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GABA mechanisms of the nucleus of the solitary tract regulates the cardiovascular and sympathetic effects of moxonidine

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ABSTRACT

The antihypertensive drugs moxonidine and clonidine are α_2-adrenoceptor and imidazoline (I_1) agonists. Previous results from our laboratory have shown that moxonidine can act in the commissural nucleus of the solitary tract (commNTS). In addition, some studies have shown that GABA or glutamate receptor blockade in the RVLM blunted the hypotension produced by these antihypertensive agents in spontaneously hypertensive rats. Therefore, in the present study we verify whether the cardiovascular and sympathetic effects produced by moxonidine in the commNTS are dependent on GABAergic or glutamatergic mechanisms. Mean arterial pressure (MAP) and splanchnic sympathetic nerve activity (sSNA) were recorded in urethane-anesthetized, and artificially-ventilated male Wistar rats (250–350 g). Injection of the GABAA antagonist bicuculline (25 pmol/50 nL) into the commNTS reduced the hypotension as well as the sympathoinhibition elicited by moxonidine. Prior injection of the glutamate receptor antagonist kynurenic acid (2.5 nmol/50 nL) into the commNTS was not effective in reducing the hypotension and sympathoinhibition elicited by moxonidine. Therefore, we conclude that the hypotensive and sympathoinhibitory effects elicited by microinjection of moxonidine into the commNTS are dependent on GABA receptors, but not ionotropic glutamate receptors.

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1. Introduction

It is well established that the nucleus of the solitary tract (NTS) is considered the site of the first synapse of the visceral sensory inputs to the brainstem (Dampney, 1994; Guyenet, 2006). One of the neurotransmitter released by these afferents in the NTS is the L-glutamate (Talman et al., 1980; Dampney, 1994). Anatomical and immunohistochemical studies have shown that, besides the baroreflex pathway, the NTS sends monosynaptic inputs to the rostral ventrolateral medulla/C1 region (RVLM/C1) (Hancock, 1988; Morilak et al., 1989; Otake et al., 1992) and these projections may convey peripheral chemoreceptor signals (Colombari et al., 1996; Koshiya and Guyenet, 1996). The existence of pressor mechanisms in the NTS is supported by the increase in arterial pressure produced by L-glutamate injections into the NTS in conscious rats (Machado and Bonagamba, 1992; Colombari et al., 1994).

The NTS and the RVLM/C1 are considered the main brainstem regions of centrally acting antihypertensive drugs (Ernsberger et al., 1997; Guyenet, 1997; Ernsberger and Haxhiu, 1997; Totola et al., 2013). Preliminary results from our laboratory depicted that moxonidine act on α_2-adrenoceptors (α_2-AR) in the commissural aspect of the NTS (commNTS) to produce hypotension and sympathoinhibition in anesthetized and conscious rats (Totola et al., 2013).

Besides the α_2-adrenergic mechanisms related to the action of antihypertensive drugs such as moxonidine and clonidine, several studies have indicated that several neurotransmitters such as GABA (γ-aminobutyric acid) and glutamate are involved in the sympathoinhibition and hypotension elicited by moxonidine or clonidine (Tingley and Arnerić, 1990; Milhaud et al., 2000). Considering the existing evidence, our hypothesis is that GABAergic and glutamatergic mechanism might be responsible to the antihypertensive effects elicited by moxonidine into commNTS. However, due to the importance of GABA as well as glutamate receptors into the NTS for cardiovascular regulation, it might be interesting to investigate whether GABAergic and glutamatergic mechanisms are also important to mediate the antihypertensive effects of moxonidine into the commNTS.

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2. Materials and methods

2.1. Animals

Experiments were performed in 37 adult male Wistar rats weighing 280–320 g. All experimental protocols were in accordance with the Ethical Principles in Animal Research of the Brazilian College of Animal Experimentation (CONCEA) and were approved by the Ethics Committee for Animal Research of the Institute of Biomedical Sciences of the University of Sao Paulo (CEUA: Authorization No. 85/2010).

2.2. Surgery and anesthesia

Rats were deeply anesthetized with halothane (5% in 100% oxygen inspired air) for general surgical procedures, such as: a) tracheostomy for artificial ventilation; b) femoral artery and vein catheterization for arterial pressure measurement and administration of fluids and drugs, respectively; c) intraparenchymal injection by removal of the occipital bone and retracting the underlying dura-mater membrane for insertion of a pipette into the medulla oblongata via a dorsal transcerebellar approach (Takakura and Moreira, 2011); d) splanchnic sympathetic nerve isolation for subsequent nerve activity monitoring. The level of anesthesia was checked by a flexor reflex to the animal's paw pinching.

Splanchnic sympathetic nerve activity (sSNA) was recorded as previously described (Takakura and Moreira, 2011). Briefly, the right splanchnic nerve was isolated via a retroperitoneal approach, and the segment distal to the suprarenal ganglion was placed on a pair of Teflon-coated silver wires that had been bared at the tip (250 μm bare diameter; A-M Systems, www.amsystems.com). The nerves and wires were embedded in adhesive material (Kwik-Cast Sealant, WPI, USP, Sarasota, FL, USA), and the wound was closed around the exiting recording wires.

Upon completion of the surgical procedures, halothane was replaced by urethane (1.2 g/kg of body weight) slowly administered intravenously (i.v.). All rats were artificially ventilated with 100% oxygen throughout the experiment. The rectal temperature was maintained at 37 °C and the end tidal-CO_2were monitored throughout the experiment with a capnometer (CWE, Inc., Ardmore, PA, USA) that was calibrated twice per experiment against a calibrated CO_2/N_2mix. The adequacy of the anesthesia was monitored during a 20-min stabilization period by testing for the absence of withdrawal response and the lack of arterial pressure change to firm toe pinch. After these criteria were satisfied, the muscle relaxant pancuronium was administered at the initial dose of 1 mg/kg i.v. and the adequacy of anesthesia was thereafter gauged solely by the lack of increase in arterial pressure to firm toe pinch. Approximately hourly supplements of one-third of the initial dose of urethane were needed to satisfy these criteria during the course of the recording period (80 min).

2.3. In vivo recordings of physiological variables

Mean arterial pressure (MAP) and the discharge of the splanchnic nerve (sSNA) and the tracheal CO_2 were recorded as previously described (Taxini et al., 2011). Before starting the experiments, the ventilation was adjusted to have the end-expiratory CO_2 at 3–4% at steady-state (60–80 cycles/s; tidal volume 1–1.2 ml/100 g). All analog data (end-expiratory CO_2, MAP and sSNA) were stored on a computer via a micro 1401 digitizer (Cambridge Electronic Design, Cambridge, UK) and were processed off-line using version 6 of the Spike 2 software (Cambridge Electronic Design) as described previously (Takakura and Moreira, 2011; Taxini et al., 2011). The integrated splanchnic nerve activity (iSNA) was obtained after the rectification and smoothing ($\sigma =$ 2 s) of the original signal, which was acquired with a 30–3000 Hz band pass. The iSNA was normalized for each animal by assigning the value of 100 to the resting SNA and the value of 0 to the minimum value recorded after the administration of the ganglionic blockade (hexamethonium: 30 mg/kg, i.v.).

2.4. Intraparenchymal injections

All drugs were purchased from Sigma-Aldrich (Sigma Chemicals Co.) unless otherwise stated. Bicuculline methiodide (Bic: 25 pmol/50 nL), kynurenic acid (kyn: 2.5 nmol/50 nL) and moxonidine hydrochloride (moxo: 5 nmol/50 nL in sterile saline pH 7.4) were pressure injected (Picospritzer III, Parker Hannifin Corp, USA) (50 nL in 5 s) through single-barrel glass pipettes (20 μm tip diameter). Injections into the commNTS were made 400 μm caudal to the *calamus scriptorius*, in the midline and 0.3 to 0.5 mm below the dorsal surface of the brainstem. A mix of propylene glycol/water 2:1 was used as vehicle for bicuculline and moxonidine because these drugs are not soluble in saline. The solution of moxonidine, bicuculline and kynurenic acid contained a 5% dilution of fluorescent latex microbeads (Lumafluor, New City, NY, USA) for later histological identification of the injection sites (Takakura and Moreira, 2011; Takakura et al., 2011).

2.5. Histology

At the end of the experiment, the rats were deeply anesthetized with urethane and perfused through the heart with PBS (pH 7.4) followed by paraformaldehyde (4% in 0.1 M phosphate buffer, pH 7.4). The brains were removed and stored in fixative for 24 h at 4 °C. The medulla was cut in 40 μm thick coronal sections with a vibrating microtome (Vibratome 1000S Plus, USA). Sections were stored at −20 °C in a cryoprotectant solution (Schreihofer and Guyenet, 1997). The injections sites were confirmed with an Axioskop 2 microscope (Zeiss, Oberkochen, Germany). The section alignment between the brains was done relative to a reference section. To align the sections around NTS level, the mid-area postrema level was identified in each brain and assigned the level 13.8 mm (Bregma −13.8 mm) according to the atlas of Paxinos and Watson (1998). The coordinates of sections rostral and caudal of this reference section were calculated with respect to the reference section, using the number of intervening sections and the section thickness.

2.6. Experimental protocol

2.6.1. Effects of the combination of bicuculline and moxonidine injected into the commNTS on arterial pressure and sympathetic outflow

All experiments were performed in rats anesthetized with urethane (1.2 g/kg, i.v.). Recordings began 10 min after the connection of the arterial line to the pressure transducer. MAP and sSNA were continuously recorded for 80 min and were analyzed every 10 min. Control (baseline) values were recorded for 10 min and were analyzed immediately before the bicuculline (25 pmol/50 nL) or vehicle injection (first treatment). These values were used as a reference to calculate the changes produced by the treatments. After 10 min, moxonidine (5 nmol/50 nL) or vehicle was injected into the commNTS and the MAP and sSNA responses were evaluated for the next hour.

2.6.2. Effects of the combination of kynurenic acid and moxonidine injected into the commNTS on arterial pressure and sympathetic outflow

All experiments were performed in rats anesthetized with urethane (1.2 g/kg, i.v.). Recordings began 10 min after the connection of the arterial line to the pressure transducer. MAP and sSNA were continuously recorded for 80 min and were analyzed every 10 min. Control (baseline) values were recorded for 10 min and were analyzed immediately before the kynurenic acid (2.5 nmol/50 nL) or vehicle injection (first treatment). These values were used as a reference to calculate the changes produced by the treatments. After 10 min, moxonidine (5 nmol/50 nL) or vehicle was injected into the commNTS and the MAP and sSNA responses were evaluated for the next hour.

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