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Short Communication

Interaction between the serotonergic and noradrenergic systems during peripheral antinociception

Danielle Diniz Aguiar, Cristina da Costa Oliveira, Júlia Alvarenga Petrocchi, Andrea Castro Perez, Igor Dimitri Gama Duarte, Marina Gomes Miranda e Castor* and Thiago Roberto Lima Romero
Department of Pharmacology, Institute of Biological Sciences, UFMG, Av. Antônio Carlos, 6627, 31.270-100, Belo Horizonte, Brazil.

ABSTRACT

Herein, we evaluated the involvement of the cannabinoid system in serotonin-mediated peripheral antinociception. After administration of intraplantar prostaglandin E_2 (PGE_2), animals demonstrating increased pain sensitivity were subjected to a paw pressure test. Serotonin induced a peripheral antinociceptive effect (250 ng); this effect was reversed by the α_1-adrenoceptor antagonist prazosin, α_2-adrenoceptor antagonist yohimbine, and the β-adrenoceptor antagonist propranolol. Reboxetin amplified the antinociceptive effects of low-dose serotonin. Our data suggest the existence of interactions between the serotonergic and noradrenergic systems during peripheral antinociception.

KEYWORDS: peripheral antinociception, serotonin, noradrenaline, analgesic interaction.

1. INTRODUCTION

The role of serotonin (5-hydroxytryptamine or 5-HT) and noradrenaline (NA) in nociception may vary depending on the subtype of activated receptors. The role of serotonin (5-hydroxytryptamine or 5-HT) and noradrenaline (NA) in nociception may vary depending on the subtype of activated receptors. Both 5-HT and NA are endogenously released at sites that express serotonergic and adrenergic receptors, such as the raphe magnus nucleus, locus coeruleus [1-3] and immune cells [1, 4].

When injected into the periphery, 5-HT and NA can induce pain by activating 5-HT_1, 5-HT_2, and 5-HT_3 receptors and α_1 and β-receptors, respectively [5-7]. Since 1969, accumulated evidence indicates that 5-HT release in the dorsal horn of the spinal cord, *via* stimulation of the periaqueductal gray matter, can activate inhibitory interneurons, resulting in the inhibition of spinal neurons [8]. At these sites, 5-HT_2 and 5-HT_3 receptors are activated by 5-HT in opioidergic inhibitory interneurons that activate opioid-dependent mechanisms [8].

Recently, our research group has verified that both 5-HT, *via* 5-HT_{1B}, 5-HT_{2A}, and 5-HT_3 receptors [9], and NA, through α_1, α_2, and β adrenergic receptors [10], could induce antinociception against prostaglandin E_2 (PGE_2)-mediated hyperalgesia when administered *via* intraplantar injection at a low dosage.

Although the modulation of peripheral pain by serotonergic and noradrenergic analgesic systems is a well-established phenomenon, little is known regarding interactions between these systems during this phenomenon at the peripheral level. Thus, in the present study, we aimed to evaluate whether serotonin-induced peripheral antinociception involves the participation of the adrenergic system.

*Corresponding author: marinacastor@ufmg.br

2. MATERIALS AND METHODS

2.1. Animals

Male Swiss mice (30-40 g) from the Bioterism Center of Federal University of Minas Gerais (CEBIO-ICB/UFMG) were used in the experiments. They were housed in standard cages and kept at a constant temperature of 23 to 25 °C with a 12 h light-dark cycle and free access to food and water. After the experimental procedures the animals were euthanized with intraperitoneal injection of 10% ketamine (Cayman Chemical) and 2% xylazine hydrochloride (Sigma Aldrich), at concentrations 3 times higher than the anesthetic concentration (180 mg/kg and 24 mg/kg of body weight respectively). Following the guidelines of the National Research Council's Guide for the Care and Use of Laboratory Animals, this project was submitted to the Ethics Committee on Animal Experiments (CETEA) of the Federal University of Minas Gerais (UFMG) and was approved with the protocol nº 50/2013.

2.2. Measure of hyperalgesia

Hyperalgesia was measured according to the protocol initially proposed by Randal & Selitto [11] and later adapted for mice by Kawabata *et al.* [12]. The nociceptive threshold (Δ) was calculated as the difference between the nociceptive threshold obtained in the beginning of the experiment (basal value) before any injection (time zero) and the response of the animal.

2.3. Experimental protocol

Serotonin was administered in the right hind paw of animals 10 minutes before the third hour of local injection of PGE_2 (peak of hyperalgesia by PGE_2). The dose and time of serotonin injection chosen were previously described by Diniz *et al.* [9]. Non-selective α_1-adrenergic receptor antagonist prazosin, non-selective α_2-adrenergic receptor antagonist yohimbine, non-selective β-adrenergic receptor antagonist propranolol and reboxetine were injected 30 minutes prior to serotonin administration. The doses and duration of action of noradrenergic antagonists chosen were previously described by Romero *et al.* [10]. All the drugs were injected in a volume of 20 µl/paw. The nociceptive threshold was always measured at the right hind paw.

2.4. Statistical analysis

The results were expressed as the mean ± S.E.M. Statistical differences between groups were calculated by one-way analysis of variance (ANOVA) followed by the Bonferroni test. Statistical significance was set at $P < 0.05$.

3. RESULTS

As shown in Figures 1A, 1B, and 1C, prazosin (0.5, 1, and 2 µg/paw), a non-selective α_1-adrenergic receptor antagonist, yohimbine (5, 10, and 20 µg/paw), a non-selective α_2-adrenergic receptor antagonist, and propranolol (150, 300, and 600 ng/paw), a non-selective β-adrenergic receptor antagonist, dose-dependently reversed serotonin-induced peripheral antinociception (250 ng/paw). None of the examined antagonists induced hyperalgesia or antinociception when injected alone or along with PGE_2.

Reboxetine (30 µg/paw), a selective NA reuptake inhibitor, potentiated peripheral antinociception induced by the lowest serotonin dose tested (125 ng/paw) (Figure 2). It should be noted that when injected alone or along with PGE_2, reboxetine did not induce any effect at a dose of 30 µg/paw.

4. DISCUSSION

In the present study, we examined the potential interactions between the serotonergic and noradrenergic systems in the event of antinociception during peripheral nociceptive pain. To induce nociception, we administered PGE_2 *via* an intraplantar injection. Prostaglandins can directly sensitize nociceptors and produce hyperalgesia *via* specific receptors in primary afferent terminals [13].

Classically, both 5-HT and NA are known for their nociceptive effects when administered peripherally [7]. Peripheral NA minimally impacts pain in healthy tissues; however, it exhibits variable effects in injured tissues, including worsening pain. In addition, the peripheral pro-nociceptive effect of NA has been associated with increased noradrenergic receptor expression, sprouting of sympathetic nerve fibers, and pro-nociceptive changes in ionic channel properties in primary afferent nociceptors [7]. Furthermore,

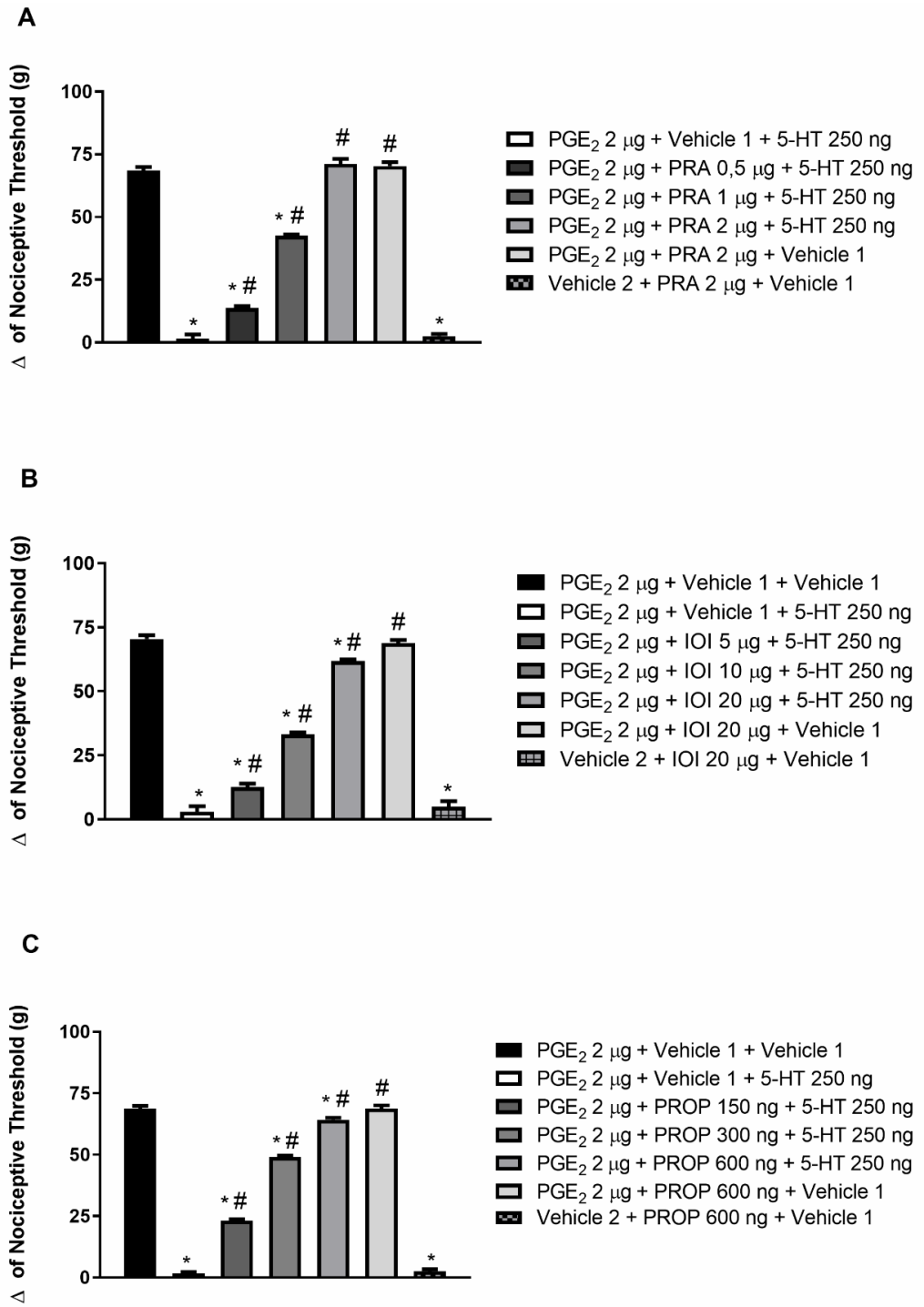

Figure 1. (A) Antagonism by prazosin (PRA) of the peripheral PGE_2-induced hyperalgesia. PRA (μg) was administered 30 minutes prior to serotonin administration (250 ng/paw). (B) Antagonism by yohimbine (IOI) of the peripheral PGE_2-induced hyperalgesia. IOI (μg) was administered 30 minutes prior to serotonin administration (250 ng/paw). (C) Antagonism by propranolol (PROP) of the peripheral PGE_2-induced hyperalgesia. PROP (μg) was administered 30 minutes prior to serotonin administration (250 ng/paw). Each column represents the average S.E.M. (n = 5). * and # indicate a statistically significant difference in relation to controls PGE_2 2 μg + Vehicle 1 + Vehicle 1 and PGE_2 2 μg + Vehicle 1 + 5-HT 250 ng, respectively ($P < 0.05$ ANOVA + Bonferroni post test).

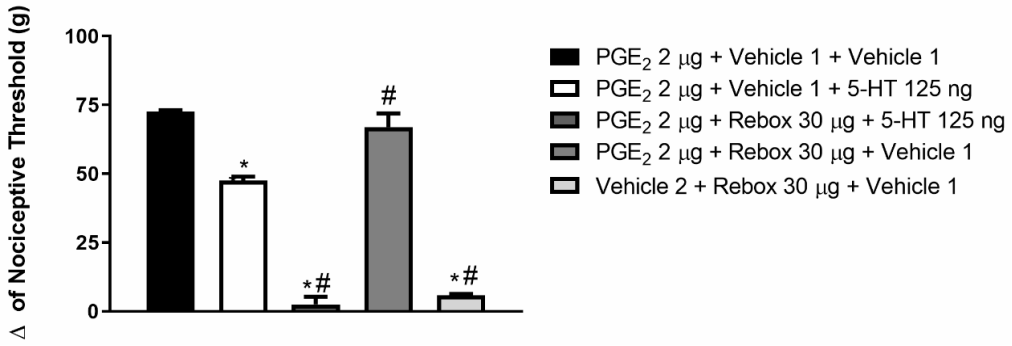

Figure 2. Intraplantar administration of Reboxetin (Rebox) enhances the peripheral antinociception induced by serotonin. Rebox (μg) was administered 30 minutes prior to serotonin administration (125 ng/paw). Each column represents the average S.E.M. (n = 5). * and # indicate a statistically significant difference in relation to controls PGE_2 2 μg + Vehicle 1 + Vehicle 1 and PGE_2 2 μg + Vehicle 1 + 5-HT 125 ng, respectively ($P < 0.05$ ANOVA + Bonferroni post test).

studies have associated the activation of α_1- and β-adrenergic receptors by NA and adrenaline, respectively, with induction of peripheral hyperalgesia [14-16].

In contrast, peripherally administered 5-HT reportedly induces pain in humans [5], as well as pain and paw edema in rats [6]. In addition, endogenous 5-HT, produced at relevant sites, plays a role in the nociceptive response induced by formaldehyde, subcutaneously injected into the rat paw [17].

However, our research group has demonstrated that both 5-HT [9] and NA [10] might confer an antinociceptive effect when administered peripherally. This antagonistic effect can be explained by the difference in doses employed, as the doses used to produce antinociception were approximately 125-fold (NA, 80 ng) and 40-fold (5-HT, 250 ng) smaller than the dose (10 μg) used to produce nociception with both substances [15, 18].

In our series of experiments, we demonstrated that the antinociceptive effect induced by intraplantar administration of 5-HT could be related to the activation of adrenergic receptors. Prazosin, a non-selective α_1-adrenergic receptor antagonist, yohimbine, a non-selective α_2-adrenergic receptor antagonist, and propranolol, a non-selective adrenergic β-receptor antagonist, reversed the antinociception induced by 5-HT in a dose-dependent manner.

Using the same nociceptive pain model as in the present study, Romero *et al*. [10] have demonstrated the involvement of adrenergic receptors in NA-induced peripheral antinociception, and this effect was reversed by administering prazosin, yohimbine, and propranolol. Likewise, using an inflammatory pain model induced by Complete Freund's adjuvant, Binder *et al*. [19] have reported that the administration of NA in inflamed tissues can induce antinociception, which was reversed by α_1-, α_2- and β-adrenergic receptor antagonists.

Adrenergic receptors are present in almost all peripheral tissues and several neuronal populations in the central nervous system. These receptors are classified into α_1 receptors and their subtypes (α_{1A}, α_{1B} and α_{1D}), α_2 receptors and their subtypes (α_{2A}, α_{2B} and α_{2C}), and β receptors and their subtypes (β_1, β_2 and β_3) [20].

α_1-adrenergic receptors couple with the G_q protein, which, once activated, increases intracellular concentrations of inositol 1,4,5-triphosphate (IP_3) and calcium ions (Ca^{2+}). α_2-adrenergic receptors couple with the $G_{i/o}$ protein, and their activation leads to adenylate cyclase inhibition, which results in a cellular reduction in cyclic 3', 5'-adenosine monophosphate (cAMP) levels, inhibition of Ca^{2+} channels, and activation of K^+ channels, along with activation of mitogen-activated protein kinases (MAPKs) [21].

β-adrenergic receptor isoforms couple with the G_s protein and stimulate adenylate cyclase, thereby

inducing the synthesis of cAMP, in addition to activating protein kinase A (PKA) [22]. α-adrenergic receptors are expressed in the dorsal root ganglion, and both α and β-adrenergic receptors are expressed in the peripheral immune system. Accordingly, adrenergic receptor ligands can modulate the excitability of primary afferent neurons *via* direct action on peripheral neurons, as well as indirectly by acting at the immune system [22].

It is well-established that 5-HT increases the release of other neurotransmitters such as NA, acetylcholine, dopamine, and histamine [23]. Studies have found that the activation of serotonergic receptors in the hippocampus, frontal cortex, hypothalamus, and presynaptic noradrenergic nerve endings can increase the release of NA in rats and rabbits [24, 25].

The literature demonstrates that excitatory transduction *via* serotonergic receptors such as 5-HT_{2A} (G_q protein-coupled) and 5-HT_3 (ligand-gated ion channel) plays an important role in serotonin-induced peripheral antinociception [9]. Additionally, it is known that immune system cells, such as macrophages [4] or other resident cells (e.g., keratinocytes or melanocytes), can synthesize and release endogenous catecholamines [26, 27]. From this dataset, together with the data obtained in the present work, we can speculate that 5-HT modulates NA release also at the peripheral level. We hypothesize that, by activating these excitatory transduction receptors, following the increased intracellular Ca^{2+} concentration, 5-HT would stimulate cellular groups to release NA, which would then bind to adrenergic receptors, inducing antinociception by direct activation of nociceptors, α_2-adrenergic receptors, or release of endogenous analgesic substances such as opioids [10] and cannabinoids [28] *via* α_1- or β-adrenergic receptors.

In the present study, we used reboxetine, a potent antidepressant that inhibits NA reuptake by selectively inhibiting the NA transporter and exhibits low affinity for serotonin transporters [29]. Several studies have reported the efficacy of reboxetine as an antinociceptive agent. For example, Obata *et al*. [30] have demonstrated that reboxetine administration could reduce the tactile hypersensitivity produced by incisional surgery in the rat paw. In addition, intravenously administered reboxetine was found to partially reverse mechanical and cold allodynia in a neuropathic pain model in rats [31].

In our model, we observed that reboxetine, at a low non-analgesic dose, potentiated the antinociceptive effect of low-dose serotonin (125 ng), demonstrating that this effect was similar to that observed with high doses administered in other experiments (250 ng). Following the inhibition of NA reuptake, additional catecholamine is available to bind with respective receptors, thus triggering its effects. Interestingly, reboxetine, when administered alone, did not reverse the nociception produced by PGE_2, demonstrating that the overall antinociceptive effect was dependent on the synergy between the serotonergic and noradrenergic systems, thus indicating that the analgesic action of serotonin depends on NA release, possibly mediated by immune cells or residents, and also on the increased intake of reboxetine-induced NA, as observed in our experimental model.

5. CONCLUSION

The results of the present study thus indicate that serotonergic and noradrenergic systems interact during serotonin-induced peripheral antinociception. To the best of our knowledge, the present study is the first one showing that inhibition of NA uptake and consequent increase in the supply of this mediator may potentiate peripheral analgesia *via* exogenous serotonin and may have good application in future practices.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

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