ORIGINAL ARTICLE

Pharmacological mechanisms involved in the antinociceptive effects of dexmedetomidine in mice

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ABSTRACT

Dexmedetomidine (DEX) is a α_2-adrenoceptor (α_2-AR) agonist used as an anesthetic adjuvant and as sedative in critical care settings. Typically, α_2-AR agonists release nitric oxide (NO) and subsequently activate NO-GMPc pathway and have been implicated with antinociception. In this study, we investigate the pharmacological mechanisms involved in the antinociceptive effects of DEX, using an acetic acid-induced writhing assay in mice. Saline or DEX (1, 2, 5, or 10 μg/kg) was intravenously injected 5 min before ip administration of acetic acid and the resulting abdominal constrictions were then counted for 10 min. To investigate the possible mechanisms related to antinociceptive effect of DEX (10 μg/kg), the animals were also pretreated with one of the following drugs: 7-nitroindazole (7-NI; 30 mg/kg ip); 1H-[1,2,4] oxadiazole [4,3-a] quinoxaline-1-one (ODQ; 2.5 mg/kg, ip); yohimbine (YOH; 1 mg/kg, ip); atropine (ATRO; 2 mg/kg, ip); glibenclamide (GLIB; 1 mg/kg, i.p.) and naloxone (NAL; 0.2 mg/kg, ip). A rotarod and open-field performance test were performed with DEX at 10 μg/kg dose. DEX demonstrated its potent antinociceptive effect in a dose-dependent manner. The pretreatment with 7-NI, ODQ, GLIB, ATRO, and YOH significantly reduced the antinociceptive affects of DEX. However, NAL showed no effecting DEX-induced antinociception. The rotarod and open-field tests confirmed there is no detectable sedation or even significant motor impairment with DEX at 10 μg/kg dose. Our results suggest that the α_2-AR and NO-GMPc pathways play important roles in the systemic antinociceptive effect of DEX in a murine model of inflammatory pain. Furthermore, the antinociceptive effect exerted by DEX appears to be dependent on K_{ATP} channels, independent of opioid receptor activity.

INTRODUCTION

Dexmedetomidine (DEX) is a highly selective α_2-adrenoceptor (α_2-AR) agonist that has been largely used for anxiolysis, sedation, and analgesia in mechanically ventilated patients in critical care settings. One therapeutic advantage of DEX is a sedative effect with minimal ventilatory impairment. DEX also decreases surgical sympathoadrenal responses and reduces the intraoperative opioid analgesic requirement [1]. The antinociceptive actions of α_2-AR agonists have been demonstrated in experimental and clinical studies [2, 3]. It is well known that α_2-AR activation of both spinal and supraspinal nuclei is involved in the antinociceptive action of α_2-AR agonists. Activation of α_2-ARs reduces the release of pronociceptive neurotransmitters, such as substance P and glutamate, from primary afferent terminals [4] and hyperpolarizes

spinal interneurons *via* G-protein-mediated activation of K^+ channels [5].

However, the mechanisms related to the antinociceptive effects of α_2-AR agonists are not entirely understood. Most of the data concerning the antinociceptive properties of α_2-AR agonists have been obtained from studies using clonidine as the α_2-AR agonist prototype. In this context, clonidine induces the release of nitric oxide (NO) [6], an important modulator of neuronal activity that plays a significant role in the peripheral and central mechanisms of antinociception [7, 8]. Moreover, clonidine-dependent NO release has been described even for intracerebroventricularly injected α_2-AR agonists in mice submitted to the tail-flick model of acute pain [9]. The antinociceptive, sedative [10], and antihypertensive [11] actions of clonidine probably share common NO-GMPc pathways. These therapeutic effects are mediated at the brain stem, mainly at the *locus coeruleus*, a key adrenergic center that sends neural projections to the spinal cord as part of the descending adrenergic analgesic system [12, 13].

The involvement of the muscarinic system in antinociception at the spinal cord level is well recognized [12, 14–17]. In this context, it has already been demonstrated that spinal muscarinic receptors mediate the analgesic action of α_2-AR agonists intrathecally administered [18], suggesting a definitive link between noradrenergic and cholinergic pathways in spinal antinociception. Inhibitory neurons are responsible for these antinociceptive effects through the release of analgesic neurotransmitters, such as endogenous opioid peptides and adenosine. Pertovaara [19] has determined that norepinephrine exerts pain relief by acting on α_2-ARs (presynaptic and postsynaptic inhibition) and by α_1-AR activation of inhibitory interneurons. Moreover, the effects of α_2-ARs on the axon terminals of excitatory neurons in the spinal dorsal horn may be involved in the spinal control of pain [19].

Different analgesic drugs, including α_2-AR agonists [20, 21], elicit an independent and complementary antinociceptive mechanism through the opening of ATP-dependent K^+ channels (K_{ATP}). In addition to the opening of K_{ATP}, analgesic drugs also activate NO, GMPc, and protein kinases in GMPc-dependent pathways. The pharmacological opening of K_{ATP} alone is not sufficient for antinociception but may enhance the antinociception achieved by other agents, such as morphine and DEX [22].

Despite the recent widespread utilization of DEX in critical care and anesthesia practice, the involvement of the NO-GMP pathway in the antinociceptive effects of DEX is not completely understood. Thus, our objective was to investigate the underlying mechanism of the DEX-induced antinociceptive effect in a classical mouse model of inflammatory pain.

MATERIAL AND METHODS

Animals

Male mice (20–30 g) were housed in the animal care facility at 23 ± 1 °C and maintained on a 12-h light/dark cycle. The animals were allowed free access to food and water until 2 h before the experiments. The animal handling and experimental procedures were approved by the Ethical Committee for Use of Experimental Animals of the State University of Rio de Janeiro (license number 00356/05) and were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). The animals were acclimatized to the laboratory for at least 2 h before the testing. The animals were used only during the protocol and were killed immediately after the algesimeter test. The control animals were evaluated concurrently and interspersed with the drug-treated animals, which prevented having all the control animal be from one group evaluated at one time during the course of the investigation. The number of animals was kept at the minimum that was compatible with consistent drug treatments and the assessor was blinded to treatment allocation at the time of behavioral assessments.

Measurement of antinociceptive activity

The antinociceptive activity of DEX was assessed by the acetic acid-induced writhing test, a classical model of chemical/inflammatory pain largely used for testing analgesic drugs in the mouse [23]. The mice were injected, via intraperitoneal (ip), with an acetic acid solution (10 mL/kg, 0,8% v/v) and placed in a $40 \times 30 \times 25$ cm Plexiglas box that was maintained in a quiet illuminated room. The resulting abdominal constrictions (writhes) were then counted for 10 min, starting at 5 min after the administration of the acetic acid solution. One writhe was defined as a contraction of the abdominal musculature followed by an extension of the forelimbs and elongation of the animal's body. Antinociceptive activity was defined as the significant reduction in the number of writhes (NOW) compared with the control group. Saline or DEX (1, 2, 5, and 10 µg/kg) was intravenously injected in the dorsal vein

of the tail 5 min before the ip administration of the acetic acid solution. In subsequent experiments, the dose of DEX with best antinociceptive response (10 μg/kg) was used for rotarod and open-field performance tests and for the pharmacological investigation of the antinociceptive effect of DEX.

Rotarod performance test

The rotarod test is an established method for evaluating motor impairment and ataxia [24]. The day before the test, the animals were twice trained to maintain equilibrium for 5 min on a roller apparatus ('Rotarod for mice', Insight, Brazil). The speed selector was set to 10 rev/min. The animals that fell from the roller twice during the 5 min of testing were discarded. Twenty-four hours later, the mice were intravenously treated with DEX (10 μg/kg), diazepam (DZP – 1 mg/kg), or vehicle, and then placed again on the roller for 5 min. A neurological deficit was defined as an animal being unable to remain on the roller for the test period and was measured by the number of times that the animal fell off the roller.

Assessment of spontaneous activity – open-field test

The procedure was similar to the method described by Barros et al. [25]. The mice received DEX (10 μg/kg), DZP (1 mg/kg), or vehicle by intravenous injection and were immediately placed individually in an observation chamber with a floor divided into 50 squares (5 × 5 cm). The total number of squares on which a mouse walked during 5 min was counted at intervals of 20 min varying from 20 to 120 min after the drug administration. The following formula based on the trapezoid rule was used to calculate the AUC: AUC = 20 × [(min 20) + (min 40) + … + (min 120)/2].

Pharmacological mechanisms involved in the antinociceptive effect of dexmedetomidine

To investigate the possible mechanisms related to antinociceptive effect of DEX, the animals were also pretreated with one of the following drugs: 7-nitroindazole (7-NI; 30 mg/kg ip), a selective inhibitor of neuronal nitric oxide synthase; 1H-[1,2,4] oxadiazole [4,3-a] quinoxaline-1-one (ODQ; 2.5 mg/kg, ip), a highly selective, irreversible heme-site inhibitor of soluble guanylyl cyclase; yohimbine (YOH; 1 mg/kg, ip), a presynaptic α_2-AR blocking agent; atropine (ATRO; 2 mg/kg, ip), a competitive muscarinic acetylcholine receptor antagonist; glibenclamide (GLIB; 1

mg/kg, i.p.), an inhibitor of ATP-sensitive potassium channels; and naloxone (NAL; 0.2 mg/kg, ip), a competitive opioid receptor antagonist. In separate groups, we assessed the possible effects of the vehicles (saline and DMSO at 7.5, 27.8, or 5 plus 5% ethylic alcohol in saline, depending on the antagonist) when injected im 25 min before the DEX treatment.

Statistical analysis

The results are expressed as the mean ± SEM for each group of six animals, and comparisons between the different groups were made with one-way analysis of variance (ANOVA). If a significant difference was detected by ANOVA, the Bonferroni test was used to identify the statistically significant differences. Differences with a $P < 0.05$ were considered significant. All calculations were made by computer-assisted analyses with a commercially available statistical package (Graphpad Instat 5.0; GraphPad Software Inc., La Jolla, CA, USA).

Drugs

The ATRO, NAL, and YOH were diluted in saline. The 7-NI and ODQ were diluted in 7.5% DMSO in saline and 27.8% DMSO in saline, respectively. The glibenclamide(GLIB) was diluted in 5% ethylic alcohol and 5% DMSO saline solution. The DEX hydrochloride was purchased from Abbott Laboratories, Rio de Janeiro, Brazil. The 7-NI, NAL, YOH, and GLIB were purchased from Sigma, St. Louis, MO, USA. The ODQ was purchased from Calbiochem-Novabiochem, Canada. The ATRO and DZP were purchased from União Química Farmacêutica Nacional Rio de Janeiro, RJ, Brazil.

RESULTS

Antinociceptive effect of dexmedetomidine

When intravenously administered in the tail vein, DEX induced a significant and dose-dependent antinociceptive effect against acetic acid-induced inflammatory peritoneal pain; doses of 1, 2, 5, and 10 μg/kg of DEX produced antinociceptive effect characterized as a significant reduction in the NOW in the writhing test (34 ± 1, 34 ± 2, 22 ± 1, and 6 ± 1, respectively. $P < 0.05$ – *Figure 1*). The dose that elicited the greatest antinociceptive effect was 10 μg/kg, and this dose was used in the pharmacological protocol to study the possible mechanisms of the antinociceptive effect of DEX.
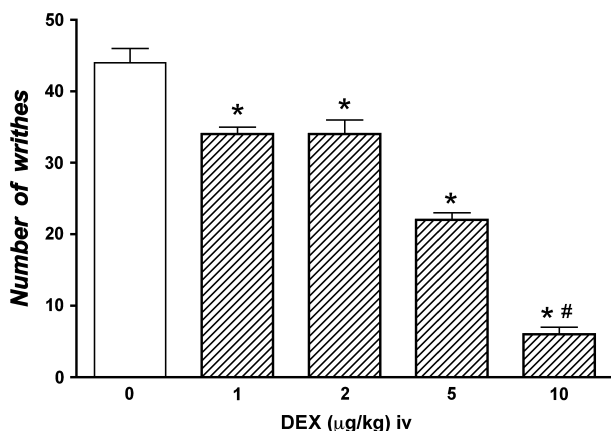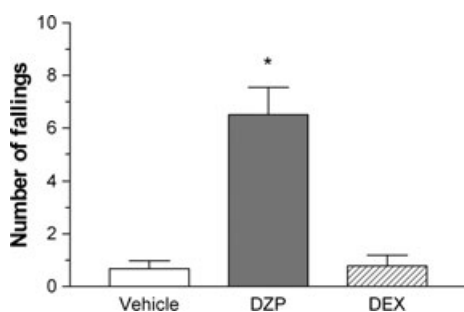
Figure 1 The antinociceptive effect of dexmedetomidine (DEX) assessed by the writhing test in mice. The results are presented as mean ± SEM; $n = 6$ animals for each group, one-way ANOVA followed Bonferroni test. *$P < 0.05$ vs. saline #$P < 0.05$ vs. DEX 0, 1, 2, and 5 µg/kg.

Rotarod performance test

In the Rotarod performance test, DEX (10 µg/kg, iv) presented a similar result when compared with the vehicle group (0.8 ± 0.4 and 0.7 ± 0.3, respectively). The positive control with DZP (1 mg/kg, iv) produced a significant increase in the number of falls compared with the vehicle and DEX groups (6.5 ± 1.1, $P < 0.05$ – Figure 2).

Assessment of spontaneous activity – open-field test

In the open-field test, the curves representing DEX (10 µg/kg, iv) and vehicle groups overlapped, while the positive control with DZP (1 mg/kg, iv)
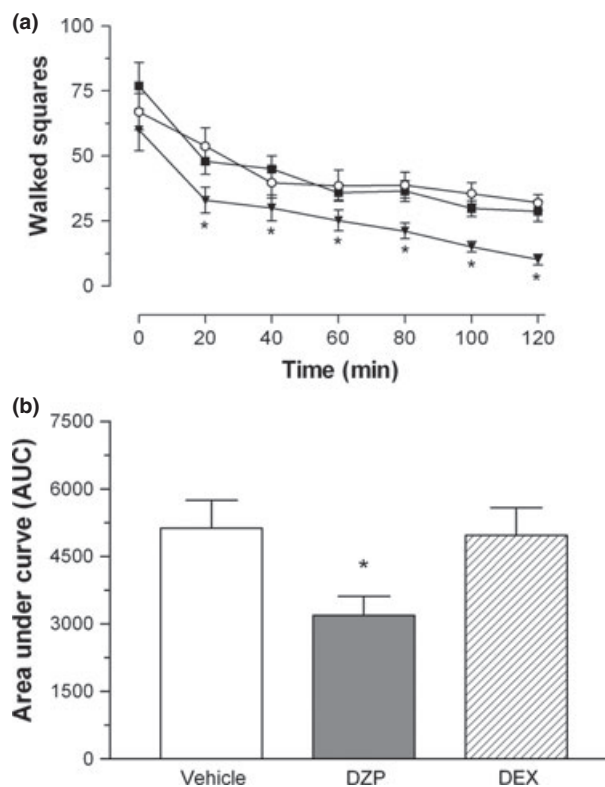

Figure 2 The effect of dexmedetomidine (DEX – 10 µg/kg), diazepam (DZP – 1 mg/kg), and the vehicle on the mice in the Rotarod test. The results are presented as mean ± SEM; $n = 6$ animals for each group, one-way ANOVA followed Bonferroni test. *$P < 0.05$ vs. vehicle and DEX.

significantly reduced the number of walked squares throughout the experiment compared with the vehicle group (*Figure 3a*).

Figure 3b shows that the area under the curve (AUC) of the DEX graph was similar to that of the vehicle (5125 ± 621 and 4964 ± 622 squares in 120 min, respectively), while the positive control with DZP significantly reduced the AUC of the number of walked squares (3193 ± 425, $P < 0.05$) throughout the experiment compared with the vehicle group.

Role of NO synthase and guanylyl cyclase on the antinociceptive effect of dexmedetomidine

Inhibiting NO synthase by 7-NI did not change the writhing tests when compared with the vehicle (36 ± 1 and 36 ± 2, respectively – *Figure 4a*). However, the 7-NI pretreatment significantly reduced the

Figure 3 The effect of dexmedetomidine (DEX), diazepam (DZP), and the vehicle on the mice in the open-field test. The mice received DEX (■, 10 µg/kg), DZP (▼, 1 mg/kg), or the vehicle (○). In (a), the graph represents the number of walked squares against time. In (b), the graph represents the area under the curve (AUC) calculated for each time-effect curve. The results are presented as mean ± SEM; $n = 6$ animals for each group, one-way ANOVA followed Bonferroni test. *$P < 0.05$ vs. vehicle.
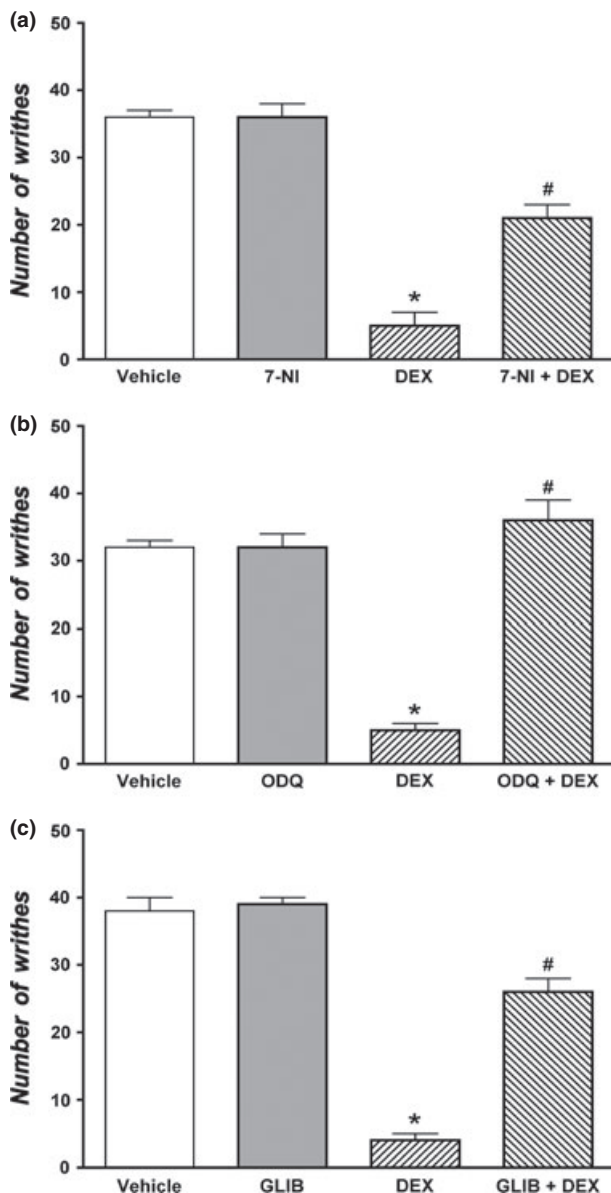
Figure 4 The effect of pretreatment with 7-NI (a), ODQ (b), and glibenclamide (c) on dexmedetomidine (DEX)-induced antinociception in mice, as assessed by the writhing test. The results are presented as mean ± SEM; *n* = 6 animals for each group, one-way ANOVA followed Bonferroni test. *P < 0.05 vs. vehicle #P < 0.05 vs. DEX.

antinociceptive effect of DEX; the NOW increased from 5 ± 2 in the DEX group to 21 ± 2 in the 7-NI + DEX group (P < 0.05 – *Figure 4a*). Soluble guanylyl cyclase was inhibited by administering 2.5 mg/kg of ODQ, which did not change the writhing test when compared with the vehicle (32 ± 1 and 32 ± 2, respectively – *Figure 4b*). By contrast, the ODQ pretreatment

significantly reduced the antinociceptive effect of DEX; the NOW increased from 5 ± 1 in the DEX group to 36 ± 3 in the ODQ + DEX group (P < 0.05 – *Figure 4b*).

Effects of glibenclamide on the dexmedetomidine-induced antinociception

The K_{ATP} channel blockade by GLIB did not change the results of the writhing test when compared with the vehicle (39 ± 1 and 38 ± 2, respectively – *Figure 4c*). However, the GLIB pretreatment significantly reduced the antinociceptive effect of DEX; the NOW increased from 4 ± 1 in the DEX group to 26 ± 2 in the GLIB + DEX group (*Figure 4c*).

The effects of atropine on dexmedetomidine-induced antinociception

When administered alone, ATRO did not exhibit any antinociceptive effects in the writhing test when compared with the vehicle (39 ± 1 and 41 ± 3, respectively – *Figure 5a*). Conversely, the ATRO pretreatment significantly reduced the DEX-induced antinociception increasing the NOW from 5 ± 1 in the DEX group to 30 ± 2 in the ATRO + DEX group (P < 0.05 – *Figure 5a*).

## Effects of yohimbine on the dexmedetomidine-induced antinociception

YOH did not exhibit any antinociceptive effects in the writhing test (*Figure 5b*). However, the YOH pretreatment significantly reduced the DEX-induced antinociception; the NOW increased from 6 ± 2 in the DEX group to 39 ± 2 in the YOH + DEX group (P < 0.05 – *Figure 5b*).

Effects of naloxone on the dexmedetomidine-induced antinociception

The opioid receptor antagonism did not exhibit any antinociceptive effects in the writhing test. NAL group presented the same NOW when compared with the saline group (43 ± 3 and 43 ± 2, respectively) and the pretreatment with NAL (NAL + DEX) group did not reduce the antinociceptive effect of DEX (4 ± 1 and 5 ± 1, respectively – *Figure 5c*).

DISCUSSION

Our results indicate that the systemic administration of DEX dose dependently reduces the pain-induced behavior triggered by injecting intraperitoneal acetic acid into mice. We also demonstrated that the antinociceptive
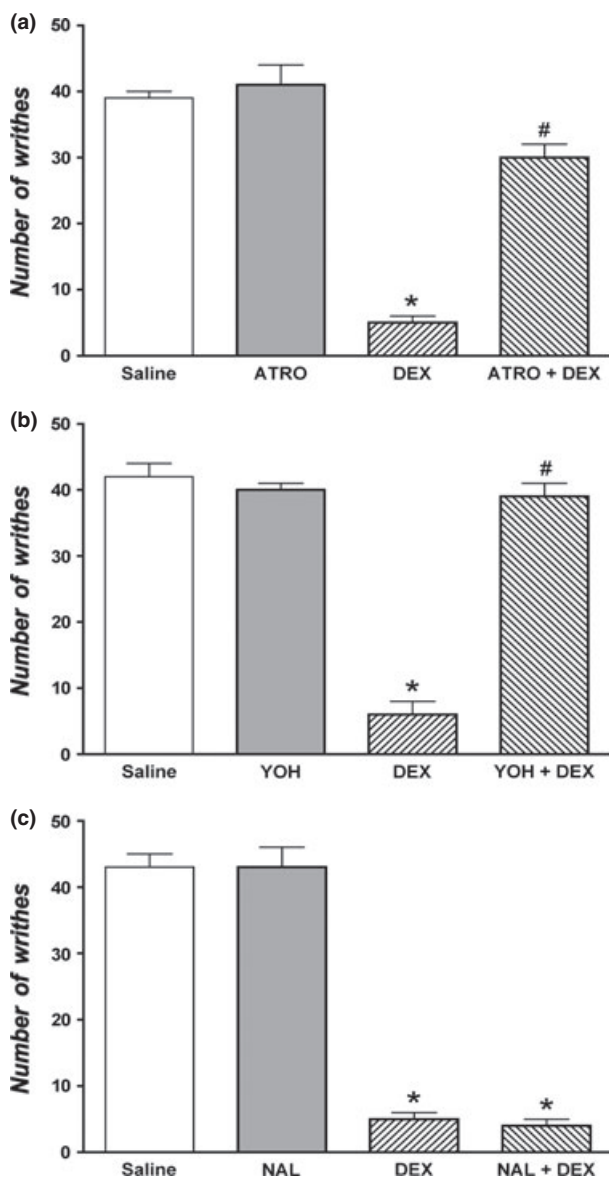
Figure 5 The effect of pretreatment with atropine (a), yohimbine (b), and naloxone (c) on dexmedetomidine (DEX)-induced antinociception in mice, as assessed by the writhing test. The results are presented as mean ± SEM; $n = 6$ animals for each group, one-way ANOVA followed Bonferroni test. *$P < 0.05$ vs. vehicle #$P < 0.05$ vs. DEX.

effects of DEX are related to the NO activity, the opening of K_{ATP} channels, and muscarinic activation. By contrast, pretreatment with NAL did not change the antinociceptive action of DEX, suggesting that opioid receptors are not involved in DEX-induced antinociception.

The antinociceptive effect of DEX has been studied over the last 15 years, and the results consistently

demonstrate that DEX induces significant antinociception in different models, such as those for cutaneous [26–28] and visceral [29, 30] pain. In this context, DEX is able to reduce spontaneous pain-related behaviors (muscle contractions, abdominal licking, and stretching) in visceral pain, such as capsaicin-induced intracolonic pain [29], and in acetic acid-induced intraperitoneal pain [30]. Consistent with these previous studies, our results indicate that systemic treatment with DEX effectively reduces the number of writhes in mice.

When studying α_2-AR agonists, it is important to determine whether the reduction in the NOW after iv DEX treatment is an antinociceptive or a sedative effect. Sedation is the main side effect of most α_2-AR agonists, and DEX was developed for its sedative effects. In our study, however, the intravenous administration of 10 µg/kg of DEX induced significant antinociceptive effects without any change in motor coordination, as evaluated by the Rotarod test, and spontaneous motor activity, as assessed by the open-field test. Moreover, pharmacological studies in rats using different routes of administration, such as intrathecal [31], intraperitoneal [28], or iv [32], strongly suggest that the dose of DEX used in our study has a significant antinociceptive effect without producing sedation or motor impairment.

In our study, the involvement of the NO in DEX-induced antinociception was demonstrated by the inhibitory effect of 7-NI, an inhibitor of neuronal NO synthase, and further strengthened by ODQ, significantly reducing the antinociceptive effect of DEX. Although the NO-cGMP pathway is an effector system that is classically coupled to the α_2-AR, the role of cGMP in the action of α_2-AR agonists is not completely understood. The decrease of cGMP elicited by α_2-AR agonists in diverse cerebral areas is related to many therapeutic effects, such as the sympatholytic, sedative-hypnotic, anxiolytic, analgesic, and anesthetic-sparing actions of these drugs [33].

K_{ATP} channels are widely distributed in the central and peripheral nervous system [34] and are involved in many different neuronal activities, such as neuroprotection, control of neurotransmitter release, and regulation of membrane excitability [34–36]. Interestingly, opening of K_{ATP} channels and the consequent cellular hyperpolarization are involved in the antinociceptive effects of drugs with dissimilar mechanisms of action, such as ketorolac [20], baclofen, and α_2-AR agonists [21]. In our study, GLIB significantly

attenuated the antinociceptive effect of DEX, confirming the important role of antinociceptive K_{ATP} channels in the analgesic effects of α_2-AR agonists. In our experimental protocol, we reduced the K_{ATP} current by the systemic administration of GLIB. It is not possible to identify the exact location of the K_{ATP} channel blockade, but there are some candidates at the spinal, brain, and even peripheral levels [37]. The loss of K_{ATP} current contributes to neuropathic pain, possibly through varied mechanisms that include increased membrane excitability, amplification of excitatory neurotransmitter release, regulation of neuronal excitability [34–36, 38], and (possibly) increased susceptibility to cell death.

A variety of drugs that modulate medullar acetylcholine (Ach) concentration and metabolism interact with the antinociceptive properties of α_2-AR agonists. Several studies have clearly demonstrated that cholinergic pathways are implicated in antinociception at the spinal level [15, 16, 39, 40]. In our study, we also demonstrated that systemic ATRO attenuates the antinociceptive effect of DEX. It is noteworthy that ATRO-induced cholinergic receptor blockade is a central effect that involves medullar and supra-medullar centers related to antinociception [41]. Moreover, cholinergic pain modulation may also involve first-order neurons (sensory neurons responsible for delivering sensory information to the central nervous system) via muscarinic acetylcholine M2 receptors. In this context, Dussor et al. [42] have demonstrated that activating peripheral M2 receptors produces antinociception *in vivo* and inhibits antinociceptive activity *in vitro*. In cultured rat dorsal root ganglia cells, DEX also inhibits muscarinic type 3 receptors and muscarine-induced intracellular Ca^{2+} elevation [43].

The effect of DEX on nonadrenergic imidazoline-preferring binding sites is another possibility to consider. It is well known that DEX has an imidazoline-like structure and some degree of imidazoline affinity [12]. In our study, we used YOH to ensure that the blockade of the antinociceptive effect of DEX was not related to activation of the imidazoline-preferring binding sites that exist in the brain and peripheral tissue. We successfully abolished the antinociceptive effect of DEX using YOH, a classical α_2-AR antagonist without the imidazoline ring. Similar results in various previous studies have demonstrated that α_2-AR antagonist block the antinociceptive effects of clonidine [8] and DEX [44]. It is interesting to note that YOH pretreatment is able to block the effects of DEX administered by the

intravenous [32], intrathecal, or intraperitoneal [44] routes.

The spinal anatomic distribution of all three α_2-AR subtypes (α_{2A}, α_{2B}, and α_{2C}) had been described using *in situ* hybridization and links the anatomic adrenergic areas along with cervical, thoracic, and lumbar spinal to the antinociception process [45]. All three α_2-AR subtypes act as presynaptic inhibitory feedback receptors to control the release of catecholamines from neurons. Alpha$_{2A}$-AR subtype activation is required for the hypnotic, analgesic, seizure-modulating, and central anti-hyperalgesia effects of α_2-AR agonists [29, 46]. Alpha$_{2B}$-AR subtype is an essential component of descending noradrenergic neurons in the spinal cord and mediates the analgesic, but not the sedative effect of nitrous oxide [47]. Alpha$_{2C}$-AR activation in the CNS is related to memory and behavioral functions that significantly interfere with the antinociception process [48, 49].

To verify the possible role of opioid receptors in the antinociceptive effects of DEX in our model of inflammatory pain, the rats were pretreated with NAL, a classical nonselective opioid receptor antagonist. NAL did not interfere with the antinociceptive effect of DEX, suggesting that opioid receptors are not involved in this effect, at least under our experimental conditions. The absence of interactions between DEX and the opioid system has also been demonstrated in previous studies performed in dogs [50] and neonatal rats [51]. Moreover, there is some pharmacological evidence showing that NAL has no effect on the antinociceptive properties of clonidine either [8]. By contrast, others' studies have found evidence that the opioid system may contribute to DEX-induced antinociception [52, 53]. In this context, Ulger et al. [32] have recently demonstrated that the antinociceptive effects of IV DEX were significantly reduced by NAL. In fact, intravenous opioid administration induces the spinal release of norepinephrine and acetylcholine, thus activating the descending spinal noradrenergic and cholinergic pathways involved in analgesia [54]. Moreover, opioid receptors and the α_2-AR belong to the superfamily of G-protein-coupled receptors and share certain pathways involved in antinociception, such as adenylyl cyclase inhibition, rectifying K^+ channel opening, and decreased conductance in voltage-gated Ca^{2+} channels. However, our results suggest that the activation of opioid receptors is not involved in the antinociceptive effect of systemic DEX in our experimental model of acetic acid-induced inflammatory pain. The controversy

involving the opioid system and the antinociceptive effect of DEX may be related to the use of completely different experimental models of nociception in different animal species.

It is important to remember that the cellular effects of DEX are not limited to its interactions with α_2-AR. Recent evidence has suggested that DEX-induced changes in neuronal activity may involve ion channels, principally those of K^+ [55, 56], Ca^{2+} [57], and Na^+ [55]. DEX causes phosphorylation of ERK_1 and ERK_2 [58, 59] in cultured mouse astrocytes and increases the expression of brain-derived neurotrophic factor and epidermal growth factor [58], both of which are involved in neuroprotection. Moreover, DEX suppresses the expression of different markers of inflammation such as cyclo-oxygenase-2, TNF-α, IL-1β, IL-6, inducible nitric oxide synthase, and interferon gamma [60]. DEX also has preconditioning and postconditioning effects against ischemic injury in neuronal cells *in vitro* [61].

In conclusion, the results of our study showed that the α_2-AR and NO pathways play important roles in the systemic antinociceptive effect of DEX in mice. Furthermore, we believe that the antinociceptive effect exerted by DEX is dependent on K_{ATP} channels and independent of opioid receptor activity, at least in our experimental model of pain-induced behavior triggered by intraperitoneal acetic acid injection in mice.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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