European Journal of Pharmacology 791 (2016) 25–36

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Cardiovascular pharmacology

Pharmacological analysis of the cardiac sympatho-inhibitory actions of moxonidine and agmatine in pithed spontaneously hypertensive rats

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ARTICLE INFO

Article history:
Received 15 July 2016
Received in revised form
3 August 2016
Accepted 22 August 2016
Available online 24 August 2016

Keywords:
Agmatine
α_2-adrenoceptor
Hypertension
I_1-imidazoline receptor
Moxonidine

Chemical compounds studied in this article:
Agmatine sulfate salt (PubChem CID: 2794990)
AGN 192403 hydrochloride (PubChem CID: 11957452)
B-HT 933 dihydrochloride (PubChem CID: 169743)
BU224 hydrochloride (PubChem CID: 11957470)
desipramine hydrochloride (PubChem CID: 65327)
gallamine triethiodide (PubChem CID: 6172)
moxonidine hydrochloride (PubChem CID: 11231255)
(±)-noradrenaline bitartrate salt (PubChem CID: 5813)
rauwolscine hydrochloride (PubChem CID: 197067)

ABSTRACT

This study shows that in spontaneously hypertensive rats (SHR) of 14-weeks-old, the sympathetically-induced, but not noradrenaline-induced tachycardic response are higher than age-matched Wistar normotensive rats. Furthermore, in SHR the sympathetically-induced tachycardic response was: (1) unaffected by moxonidine (3 μg/kg min); (2) partially inhibited by B-HT 933 (30 μg/kg min), both at the lowest doses; and (3) completely inhibited by the highest doses of B-HT 933 (100 μg/kg min), moxonidine (10 μg/kg min) or agmatine (1000 and 3000 μg/kg min) while the noradrenaline-induced tachycardic responses remained unaffected by the above compounds, except by 3000 μg/kg min agmatine. In SHR, 300 μg/kg rauwolscine failed to block the sympatho-inhibition to 100 μg/kg min B-HT 933 or 10 μg/kg min moxonidine, but 1000 μg/kg rauwolscine abolished, partially antagonized, and did not modify the sympatho-inhibition to the highest doses of B-HT 933, moxonidine, and agmatine, respectively, 3000 μg/kg AGN 192403 or 300 μg/kg BU224 given alone had no effect in the moxonidine- or agmatine-induced sympatho-inhibition, and the combination rauwolscine plus AGN 192403 but not plus BU224, abolished the sympatho-inhibition to the highest doses of moxonidine and agmatine. In conclusion, the sympathetically-induced tachycardic responses in SHR are inhibited by moxonidine and agmatine. The inhibition of moxonidine is mainly mediated by prejunctional α_2-adrenoceptors and to a lesser extent by I_1-imidazoline receptors, while the inhibition of agmatine is mediated by prejunctional α_2-adrenoceptors and I_1-imidazoline receptors at the same extent. Notwithstanding, the inhibitory function of α_2-adrenoceptors seems to be altered in SHR compared with Wistar normotensive rats.

1. Introduction

Sympathetic nervous system modulates the cardiovascular function via α_2-adrenoceptors, which regulate the releasing of noradrenaline in sympathetic neurons (Hein et al., 1999). Notwithstanding, in spontaneously hypertensive rats (SHR), the α_2-adrenoceptors are down-regulated in the hypothalamus, cerebral cortex (Olmos et al., 1991), rostral ventrolateral medulla (Gulati, 1991), stellate ganglion (Zugck et al., 2003) but not in the heart atria (El-Ayoubi et al., 2004) and their inhibitory role is impaired in sympathetic cardiac neurons (Shanks et al., 2013b; Zugck et al., 2003). The alterations in the expression and/or function of α_2-adrenoceptors can contribute to the establishment and maintenance of hypertension (Grassi et al., 2010).

Imidazoline compounds can bind to I_1-imidazoline receptors and α_2-adrenoceptors. Both receptors coexist in many types of tissues (Burke et al., 1995; Chen et al., 2003; Lacombe et al., 1993). For instance, the I_1-imidazoline receptors, in addition to α_2-adrenoceptors, inhibit the noradrenaline release in sympathetic neurons (Raasch

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http://dx.doi.org/10.1016/j.ejphar.2016.08.017
0014-2999/

et al., 2003a, 2003b, 2002). It is to draw attention that expression of imidazoline receptors in SHR is up-regulated in peripheral tissues (El-Ayoubi et al., 2003, 2004; Mar et al., 2013), in contrast to the α_2-adrenoceptors, and remains unchanged in the central nervous system (Olmos et al., 1992).

Moxonidine, an imidazoline compound, and agmatine, the endogenous ligand for imidazoline receptors, inhibit the cardioaccelerator sympathetic outflow mainly by α_2-adrenoceptors and, to a lesser extent, by I_1-imidazoline receptors in Wistar normotensive rats (Cobos-Puc et al., 2009). However, the expression and/or functionality of α_2-adrenoceptors and imidazoline receptors are modulated differentially during systemic arterial hypertension. If these changes could modify the mechanisms involved in the cardiac sympatho-inhibition of moxonidine and agmatine in SHR is unknown. On this basis, the present study has investigated the mechanisms of the cardiac sympatho-inhibitory actions induced by moxonidine and agmatine in SHR. For this purpose, we used B-HT 933, as control response of α_2-adrenoceptors and selective antagonists as rauwolscine (α_2-adrenoceptor), AGN 192403 (I_1-imidazoline receptor) and BU224 (I_2-imidazoline receptor) given alone or in combination and the results obtained were analyzed considering previous findings in Wistar normotensive rats.

2. Materials and methods

2.1. Animals

This study included a total of 294 male rats that comprised 282 SHR and 12 Wistar normotensive rats. The animals were maintained at a 12/12 h light–dark cycle (with light beginning at 7 a.m.) and housed in a special room at constant temperature (22 ± 2 °C) and humidity (50%), with food and water freely available in their home cages. All animal procedures and the protocols of the present investigation were approved by our Institutional Ethics Committee and followed the regulations established by the Mexican Official Norm for the Use and Welfare of Laboratory Animals (NOM-062-ZOO-1999), in accordance with the Guide for the Care and Use of Laboratory Animals in the U.S.A.

2.2. Measurements of systolic blood pressure and heart rate in conscious rats

Measurements of systolic blood pressure and heart rate in conscious rats were done using the tail plethysmographic method one week before that the pharmacological treatments were performed. For this procedure, rats were habituated at least three days before by placing the animal into the restrainer for habituation and some measurements were made for habituating the rat to the pressure on its tail. The rats were placed in a quiet, temperature-controlled (32 °C) environment for 30 min. Then, the tail was inserted through the cuff which contained a photoelectric pulse detector (heart rate and pressure meter model 72, IITC Inc., Landing, N.J., U.S.A.) equipped with a pulse amplifier (pulse amplifier model 59, IITC Inc., Landing, N.J., U.S.A.) connected to a tachograph (7P4, Grass Instrument Co., Quincy, Ma, U.S.A.). Three consecutive systolic blood pressure and heart rate readings were recorded when the first oscillation appeared during the gradual reduction of the cuff pressure. The values reported are the average of three determinations. Systolic blood pressure values above of 150 mmHg were regarded as hypertensive (Wolfensohn and Llyod, 2013).

2.3. General methods

After anesthesia with ether and cannulation of the trachea, the rats were pithed by inserting a stainless-steel rod through the orbit and foramen magnum into the vertebral foramen (Shipley and Tilden, 1947). The animals were artificially ventilated with room air using an Ugo Basile pump (52 strokes/min and a stroke volume of 20 ml/kg), as previously established by Kleinman and Radford (1964). After bilateral vagotomy, catheters were placed in the left and right femoral veins, for the infusion of agonists and administration of antagonists, respectively and the left carotid artery. The latter was connected to a pressure transducer (P23 XL, Grass Instrument Co., Quincy, Ma, U.S.A.) for recording arterial blood pressure. Blood pressure and heart rate were recorded simultaneously by using a data acquisition unit (MP150A-CE) and Acknowledge software v3.8.1 (Biopac Systems Inc., Goleta, CA, U.S.A.).

At this point, the 294 animals were divided into two main groups so that the effects produced by i.v. continuous infusions of saline or the several compounds could be investigated on the tachycardic responses elicited by either: (i) selective preganglionic (C_7-T_1) stimulation of the cardiac sympathetic outflow (group 1; n=234); or (ii) i.v. bolus injections of exogenous noradrenaline (group 2; n=60). The tachycardic responses evoked by sympathetic stimulation and exogenous noradrenaline were: (i) elicited using a sequential schedule, in 0.5 log unit increments at 3- to 5 min intervals; and (ii) completed in about 30 min without significant changes in resting heart rate. The body temperature of each rat was maintained at 37 °C by a lamp and monitored with a rectal thermometer (Cobos-Puc et al., 2009).

2.4. Experimental protocols

2.4.1. Electrical stimulation of the cardioaccelerator sympathetic outflow

In the group 1, the pithing rod was replaced by an enameled electrode except for 1 cm length 7 cm from the tip, so that the uncovered segment was situated at C_7-T_1 of the spinal cord to allow selective stimulation of the cardiac sympathetic outflow (Gillespie et al., 1970). Prior to electrical stimulation, the animals received gallamine (25 mg/kg, i.v.) to avoid electrically-induced muscular twitching. Since the sympatho-inhibitory responses are particularly more pronounced at lower frequencies of stimulation, all the animals were systematically pretreated with desipramine (50 μg/kg, i.v., a noradrenaline-reuptake inhibitor) before each stimulus–response curve, as previously described (Cobos-Puc et al., 2009). After a stable haemodynamic condition for at least 30 min, baseline values of diastolic blood pressure and heart rate were determined. In this point, the group 1 was divided into two subgroups (n=36 and 198, respectively). The first subgroup was divided into two sets (n=24 and 12, respectively). The first set was divided into four subsets (n=6 each) that included SHR of 8, 10, 12, and 14 weeks of age. The second set was divided into two subsets (n=6 each), which included SHR and Wistar normotensive rats at the age of 14 weeks. Then, a stimulus–response curve was performed as follow; the preganglionic cardiac sympathetic outflow was stimulated to elicit tachycardic responses by applying trains of 10 s trains of monophasic, rectangular pulses (2 ms, 50 V), at increasing frequencies of stimulation (0.03, 0.1, 0.3, 1.0 and 3.0 Hz). When the heart rate had returned to baseline levels, the next frequency was applied; this procedure was systematically performed until the stimulus–response curve had been completed (about 30 min).

For the pharmacological analysis, SHR at the age of 14 weeks were chosen because until this age have not yet developed cardiac hypertrophy (Louis et al., 1969). Hence, the second subgroup was divided into five sets (n=48, 42, 30, 42, and 36, respectively). The