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Involvement of central α_1-adrenoceptors on renal responses to central moxonidine and α-methylnoradrenaline

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ABSTRACT

Moxonidine (α_2-adrenoceptor/imidazoline receptor agonist) injected into the lateral ventricle induces diuresis, natriuresis and renal vasodilation. Moxonidine-induced diuresis and natriuresis depend on central imidazoline receptors, while central α_1-adrenoceptors are involved in renal vasodilation. However, the involvement of central α_1-adrenoceptors on diuresis and natriuresis to central moxonidine was not investigated yet. In the present study, the effects of moxonidine, α-methylnoradrenaline (α_2-adrenoceptor agonist) or phenylephrine (α_1-adrenoceptor agonist) alone or combined with previous injections of prazosin (α_1-adrenoceptor antagonist), yohimbine or RX 821002 (α_2-adrenoceptor antagonists) intracerebroventricularly (i.c.v.) on urinary sodium, potassium and volume were investigated. Male Holtzman rats ($n=5$–18/group) with stainless steel cannula implanted into the lateral ventricle and submitted to gastric water load (10% of body weight) were used. Injections of moxonidine (20 nmol) or α-methylnoradrenaline (80 nmol) i.c.v. induced natriuresis (196 ± 25 and 171 ± 30, respectively, vs. vehicle: 101 ± 9 μEq/2 h) and diuresis (9.0 ± 0.4 and 12.3 ± 1.6, respectively, vs. vehicle: 5.2 ± 0.5 ml/2 h). Pre-treatment with prazosin (320 nmol) i.c.v. abolished the natriuresis (23 ± 4 and 76 ± 11 μEq/2 h, respectively) and diuresis (5 ± 1 and 7.6 ± 0.8 ml/2 h, respectively) produced by i.c.v. moxonidine or α-methylnoradrenaline. RX 821002 (320 nmol) i.c.v. abolished the natriuretic effect of α-methylnoradrenaline, however, yohimbine (320 nmol) did not change renal responses to moxonidine. Phenylephrine (80 nmol) i.c.v. induced natriuresis and kaliuresis that were blocked by prazosin. Therefore, the present data suggest that moxonidine and α-methylnoradrenaline acting on central imidazoline receptors and α_2-adrenoceptors, respectively, activate central α_1-adrenergic mechanisms to increase renal excretion.

1. Introduction

Moxonidine and clonidine are well known anti-hypertensive drugs (Ernsberger et al., 1997; Haxhiu et al., 1994). This therapeutic property is widely assumed to depend on reduction in sympathetic activity, but it may also result from attenuation in extracellular volume because these drugs inhibit fluid intake and increase renal water and sodium loss (Callera et al., 1993; De Luca and Menani, 1997; De Paula et al., 1996; Ferrari et al., 1990; Fregly et al., 1981; Fregly et al., 1984a,b; Le Douarec et al., 1971; Menani et al., 1999; Penner and Smyth, 1994a,b, 1995a,b).

Moxonidine and clonidine bind to α_2-adrenoceptors and imidazoline receptors that are distributed throughout the central nervous system (French, 1995; Ruggiero et al., 1998; King et al., 1995), and may produce cardiovascular and renal effects related to a preferential binding according to the area under investigation. For example, whereas α_2-adrenoceptors mediate the hypotension produced by clonidine (Guyenet, 1997), imidazoline receptors located in the rostral ventrolateral medulla mediate the anti-hypertensive response to moxonidine (Ernsberger et al., 1997; Haxhiu et al., 1994). However, both imidazoline receptors and α_2-adrenoceptors are apparently involved with the natriuretic and diuretic action of moxonidine (El-Ayoubi et al., 2005; Smyth and Penner, 1999). Also, moxonidine reduces arterial pressure, heart rate and mesenteric and hindlimb vascular resistances when injected into the 4th ventricle, but produces no change in arterial pressure or heart rate when injected into the lateral ventricle (Moreira et al., 2004; Nurminen et al., 1998).

Moxonidine into the lateral ventricle also induces renal vasodilation and increases renal blood flow, effects completely blocked by prior injection, into the lateral ventricle, of prazosin, an α_1-adrenoceptor antagonist, but not of yohimbine, an α_2-adrenoceptor antagonist (Moreira et al., 2007). The increase in renal blood flow might contribute to the renal excretory effects of central moxonidine, effects perhaps mediated not only by α_2-adrenoceptors/imidazoline receptors as suggested previously (El-Ayoubi et al., 2005; Smyth and Penner, 1999), but also by the central α_1-adrenoceptors.

To the best of our knowledge, no study has investigated the involvement of central α_1-adrenoceptors on diuretic and natriuretic

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effects of central moxonidine. In addition, except for idazoxan (an α_2-adrenoceptor/imidazoline receptor antagonist), no study has consistently investigated the effects of central combination of specific α_2-adrenoceptor antagonists and moxonidine on renal excretion.

Therefore, in the present study, we tried a better characterization of forebrain α-adrenoceptors involved with the diuretic and natriuretic effects of moxonidine injected intracerebroventricularly (i.c.v.), by combining it with selective antagonists like prazosin, an α_1-adrenoceptor antagonist, or RX 821002 or yohimbine, α_2-adrenoceptor antagonists, via the same route. We also tested the effects of α-methylnoradrenaline, a non-imidazoline α_2-adrenoceptor agonist, and phenylephrine, an α_1-adrenoceptor agonist, alone or combined with the antagonists. All tests were done in rats that received a water load (10% of body weight) to increase basal urine volume.

2. Materials and methods

2.1. Animals

Male Holtzman rats (280 to 320 g) obtained from the Animal Facility of São Paulo State University were housed in individual stainless steel metabolic cages with free access to normal sodium diet (Guabi Rat Chow, Paulinia, SP, Brazil) and water. Temperature was maintained at 23 ± 2 °C, and humidity was maintained at 55 ± 10% on a 12:12 light–dark cycle with light onset at 7:30 AM. The experimental protocols were approved by the Ethical Committee for Animal Care and Use from Dentistry School of Araraquara — UNESP and followed the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication no. 80-23, 1996).

2.2. Cranial surgery

Rats were anesthetized with ketamine (80 mg/kg of body weight) combined with xylazine (7 mg/kg of body weight) and placed in a Kopf stereotaxic instrument under aseptic conditions. Their skull was leveled between bregma and lambda. A stainless steel 23-gauge cannula was implanted into the lateral ventricle following the coordinates: 0.3 mm caudal to bregma, 1.5 mm lateral to the midline, and 4.0 mm below the dura mater. The tip of the cannula was positioned at a point 2 mm above the lateral ventricle. The cannula was fixed to the cranium by dental acrylic resin and jeweler screws. A 30-gauge metal obturator filled the cannula between tests. The rats were allowed to recover 6 days before drug injections into the lateral ventricle.

2.3. Injections into the lateral ventricle

Injections into the lateral ventricle were made using 10-μl Hamilton syringes connected by polyethylene tubing (PE-10) to 30-gauge injection cannulas. At time of testing, the obturator was removed and the injection cannula (2 mm longer than the guide cannulas) was introduced in the brain. The injection volume into the lateral ventricle was from 1 to 3 μl. The obturator was replaced into the cannula after injection, and the rats were returned to their home cages.

2.4. Drugs

Moxonidine hydrochloride (5, 10 and 20 nmol/1 μl, a donation of Solvay Pharma, Hannover, Germany), RX 821002 hydrochloride (2-methoxyidazoxan, 160 and 320 nmol/1 μl, Research Biomedical International, RBI, Natick, MA, USA), yohimbine hydrochloride (320 nmol/3 μl, Research Biomedical International, RBI, Natick, MA, USA), α-methylnoradrenaline (40, 80 and 160 nmol/1 μl, Sigma Chem., St Louis, MO, USA), prazosin (80 nmol/1 μl and 320 nmol/3 μl, Sigma Chem., St Louis, MO, USA) and phenylephrine (40, 80 and 160 nmol/1 μl, Sigma Chem., St Louis, MO, USA) were injected into the lateral ventricle.

Moxonidine was dissolved in acidified saline (pH adjusted to 4.6). Yohimbine, prazosin, phenylephrine, RX 821002 and α-methylnoradrenaline were dissolved in a mix of propylene glycol and water 2:1. Acidified isotonic saline or vehicle was injected as control.

2.5. Urinary excretion

Rats had water, but no food available, for 14–16 h. Then, each animal received two gastric water loads (5% of body weight each) at one-hour interval, in order to increase urine flow and thus allow reliable urine sampling. The intragastric load was done by gavage, using a polypropilene flexible tube. Thirty minutes after the first water load, prazosin, yohimbine, RX 821002 or vehicle was injected into the lateral ventricle followed 15 min later by an injection of moxonidine, α-methylnoradrenaline, phenylephrine or vehicle into the same place. Urine samples were collected in 0.1 ml graduated polypropylene tubes every 30 min during 2 h starting immediately after the second water load. The concentration of sodium and potassium in urine was measured in a sodium–potassium analyzer (Nova 1, Nova Biomedical). The amount of sodium or potassium excreted was calculated as a product of the ion concentration vs. the respective urine volume.

The effects of moxonidine (5, 10 and 20 nmol), α-methylnoradrenaline (40, 80 and 160 nmol), phenylephrine (40, 80 and 160 nmol) or vehicle were tested in three respective different groups of animals. Each group received single i.c.v. injections of each agonist, at different doses, or vehicle. The rats were submitted to a total of four tests with at least a 48 hour-interval between two tests. In each test, the group was divided in two, and each half of the group received one of the three doses of the agonist or vehicle (treatment) at random, so

Table 1
Summary of the combinations of treatments per test into the lateral ventricle

First treatment	Second treatment
Group 1	
Vehicle	Vehicle
Vehicle	Moxo (20 nmol)
RX 821002 (160 nmol)	Moxo (20 nmol)
RX 821002 (160 nmol)	Vehicle
Group 2	
Vehicle	Vehicle
Vehicle	Moxo (20 nmol)
Yohimbine (320 nmol)	Moxo (20 nmol)
Yohimbine (320 nmol)	Vehicle
Group 3	
Vehicle	Vehicle
Vehicle	Moxo (20 nmol)
Prazosin (320 nmol)	Moxo (20 nmol)
Prazosin (320 nmol)	Vehicle
Group 4	
Vehicle	Vehicle
Vehicle	α-Methylnor (80 nmol)
RX 821002 (320 nmol)	α-Methylnor (80 nmol)
RX 821002 (320 nmol)	Vehicle
Group 5	
Vehicle	Vehicle
Vehicle	α-Methylnor (80 nmol)
Prazosin (320 nmol)	α-Methylnor (80 nmol)
Prazosin (320 nmol)	Vehicle
Group 6	
Vehicle	Vehicle
Vehicle	Phenyl (80 nmol)
Prazosin (80 nmol)	Phenyl (80 nmol)
Prazosin (80 nmol)	Vehicle