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Angiotensin-(1–7) through Mas receptor activation induces peripheral antinociception by interaction with adrenoreceptors

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ABSTRACT

Angiotensin-(1–7) [Ang-(1–7)] develops its functions interacting with Mas receptor. Mas receptor was recently identified in the DRG and its activation by Ang-(1–7) resulted in peripheral antinociception against PGE_2 hyperalgesia in an opioid-independent pathway. Nevertheless, the mechanism by which Ang-(1–7) induce peripheral antinociception was not yet elucidated. Considering that endogenous noradrenaline could induce antinociceptive effects by activation of the adrenoceptors the aim of this study was verify if the Ang-(1–7) is able to induce peripheral antinociception by interacting with the endogenous noradrenergic system. Hyperalgesia was induced by intraplantar injection of prostaglandin E_2 (2 μg). Ang-(1–7) was administered locally into the right hindpaw alone and after either agents, α_2-adrenoceptor antagonist, yohimbine (5, 10 and 20 μg/paw), α_{2C}-adrenoceptor antagonist rauwolscine (10, 15 and 20 μg/paw), α_1-adrenoceptor antagonist prazosin (0.5, 1 and 2 μg/paw), β-adrenoceptor antagonist propranolol (150, 300 and 600 ng/paw). Noradrenaline (NA) reuptake inhibitor reboxetine (30 μg/paw) was administered prior to Ang-(1–7) low dose (20 ng) and guanetidine 3 days prior to experiment (30 mg/kg/animal, once a day), depleting NA storage. Intraplantar Ang-(1–7) induced peripheral antinociception against hyperalgesia induced by PGE_2. This effect was reversed, in dose dependent manner, by intraplantar injection of yohimbine, rauwolscine, prazosin and propranolol. Reboxetine intensified the antinociceptive effects of low-dose of Ang-(1–7) and guanethidine, which depletes peripheral sympathomimetic amines, reversed almost 70% the Ang-(1–7)-induced peripheral antinociception. Then, this study provides evidence that Ang-(1–7) induce peripheral antinociception stimulating an endogenous noradrenaline release that activates peripheral adrenoceptors inducing antinociception.

Introduction

Angiotensin-(1–7) [Ang-(1–7)] is a biologically active member of the renin–angiotensin system (RAS). Ang-(1–7) is formed through an ACE-independent pathway and may have similar or opposite activities to angiotensin II [1]. The initial reports of angiotensin involved its ability to convert enzyme-2, which generates Ang-(1–7) from Angiotensin I or Angiotensin II [2–5] and interacts with a G protein-coupled receptor (GPCR), the Mas receptor, which is associated with several Ang-(1–7) activities [6]. Thus, several physiological roles of Ang-(1–7) have been described [6].

In the central nervous system, Ang-(1–7) acts as an important neuromodulator, particularly in the hypothalamus, the dorsomedial and ventrolateral medulla and in areas that are related to the tonic and reflex control of arterial pressure [7]. Several studies have described Angiotensin II as a regulator of many sensory modalities, including nociception [8,9], and RAS as an important agent that decreases pain sensitivity [10]. Considering the involvement of Angiotensin II in nociception, our group has demonstrated that Ang-(1–7) induces peripheral antinociception in PGE_2–induced hyperalgesia through an opioid-independent pathway [11]. Although, Ang-(1–7) was able to induce an increase in nitrite levels, activating nNOS (neuronal nitric oxide synthase) at peripheral sites inducing antinociception through NO release. NO in turn activated cGMP causing the opening of K+ channels in the peripheral sensitive neuron and thus analgesia [12].

NA is an adrenergic agonist that is involved in the intrinsic control of pain-inducing pro-nociceptive effects in the primary afferent nociceptors [13] or of peripheral antinociception through interactions with the α_2-, α_1- and β-adrenoceptors [14,15]. Moreover, NA can induce peripheral antinociception directly by the activation of the NO/cGMP analgesic pathway and subsequent

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activation of K^+ channels [16] or indirectly by opioid release from immune cells, which are recruited during inflammatory processes [14]. Our group recently showed that analgesic molecules, such as opioids and cannabinoids, induce peripheral antinociception by releasing NA, which activates the α_2-, α_1- and β-adrenoceptors [15,17]. Opioid receptor agonists have been shown to induce peripheral antinociception by interacting with the μ-, δ- and κ-opioid receptors via noradrenaline release at the supraspinal, spinal and peripheral sites [15,18–20,53]. Additionally, endocannabinoids, such as AEA and PEA, interact with adrenergic receptors to induce NA release, which results in peripheral antinociception [17]. Considering that Ang-(1–7) can activate NO/cGMP pathway in the same way of NA, and that analgesic molecules induce peripheral antinociception by interacting with the adrenergic system, could the Ang-(1–7) be releasing NA to induce peripheral analgesia? Then, the aim of present study was to investigate role of the noradrenergic system in Ang-(1–7)-induced peripheral antinociception.

Methods

Animals

The experiments were performed on 180–220 g male Wistar rats from CEBIO-UFMG (Animal House of the Federal University of Minas Gerais). The animals were housed in a temperature-controlled room (23 °C) on an automatic 12 h light/dark cycle (6:00 AM–6:00 PM of light phase). All tests were conducted during the light phase (8:00 AM–3:00 PM). Food and water were freely available until the beginning of the experiments.

Measurement of the hyperalgesia

Hyperalgesia was induced by subcutaneous injection of PGE_2 (2 μg) into the plantar surface of the mice hind paw. Hyperalgesia was measured according to the rat paw pressure test [21], which has been adapted to mice [22]. An analgesimeter equipped with a cone-shaped paw-presser with a rounded tip was used (Ugo-Basile) to apply a linearly increasing force to the hind paw. The weight in grams (g) required to elicit the nociceptive response of paw flexion was determined to be the nociceptive threshold. A cutoff value of 160 g was used to reduce the possibility of damage to the paws. The nociceptive threshold (Δ) was calculated as the difference between the nociceptive threshold obtained in the beginning of the experiment (basal value) and the threshold measured in the third hour following PGE_2 injection, and also was expressed in grams. A nociceptive threshold value of $\Delta > 0$ indicated hyperalgesia induced by PGE_2 injection, whereas decreases in this value indicated the anti-hyperalgesic effect of the tested drug. The animals were habituated once a day for the period of 2 days before the test. This habituation involves subjecting the animal to the same situation that will be experienced on the day of the experiment. The right hind paw of the animal was pressed by analgesimeter three times, enough it does not express one more flight reaction simply by being positioned on the device. This procedure is important because it allows better observation of the animal nociceptive response, that during the test must remain quiet, avoiding an aversive reaction by stress.

The experimental protocol was approved by the Ethics Committee on Animal Experimentation of the Federal University of Minas Gerais (CETEA/UFMG).

Experimental protocol

Ang-(1–7) was administered subcutaneously in the right hind paw 2 h and 50 min after local injection of prostaglandin E_2. Yohimbine, rauwolscine, BRL 44 480, imiloxan, RX 821002, prazosin, propranolol, reboxetine, were injected 30 min prior to Ang-(1–7). Guanetidine, which depletes peripheral sympathomimetic amines, was administered, once a day for 3 days prior to experiment.

The protocols concerning dose and time of administration of each drug used in this study were obtained through literature data and pilot experiments.

Chemicals

The drug used as hyperalgesic agent was PGE_2 (Cayman, USA) (2 μg). Angiotensin-(1–7) (Bachem, German) (4 μg) was used as the antinociceptive drugs. They were injected in the right hind paw a volume of 100 μl/paw. The α_2 adrenoceptor antagonist yohimbine 5, 10 and 20 μg/paw (YOH; Sigma), the α_{2A} adrenoceptor antagonist BRL 44 480 (2-[(4,5-Dihydro-1H-imidazol-2-yl)methyl]-2,3-dihydro-1-methyl-1H-isoindole maleato) (BRL; Tocris), the α_{2B} adrenoceptor antagonist imiloxan (RS 21361/2-(1-Ethyl-2-imidazoyl)methyl-1,4-benzodioxan hydrochloride) (IMI; Tocris), the α_{2C} adrenoceptor antagonist rauwolscine (Corynanthidine/α-Yohimbine/17a-Hydroxy-20a-yohimban-16b-carboxylic acid, methyl ester hydrochloride) (RAU; Tocris), the α_{2D} adrenoceptor antagonist RX 821002(2-(2,3-Dihydro-2-methoxy-1,4-benzodioxin-2-yl)-4,5-dihydro-1H-imidazole hydrochloride) (RX; Tocris), the α_1 adrenoceptor antagonist prazosin (PRA; Sigma), the β adrenoceptor antagonist propranolol (PROP; Sigma) and the noradrenaline reuptake inhibitor reboxetine (REB; Pfizer; USA) were dissolved in saline and were injected into right hind paw in a volume of 50 μl/paw. The stock solution of PGE_2 was prepared in ethanol, and further dilutions were made in saline; the final concentration of ethanol was 2%. Ang-(1–7), was dissolved in saline.

Guanethidine sulfate (Sigma, USA) 30 mg/Kg was dissolved in saline and injected intraperitoneally to depletion of the peripheral noradrenaline.

Statistical analysis

The statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Bonferroni's test for multiple comparisons. Probabilities less than 5% ($P < 0.05$) were considered statistically significant.

Results

Ang-(1–7) induced peripheral antinociception via interactions with α_{2C}-adrenoceptor

Using a non-inflammatory pain model, the injection of 4 μg of Ang-(1–7) into the right hind paw was shown to produce peripheral antinociceptive responses in animals with PGE_2-induced hyperalgesia [11]. Using the same model, the participation of the α_2-adrenoceptor in Ang-(1–7)-induced peripheral antinociception was examined using the nonselective α_2-adrenoceptor antagonist yohimbine (5, 10 and 20 μg/paw). Yohimbine antagonized the peripheral effects of Ang-(1–7) (4 μg/paw) in a dose-dependent manner and did not induce hyperalgesia or antinociception when administered alone (Fig. 1A). We also investigated the selectivity of the α_2 subtypes involved in Ang-(1–7)-induced peripheral antinociception and observed the involvement of the α_{2C}-adrenoceptor subtype, as shown by the blockage of this effect in a dose-dependent manner by the α_{2C}-adrenoceptor antagonist rauwolscine (10, 15 and 20 μg/paw) (Fig. 1B). The α_{2A}-, α_{2B}- and α_{2D}-subtype antagonists exhibited no effects (Fig. 1C).