Xylazine Induces Peripheral Antinociception by $\alpha_1$, $\beta$-Adrenoceptors and $\mu$, $\delta$-Opioid Receptors Activation

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Abstract

Objective: Using a non-inflammatory model of hyperalgesia, this study investigated the participation of $\alpha_1$ and $\beta$ adrenoceptors and opioid receptor subtypes in xylazine-induced peripheral antinociception in this event.

Materials & Methods: Nociceptive threshold was measured using the rat pressure test in animals that were injected with prosta glandin $E_2$, xylazine, prazosin, propranolol, clocinamox, naltrindole or nor-binaltorphimine. Male Wistar rats were used in which hyperalgesia induced by intraplantar injection of prosta glandin $E_2$ (2 µg). Results: Xylazine was administered locally (100 µg) into the right hind paw of animals, alone and after the $\alpha_1$-adrenoceptor antagonist prazosin (0.5, 1 and 2 µg/paw), the $\beta$-adrenoceptor antagonist propranolol (150, 300 and 600 µg/paw), the $\mu$-opioid antagonist clocinamox (10, 20, and 40 µg/paw), the $\delta$-opioid antagonist naltrindole (15, 30 and 60 µg/paw) or the $\kappa$-opioid antagonist nor-binaltorphimine (100 µg/paw). Nociceptive threshold was measured using the rat pressure test. Intraplantar injection of xylazine induced peripheral antinociception against hyperalgesia induced by PGE$_2$. This effect was prevented, in a dose dependent manner, by intraplantar injection of prazosin, propranolol, clocinamox and naltrindole. However, injection of nor-binaltorphimine did not alter xylazine antinociception.

Conclusion: The present data provide evidence that xylazine produces peripheral antinociception by activation of $\alpha_1$ and $\beta$-adrenoceptor as well as involving $\mu$ and $\delta$ but not $\kappa$ opioid receptors, either directly or indirectly.

Keywords: Xylazine; Adrenoceptor; Opioid receptor; Peripheral antinociception

Introduction

Xylazine is an $\alpha_2C$ receptor agonist that produces supraspinal, spinal and peripheral antinociception [1-3]. It has wide practical use in veterinary medicine, in experimental animal testing and in studies of adrenoceptors in pain [4]. Xylazine induces sedation, antinociception and muscle relaxation without inducing general anesthesia[4].

Interaction between adrenergic receptors and endogenous opioid systems (receptors, peptides) have been frequently reported in scientific literature [2,5]. The antinociceptive effect of $\alpha_2C$ adrenoceptor agonists has been related to opioid receptor interaction and can be blocked by opioid antagonists [2,5]. In the same way, the antinociceptive effect of adrenoceptor agonists via $\alpha_2$- and $\beta$-adrenoceptors has been also associated with the opioid systems. Binder et al. [5] suggested that noradrenaline (NA) activating $\alpha_2$-, $\alpha_2$- and $\beta$-adrenoceptors on immune cells leads to the release of the endogenous opioid $\beta$-endorphin, that subsequently produces analgesia during inflammation, but not in absence of inflammation. However, our group showed that in the absence of an inflammatory process, xylazine induced peripheral antinociceptive effects directly by activation of $\alpha_2$-,adrenoceptor or indirectly by activation of opioid receptors in nociceptors. Nonetheless, it is not clear if xylazine can interact with other adrenoceptors to induce peripheral antinociception nor which opioid receptor participate in this peripheral antinociceptive effect. The goal of the present study to investigate the possible participation of $\alpha_1$ and $\beta$ adrenoceptors and opioid systems in xylazine-induced peripheral antinociception.

Methods

Animals

155 male Wistar rats 160-200 g (from CEBIO-UFMG) were used. The rats were housed in a temperature-controlled room (23 ± 1°C) on an automatic 12h light/dark cycle (06:00-18:00 h). All tests were conducted during the light phase (08:00-15:00 h). Food and water were freely available until the onset of the experiments.

The Ethics Committee on Animal Experimentation (CETEA) of Federal University of Minas Gerais (UFMG) previously approved the animal procedures and protocols and the Guide for the Care and Use of Laboratory Animals (published by the National Academy of Science, National Academy Press, Washington, D.C.) was also observed.

Hyperalgesia measurement

To induce hyperalgesia prostaglandin $E_2$ (2 µg) was injected subcutaneously into the plantar surface of the hind paw. Nociceptive threshold was measured according to the paw pressure test described by Randall and Sellito [6].

Drug administration

PGE$_2$ (Cayman, USA) injected volume was 100 µl/paw, while prazosin (Sigma, USA), propranolol (Sigma), clocinamox (Tocris, USA), naltrindole (Tocris), nor- binaltorphimine (Tocris), injected volume were 50 µl/paw. All drugs were dissolved in isotonic saline, with the exception of PGE$_2$, that was dissolved in 2% ethanol in saline.

Experimental protocol

PGE$_2$ was injected subcutaneously in the right hind paw 2 h and 55 min prior to xylazine injection. Prazosin, propranolol, clocinamox, naltrindole or nor- binaltorphimine were administered 30 min prior to xylazine injection. All the threshold measurements were done before the any injection to verify the basal threshold and more once after all treatments in the 3 h after PGE$_2$ injection (linking the peak of action of all drugs studied). The A of threshold was calculated by difference between the basal measurement and the after treatment measurement.

Statistical analysis

One-way analysis of variance (ANOVA) was used to statistical analyzes and the post-hoc Bonferroni test for multiple comparisons. Probabilities of less than 5 % (P<0.05) were considered to be statistically significant.

Results

Xylazine produces antinociception by $\alpha_1$ and $\beta$-adrenoceptors

The 100 µg dose of xylazine was chosen based in previous study of our group showing that it was able to induce peripheral antinociception against PGE2-induced hyperalgesia with only local effect, once the
contralateral injection of this dose of xylazine (in left paw) not induced antinociception effect in the tested paw (right paw) [2].

Participation of the α1-adrenoceptor in the peripheral antinociceptive effect induced by xylazine was verified using the selective α1-adrenoceptor antagonist prazosin (0.5, 1 and 2 μg/paw; [F(1,28)=599.2; p<0.0001]), which was shown to antagonized the peripheral effect of xylazine (100 μg/paw) in a dose-dependent manner and without inducing hyperalgesia or antinociception when administered alone (Figure 1A). In the same way, the β-adrenoceptor antagonist propranolol (150, 300 and 600 ng/paw; [F(1,28)=779.9; p<0.0001]) blocked the peripheral antinociceptive effect of xylazine (100 μg/paw) against PGE2, dose-dependent manner and no effect was observed when it was injected alone into normal or hyperalgesic paws (Figure 1B).

**Xylazine antinociception was reversed by μ and δ opioid receptors antagonists**

The μ opioid receptor antagonist, clocinnamox (10, 20 and 40 μg/paw; [F(2,12)=120.1; p<0.0001]) and the δ opioid receptor antagonist naltrindole (15, 30 and 60 μg/paw; [F(2,12)=208.6; p<0.0001]) were able to reverse, in a dose-depend manner, the peripheral xylazine-induced antinociception (100 μg/paw) (Figure 2A and 2B, respectively). In contrast, administration of κ opioid receptor antagonist, nor-binaltorphimine (Nor Bni; 100 μg/paw; [F(1,12)=190.1; p>0.05], were ineffective to antagonize xylazine antinociception (Figure 2C).

#### Figure 1:

![Image of Figure 1](image)

Prazosin (PRA; μg/paw) and Propranolol (PROP; ng/paw) were administered 30 min prior to xylazine (XYL; μg/paw). Each column represents the mean ± S.E.M. (n=5). # and * indicate a significant differences compared to (PGE2 + veh 1 + veh 1) and (PGE2 + Xyl 100 + veh 1)-injected groups, respectively (P<0.05, ANOVA + Bonferroni test). Veh 1=Saline, Veh 2=Ethanol 2% in saline.

**Figure 1:** Prazosin (A), propranolol (B) effect in the peripheral antinociception produced by xylazine in hyperalgesic paws (PGE2, 2 μg).

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Clocinnamox (CLOC; μg/paw), naltrindole (NDT; μg/paw) and nor-binaltorphimine (NOR BNI; μg/paw) were administered 30 min prior to xylazine (XYL; μg/paw). Each column represents the mean ± S.E.M. (n=5). # and * indicate a significant differences compared to (PGE2 + veh 1 + veh 1) and (PGE2 + Xyl 100 + veh 1)-injected groups, respectively (P<0.05, ANOVA + Bonferroni test). Veh 1=Saline, Veh 2=Ethanol 2% in saline.

**Figure 2:** Clocinnamox (A), naltrindole (B) and nor-binaltorphimine (C) effect in the peripheral antinociception produced by xylazine in hyperalgesic paws (PGE2, 2 μg).

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**Discussion**

Xylazine is a α2 adrenoceptor agonist largely used for anesthesia and analgesia procedures in veterinary medicine because of its antinociceptive effect. Our group previously demonstrated the efficacy of xylazine at peripheral antinociception in the rat paw PGE2-induced hyperalgesia [2]. We also demonstrated that the specific α2 adrenoceptor subtype involved in the antinociceptive effect of xylazine is the α2C receptor [2]. However, participation of α1 and β adrenoceptors in peripheral antinociception induced by xylazine had never been examined.

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Interestingly, in the present study, we observed that xylazine was also able to induce antinociception via $\alpha_1$ and $\beta$ adrenoceptors, indicating a non-selective interaction of xylazine with several adrenoceptors at peripheral sites.

Both $\alpha_1$ and $\beta$ adrenoceptors have been associated to peripheral hyperalgesia by excitatory transduction [7]. Moreover, studies involving $\alpha_2$ and $\beta$ adrenergic receptors with antinociceptive events are also observed [3, 5]. Using cell cultures from dorsal root ganglion (DRG) of rats, Pluteanu [8] verified that the administration of adrenaline or isoprenaline was capable of inducing, by persistent depolarization, a neuronal hyperpolarization. These data have led researchers to conclude that activation of $\alpha_1$ and $\beta$ adrenoceptors induce hyperpolarization in the DRG with a resultant decrease in neuronal excitability.

Interactions between adrenergic and opioid systems producing antinociception has been reported in peripheral nervous system [5]. Previously, our group also showed a role of the opioid system in the antinociception induced by xylazine, because naloxone, a non-selective opioid antagonist, reversed the xylazine effect [2]. It was also demonstrated the participation of $\mu$-opioid receptor in the mechanism of central antinociception induced by xylazine [3]. In the present study, we investigated which opioid receptor was involved in peripheral antinociception induced by xylazine, using selective opioid antagonists. Both $\mu$ and $\delta$ opioid receptors were involved in the antinociceptive effect of xylazine, but there was no involvement of $\kappa$ opioid receptor in this event.

Binder et al. [5] suggested that the peripheral antinociceptive effect of NA could be dependent on opioid release from the immune system because neither noradrenaline nor isoproterenol produced any effect in noninflamed paws. However, in the absence of inflammation xylazine induced antinociception centrally [3] and peripherally [2] interacting with the opioid system. It indicates that, in this event, immune system is not the fundamental or unique factor responsible for the peripheral antinociception induced by adrenergic agonists. Perhaps, opioid peptides are released also by resident cells. This hypothesis is possible because resident cells such as keratinocytes, express adrenergic receptors [9] and are able to synthesize and release $\beta$-endorphin when stimulated by drugs that increase calcium concentration [10] like $\alpha_1$ or $\beta$ adrenergic agonists. However, the present results cannot discard the direct interaction between xylazine and opioids receptors subtypes. Therefore, this study are consistent with both mechanism, and that without further data, like binding assay or antibody to $\beta$-endorphin use, we cannot distinguish which mechanism is involved.

Considering the present data, xylazine is not a selective $\alpha_1$ agonist, because it was able to induce peripheral antinociception by $\alpha_1$ and $\beta$ adrenoceptors. Interaction with opioids receptor subtypes $\mu$ and $\delta$ seems to be one of the mechanisms by which xylazine produce its effect. This interaction could be by an increase of endogenous opioid release, such as $\beta$-endorphin, nonetheless future experiments are needed to clarify this interaction.

**Author Contributions**

**Thiago Roberto Lima Romero**: Substantial contributions to conception and design, acquisition of data, analysis and interpretation of data and at study writing.

**Marina Gomes Miranda e Castor**: Substantial contributions to acquisition of data, analysis and interpretation of data.

**Andrea Castro Perez**: Substantial contributions to acquisition of data and interpretation of data.

**Igor Dimitri Gama Duarte**: Substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; revising it critically for important intellectual content and final approval of the version to be published.

**Competing Interests**

The authors declare that they have no competing interests.

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