments did not show an enhancement of the inhibition of the wind-up phenomenon induced by the administration of medetomidine. Wind-up is a centrally-mediated phenomenon, mainly dependent on the activity of NMDA and neurokinin receptors (see for review Herrero et al., 2000). Therefore, it can be suggested that the enhancement of the antinociception observed in our experimental conditions might be due either to a peripheral action of the compounds, or to an action independent of the mechanisms underlying the phenomenon of wind-up. The latter seems to be more probable, since medetomidine inhibited dose-dependently wind-up, when injected alone, and PAR crosses the blood brain barrier easily (Bannwarth et al., 1989).

In conclusion, the systemic administration of subeffective doses of NCX701 enhances the antinociceptive activity mediated by the i.v. injection of the α_2-adrenoceptor agonist medetomidine, reducing the ID_{50} and the dose needed to achieve a full inhibition of naturally-evoked nociceptive responses. In addition, a significant increment of the duration of the antinociception is observed, and the effect is not reversed by the administration of atipamezole. These results might open interesting new perspectives in the treatment of inflammation-induced pain.

Acknowledgments

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