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# Evidence that some imidazoline derivatives inhibit peripherally the vasopressor sympathetic outflow in pithed rats

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ABSTRACT

Imidazoline derivatives (e.g. clonidine and moxonidine) and $\alpha_2$-adrenoceptor agonists (e.g. B-HT 933) have been shown to inhibit sympathetically-induced [$^3$H]noradrenaline release in several isolated blood vessels. The present study has compared the potential capability of agonists at imidazoline $I_{1/2}$ receptors and/or $\alpha_{1/2}$-adrenoceptors to inhibit the sympathetically-induced vasopressor responses in pithed rats. For this purpose, male Wistar rats were pithed and prepared for measurement of diastolic blood pressure and heart rate. Then, the vasopressor responses induced by either selective electrical stimulation (2 ms, 60 V; 0.03, 0.1, 0.3, 1 and 3 Hz) of the vascular sympathetic outflow ($T_7$–$T_9$) or i.v. bolus injections of exogenous noradrenaline (0.03, 0.1, 0.3, 1 and 3 μg/kg) were determined before and during i.v. continuous infusions of the agonists B-HT 933 ($\alpha_2$), clonidine ($\alpha_2$, $I_1$), moxonidine ($\alpha_2$, $I_1$), cirazoline ($\alpha_1$, $I_2$), agmatine (putative endogenous ligand of imidazoline receptors) and methoxamine ($\alpha_1$), or equivalent volumes of physiological saline. Electrical sympathetic stimulation elicited frequency-dependent vasopressor responses which were significantly inhibited during the continuous infusions of B-HT 933, clonidine, moxonidine, cirazoline and agmatine, but not of physiological saline. Interestingly, the vasopressor responses to exogenous noradrenaline, which remained unaffected during the infusions of physiological saline, B-HT 933, moxonidine, cirazoline and agmatine, were significantly blocked during the infusions of clonidine or methoxamine. These results suggest that B-HT 933, moxonidine, cirazoline and agmatine induced a prejunctional inhibition of the vasopressor sympathetic outflow in pithed rats, whilst clonidine inhibited the vasopressor sympathetic outflow by both prejunctional and postjunctional mechanisms.



## 1. Introduction

Imidazoline derivatives such as clonidine and moxonidine produce hypotension by decreasing the sympathetic outflow; this effect is mainly mediated by a central mechanism which involves activation of $\alpha_2$-adrenoceptors and/or imidazoline $I_1$ receptors (Bousquet et al., 1984; Szabo, 2002). Moreover, in some isolated preparations imidazoline derivatives are capable of inhibiting [$^3$H]noradrenaline release elicited by sympathetic stimulation via activation of prejunctional imidazoline receptors; such preparations include the pulmonary arteries (Molderings et al., 1991), aorta (Molderings and Göthert, 1995) and atria (Gaiser et al., 1999) of the rabbit as well as right atria and pulmonary arteries (Molderings et al., 1997) of humans. However, very few studies have shown the above peripheral sympatho-inhibition in the systemic vasculature. In this respect: (i) in pithed rabbits with electrical stimulation of the spinal cord, i.v. moxonidine or rilmenidine produced hypotension and bradycardia (Szabo et al., 1999); and (ii) in pithed spontaneously hypertensive rats (SHR) pretreated with phenoxibenzamine (an α-adrenoceptor antagonist), moxonidine decreased plasma concentrations of noradrenaline (Raasch et al., 2003).

On this basis, the present study in pithed rats was designed to determine the capability of the agonists B-HT 933 ($\alpha_2$), clonidine ($\alpha_2$, $I_1$), moxonidine ($\alpha_2$, $I_1$), cirazoline ($\alpha_1$, $I_2$), agmatine (putative endogenous ligand of imidazoline receptors) or methoxamine ($\alpha_1$) to inhibit the vasopressor responses to either: (i) sympathetic stimulation; or (ii) exogenous noradrenaline.

## 2. Materials and methods

### 2.1. Animals

Male Wistar normotensive rats (250–300 g) were used in the present experiments. The animals were maintained at a 12/12-h light–dark cycle (lights on at 7 a.m.) and housed in a special room at a constant temperature (22±2 °C) and humidity (50%), with food and water freely available in their home cages.

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### 2.2. General methods

Experiments were carried out in a total of 80 rats. After anaesthesia 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 air using an Ugo Basile model 7025 pump (56 strokes/min), as previously established (Kleinman and Radford, 1964). After bilateral vagotomy, catheters were placed in the left and right femoral veins, for the infusion of agonists and for the administration of antagonists, respectively, and the left carotid artery, connected to a Grass pressure transducer (P23 XL), for the recording of blood pressure. Heart rate was measured with a tachograph (7P4, Grass Instrument Co., Quincy, MA, U.S.A) triggered from the blood pressure signal. Both blood pressure and heart rate were recorded simultaneously by a model 7 Grass polygraph (Grass Instrument Co., Quincy, MA, U.S.A). At this point, the 80 rats were divided into two main groups, so that the effects produced by i.v. continuous infusions of physiological saline as well as the agonists B-HT 933, clonidine, methoxamine, agmatine, moxonidine and cirazoline could be investigated on the vasopressor responses induced by either selective preganglionic ($T_7$–$T_9$) stimulation of the vasopressor sympathetic outflow (group 1; $n=40$) or i.v. bolus injections of exogenous noradrenaline (group 2; $n=40$). The vasopressor stimulus–response curves (S–R curves) and dose–response curves (D–R curves) elicited by, respectively, sympathetic stimulation and exogenous noradrenaline were completed in about 30 min without any change in resting diastolic blood pressure or heart rate. Moreover, the vasopressor sympathetic stimuli as well as the noradrenaline bolus injections were given using a sequential schedule, in 0.5 log unit increments at 3- to 5-min intervals. The body temperature was maintained at 37 °C by a lamp and monitored with a rectal thermometer. The Ethical Committee of Cinvestav (CICUAL) dealing with the use of animals in scientific experiments approved the protocols of this investigation.

### 2.3. Experimental protocols

#### 2.3.1. Protocol 1: electrical stimulation of the vasopressor sympathetic outflow

In the first group of rats ($n=40$), the pithing rod was replaced by an electrode enamelled except for a 1-cm length 9 cm from the tip, so that the uncovered segment was situated at $T_7$–$T_9$ of the spinal cord to stimulate the thoracic sympathetic nerves supplying the systemic vasculature (Gillespie et al., 1970; Villalón et al., 1995). Prior to electrical stimulation, the animals received gallamine (25 mg/kg, i.v.) to avoid electrically-induced muscular twitching. Furthermore, since the sympatho-inhibitory responses to monoamines are particularly more pronounced at lower stimulation frequencies (Langer, 1980), the animals were pretreated with 50 μg/kg (i.v.) of desipramine (a noradrenaline-reuptake inhibitor) before each S–R curve, as previously described (Villalón et al., 1995, 1998). After stable haemodynamic conditions had been maintained for at least 20 min, baseline values of diastolic blood pressure and heart rate were determined. Then, the preganglionic sympathetic outflow was stimulated to elicit vasopressor responses by applying 10-s trains of monophasic, rectangular pulses (2 ms duration and 60 V), at increasing frequencies of stimulation (0.03, 0.1, 0.3, 1 and 3 Hz). When the diastolic blood pressure had returned to baseline levels, the next frequency was applied; this procedure was performed systematically until the S–R curve had been completed (about 30 min). Subsequently, this group of rats was subdivided into eight subgroups ($n=5$ each) that received, by a WPI model sp100i pump (World Precision Instruments Inc., Sarasota, FL, U.S.A.), i.v. continuous infusions of, respectively: (i) physiological saline (control, three times; 0.02 ml/min); (ii) B-HT 933 (10 and 30 μg/kg min); (iii) B-HT 933 (18 μg/kg min); (iv) clonidine (0.56, 1 and 1.8 μg/kg min); (v) moxonidine (1, 3 and 10 μg/kg min); (vi) cirazoline (0.56, 1 and 1.8 μg/kg min); (vii) agmatine (1000 and 3000 μg/kg min); and (viii) methoxamine (9.6 and 15 μg/kg min). Ten minutes later, an S–R curve was elicited again during the above infusions to analyse their effects on the sympathetically-induced vasopressor responses. Once the S–R curve had been completed, the infusion was stopped. The intervals between the different doses of the infusions ranged between 10 and 15 min, as in each case we waited until diastolic blood pressure had returned to baseline values.

#### 2.3.2. Protocol 2: administration of exogenous noradrenaline

In the second group of rats ($n=40$), the pithing rod was left throughout the experiment. After determining baseline values of diastolic blood pressure and heart rate, vasopressor responses were elicited by i.v. bolus injections of exogenous noradrenaline (0.03, 0.1, 0.3, 1 and 3 μg/kg). This procedure was performed until the D–R curve was completed. Then, this group of rats was subdivided into eight subgroups ($n=5$ each) that received, as described above, i.v. continuous infusions of, respectively: (i) physiological saline (three times; 0.02 ml/min); (ii) B–HT 933 (10 and 30 μg/kg min); (iii) B-HT 933 (18 μg/kg min); (iv) clonidine (0.56, 1 and 1.8 μg/kg min); (v) moxonidine (1, 3 and 10 μg/kg min); (vi) cirazoline (0.56, 1 and 1.8 μg/kg min); (vii) agmatine (1000 and 3000 μg/kg min); and (viii) methoxamine (9.6 and 15 μg/kg min). Ten minutes later, a D–R curve was elicited again (during the above infusions).

### 2.4. Data presentation and statistical analysis

All data in the text and figures are presented as mean ± s.e.m. The peak changes in diastolic blood pressure produced by either electrical stimulation or exogenous noradrenaline in saline- and agonist-infused animals were determined. The difference between the changes in diastolic blood pressure within one subgroup of animals was evaluated by Student–Newman–Keuls' test, once a two-way repeated-measures analysis of variance (randomized block design) had revealed that the samples represented different populations (Steel and Torrie, 1980). Statistical significance was accepted at $P<0.05$.

### 2.5. Drugs

Apart from the anaesthetic (diethyl ether), the drugs used in the present study (obtained from the sources indicated) were: gallamine triethiodide, (−)-noradrenaline bitartrate, 6-ethyl-5,6,7,8-tetrahydro-4H-oxazolo[4,5-d]azepin-2-amine) dihydrochloride (B-HT 933), clonidine hydrochloride, methoxamine hydrochloride, agmatine sulfate, moxonidine hydrochloride, cirazoline hydrochloride and desipramine hydrochloride (Sigma Chemical Co., St Louis, MO, U.S.A.); all compounds were dissolved in physiological saline. In the case of noradrenaline, ascorbic acid (1%) was used to prevent oxidation. These vehicles had no effect on baseline diastolic blood pressure or heart rate (not shown). Fresh solutions were prepared for each experiment. The doses mentioned in the text refer to the free base of substances except in the case of gallamine, where it refers to the corresponding salt.

## 3. Results

### 3.1. Systemic haemodynamic variables

The baseline values of diastolic blood pressure and heart rate in the 80 rats were 51 ± 2 mm Hg and 285 ± 3 bpm, respectively. After desipramine (50 μg/kg, i.v.), these variables significantly ($P<0.05$) and transiently increased to 69 ± 2 mm Hg and 290 ± 2 bpm, respectively, returning to baseline values after 10 min. The subsequent treatments with desipramine did not change the baseline values of diastolic blood pressure or heart rate. Moreover, the i.v. continuous infusions of physiological saline or agmatine failed to modify the baseline values of diastolic blood pressure (see Table 1) or heart rate (not shown). In