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Pharmacological evidence that metformin blocks the vasopressor responses mediated by stimulation of α_1- and α_2-adrenoceptors in pithed rats

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ARTICLE INFO

Chemical compounds studied in this article:
Gallamine triethiodide (PubChem ID: 6172)
Methoxamine hydrochloride (PubChem ID: 6081)
(±)-noradrenaline bitartrate (PubChem ID: 297812)
5-Bromo-N-(2-imidazolin-2-yl) – 6-quinoxalinamine, 5-Bromo-N-(4,5-dihydro-1H-imidazol-2-yl) – 6-quinoxalinamine (UK 14,304, brimonidine) (PubChem ID: 2435)
1,1-dimethyl biguanide hydrochloride (metformin) (PubChem ID: 14219)

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ABSTRACT

It has been reported that metformin reduces blood pressure although the mechanisms have not been described. Indeed, several mechanisms could be implicated including the interaction with α-adrenoceptors or inhibition of sympathetic outflow. Therefore, this study was designed to determine the capability of metformin to block the vasopressor responses induced by $\alpha_{1/2}$-adrenoceptor agonists or selective electrical stimulation of sympathetic outflow. For this purpose, Wistar male rats were anesthetized, pithed and cannulated for selective preganglionic stimulation of the vasopressor sympathetic outflow or drugs administration. The effect of i.v. bolus injection of metformin (180 and 310 mg/kg) or its vehicle (bidistilled water) was studied on the vasopressor responses induced by: (1) selective sympathetic stimulation (0.03–3 Hz); (2) exogenous noradrenaline (0.03–3 μg/kg); (3) methoxamine (1–100 μg/kg); and (4) UK 14,304 (0.1–30 μg/kg). The tachycardic responses to noradrenaline were also investigated in presence of metformin. The vasopressor responses induced by selective electrical stimulation of sympathetic outflow were diminished by metformin (180 and 310 mg/kg) and remained unchanged in presence of vehicle. Moreover, the vasopressor responses induced by exogenous noradrenaline, methoxamine and UK 14,304 were dose-dependently inhibited by i.v. bolus injections of metformin (180 and 310 mg/kg) and were not affected by vehicle. Metformin practically did not block the tachycardic responses to noradrenaline except at the dose of 3 μg/kg. Taken together, these results demonstrate that metformin is capable to block vascular $\alpha_{1/2}$-adrenoceptors but not cardiac β-adrenoceptors. Thus, this mechanism could contribute, at least in part, on the hypotensive responses induced by metformin.

1. Introduction

Metformin is the first-line drug used for the treatment of type 2 diabetes mellitus. This biguanide also reduces blood pressure in hypertensive rats (Bhalla et al., 1996; Verma et al., 1994). Clinical studies have demonstrated that chronic administration of metformin reduced blood pressure (Giugliano et al., 1993; Landin et al., 1991) or produced no effect in blood pressure (Fanghanel et al., 1996; He et al., 2012). Notwithstanding, the mechanisms underlying the antihypertensive effect of this drug remains elusive. In this respect, several mechanisms have been proposed to explain this effect: (1) attenuation of agonist-induced increases in intracellular calcium concentration and increases of nitric oxide production in vascular smooth muscle (Bhalla et al., 1996); (2) acute inhibition of the sympathetic neurotransmission (Lee and Peuler, 1999; Petersen and DiBona, 1996; Petersen et al., 1997); (3) activation of voltage-gated K^+ channels (Peuler et al., 1999); (4) restoring the expression of calcium-activated K^+ channels (Zhao et al., 2014); (5) decreases of reactive oxygen species (Ouslimani et al., 2005); (6) re-establishment of endothelial function (Ghosh et al., 2015; Majithiya and Balaraman, 2006; Sena et al., 2011); and (7) decreases in plasma concentration of noradrenaline and dopamine (Kosegawa et al., 1996).

Considering the potential sympatho-inhibition of metformin, it has been demonstrated that the hypotension to metformin was unaffected by propranolol, atropine or N^G-methyl-arginine and blocked by hexamethonium, phentolamine or its respective combination, suggesting that metformin produced hypotension by inhibiting the sympathetic tone (Muntzel et al., 1997). However, metformin may directly block $\alpha_{1/2}$-adrenoceptors. In this context, it has been demonstrated that metformin blocked the contraction induced by noradrenaline (Peuler et al., 1997), phenylephrine and 5–Hydroxitryptamine (Lee and Peuler, 1999) in rat tail artery.

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Despite the above findings, unfortunately, the potential α_1- and α_2-adrenoceptors blocking properties of metformin have not been comprehensively explored. Thus, the main objective of this study was to determine the effect of metformin on the vasopressor responses induced by sympathetic stimulation or by noradrenaline ($\alpha_{1/2}$), methoxamine (α_1) and UK 14,304 (α_2) in pithed rats.

2. Materials and methods

2.1. Animals

72 male Wistar rats (300–350 g) were housed in plastic cages and maintained under standardized condition (22 ± 2 °C, 50% humidity and 12/12 h light-dark cycle) and provided with food and water ad libitum. All animal procedures and the protocols of the present study were approved by our Institutional Ethics Committee (Cicual-Cinvestav) following 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 U.S.A.

2.2. Pithed rat

Animals were anesthetized with isoflurane (3%). The trachea was cannulated. In order to destroy the central nervous system, animals were pithed by inserting a stainless-steel rod through the orbit and *foramen magnum* into the vertebral foramen (Centurión et al., 2009; Gillespie and Muir, 1967). Under these conditions, the animals were unconscious. Therefore, the animals were artificially ventilated with room air using a positive pressure pump (7025 rodent ventilator, Ugo Basile, Comerio, VA, Italy) at 56 strokes/min and a stroke volume of 20 ml/kg, as previously described (Kleinman and Radford, 1964). After bilateral vagotomy, catheters were placed in: (1) the left and the right femoral veins for drug administration and (2) the left carotid artery. The latter was connected to a pressure transducer (RX104A, Biopac Systems Inc., Goleta, CA, USA) to record the arterial blood pressure and heart rate. Blood pressure and heart rate were recorded simultaneously using a data acquisition unit (MP150A-CE, Biopac Systems Inc., Goleta, CA, USA) and Acknowledge software v4.0.1 (Biopac Systems Inc., Goleta, CA, USA). Diastolic blood pressure was determined, as this is the blood pressure when the left ventricle is relaxed and thus could indirectly represent the systemic vascular resistance that regulates arterial blood pressure and blood flow to organs.

2.3. Experimental design

After this procedure, the 72 rats were divided into two main sets. In the set 1 (n = 18), the vasopressor responses produced by selective preganglionic (T_7-T_9) stimulation of the vasopressor sympathetic outflow was determined. The set 2 (n = 54) received i.v. bolus injections of exogenous noradrenaline, methoxamine or UK 14,304. The vasopressor sympathetic stimuli (0.03, 0.1, 0.3, 1 and 3 Hz) as well as i.v. bolus injections of: (1) exogenous noradrenaline (0.03, 0.1, 0.3, 1, and 3 μg/kg); (2) methoxamine (1, 3, 10, 30 and 100 μg/kg); and (3) UK 14,304 (0.1, 0.3, 1, 3, 10 and 30 μg/kg) were given using a sequential schedule of 0.5 log unit increments. The interval between the different stimulation frequencies or doses of the compounds mentioned above was dependent on the duration of the resulting response (approx. 10 min) until diastolic blood pressure returned to baseline values. The body of each pithed rat was maintained at 37 °C by a lamp and monitored with a rectal thermometer. The vasopressor stimulus-response or dose-response curves elicited by, respectively, sympathetic stimulation or exogenous noradrenaline, methoxamine and UK 14,304 were completed in about 60 min.

2.3.1. Protocol 1. Stimulation of the vasopressor sympathetic outflow

In the first set of rats (n = 18), the stainless-steel rod was replaced by an enameled bipolar electrode except for 1 cm length 9 cm from the tip. The uncovered segment was situated in the T_7-T_9 region of the spinal cord and allows the selective stimulation of the sympathetic nerves supplying the systemic vasculature (Gillespie and Muir, 1967). The animals received an i.v. bolus of gallamine (25 mg/kg) before electrical stimulation in order to avoid electrically-induced muscle twitching. Blood pressure and heart rate were determined after a stable haemodynamic condition (approx. 10 min). Then, the preganglionic vasopressor sympathetic outflow was stimulated with an S88X square pulse stimulator (Grass Technologies, Warwick, RI, U.S.A.) by applying 10 s trains of monophasic, rectangular pulses (2 ms, 60 V), at increasing frequencies (0.03, 0.1, 0.3, 1 and 3 Hz). These frequencies deliver, respectively 1, 1, 3, 10 and 30 pulses/train during 10 s.

Then, the first set of rats (n = 18) was divided into three subgroups (n = 6 each) that received i.v. bolus injections of: (1) vehicle (bidistilled water; 1 ml/kg); (2) metformin (180 mg/kg); and (3) metformin (310 mg/kg). Ten minutes later, a stimulus-response curve was constructed again under metformin (180 or 310 mg/kg) or its vehicle in order to analyse their effects on the vasopressor responses induced by sympathetic stimulation. The doses of metformin were chosen from preliminary experiments in which a lower dose (100 mg/kg) than 180 mg/kg did not produce an effect (data not shown) while higher doses than 310 mg/kg may have produced non-selective effects.

2.3.2. Protocol 2. Administration of exogenous noradrenaline, methoxamine or UK 14,304

The second set of rats (n = 54) was prepared as described above, but the pithing rod was left throughout the experiment. After determining baseline values of diastolic blood pressure and heart rate (approx. 10 min), the animals were divided into three subgroups (n = 18 each). The vasopressor responses were elicited by i.v. bolus injections of: (1) exogenous noradrenaline (0.03, 0.1, 0.3, 1 and 3 μg/kg); (2) methoxamine (1, 3, 10, 30 and 100 μg/kg) and (3) UK 14,304 (0.1, 0.3, 1, 3, 10 and 30 μg/kg). Next, the three subgroups were divided into three subsets (n = 6 each) that received, respectively, an i.v. bolus injection of: (1) vehicle (bidistilled water; 1 ml/kg); (2) metformin (180 mg/kg); and (3) metformin (310 mg/kg). Ten minutes later, a dose-response curve was performed again under the above administrations. When diastolic blood pressure had returned to baseline values, the next dose was applied; this procedure was performed until the dose-response curve had been completed.

2.4. Drugs

Apart from the anesthetic isoflurane (Fluriso™, Vet Ones, Boise, ID, U.S.A.), the compounds used in the present study (obtained from the sources indicated) were: gallamine triethiodide; (±)-noradrenaline bitartrate, methoxamine hydrochloride; 5-Bromo-N-(2-imidazolin-2-yl) − 6-quinoxalinamine, 5-Bromo-N-(4,5-dihydro-1H-imidazol-2-yl) − 6-quinoxalinamine (UK 14,304, brimonidine); and 1,1-dimethyl biguanide hydrochloride (metformin) (Sigma Chemical Co., St. Louis, MO, U.S.A.). All the compounds were dissolved in bidistilled water.

2.5. Data presentation and statistical evaluation

All data in the text and Figs. are presented as mean ± S.E.M. The maximum changes in diastolic blood pressure and heart rate to either electrical stimulation or administration of exogenous noradrenaline, methoxamine or UK 14,304 were determined. Area under the curve (AUC) was calculated by using the trapezoid rule. Statistical analysis of the difference between the changes in diastolic blood pressure within one subgroup of animals was performed using a two-way repeated measures analysis of variance (Two-Way RM ANOVA). A Tukey's post-hoc test was used when necessary. Further, differences in the effects