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COMMISSURAL NUCLEUS OF THE SOLITARY TRACT REGULATES THE ANTIHYPERTENSIVE EFFECTS ELICITED BY MOXONIDINE

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Abstract—The rostral ventrolateral medulla (RVLM) contains the presympathetic neurons involved in cardiovascular regulation that has been implicated as one of the most important central sites for the antihypertensive action of moxonidine (an α2-adrenergic and imidazoline agonist). Here, we sought to evaluate the cardiovascular effects produced by moxonidine injected into another important brainstem site, the commissural nucleus of the solitary tract (commNTS). Mean arterial pressure (MAP), heart rate (HR), splanchnic sympathetic nerve activity (sSNA) and activity of putative sympathoexcitatory vasomotor neurons of the RVLM were recorded in conscious or urethane-anesthetized, and artificial ventilated male Wistar rats. In conscious or anesthetized rats, moxonidine (2.5 and 5 nmol/50 nl) injected into the commNTS reduced MAP, HR and sSNA. The injection of moxonidine into the commNTS also elicited a reduction of 28% in the activity of sympathoexcitatory vasomotor neurons of the RVLM. To further assess the notion that moxonidine could act in another brainstem area to elicit the antihypertensive effects, a group with electrolytic lesions of the commNTS or sham and with stainless steel guide-cannulas implanted into the 4th V were used. In the sham group, moxonidine (20 nmol/1 μl) injected into 4th V decreased MAP and HR. The hypotension but not the bradycardia produced by moxonidine into the 4th V was reduced in acute (1 day) commNTS-lesioned rats. These data suggest that moxonidine can certainly act in other brainstem regions, such as commNTS to produce its beneficial therapeutic effects, such as hypotension and reduction in sympathetic nerve activity.

Key words: commissural NTS, sympathetic activity hypertension, moxonidine.

INTRODUCTION

It is well establish that the nucleus of the solitary tract (NTS) is considered the site of the first synapse of the visceral sensory inputs in the brainstem, including those related to baro-, chemo- and cardiopulmonary reflexes (Dampney, 1994; ; Guyenet, 2006). The neurotransmitter released by these afferents in the NTS is suggested to be L-glutamate (Talman et al., 1980; Dampney, 1994). From the NTS, the baroreceptor afferent signals project to the caudal ventrolateral medulla (CVLM) (Sved et al., 2000; Schreihofer and Guyenet, 2002). Through GABAergic mechanisms, the CVLM inhibits the presympathetic neurons in the rostral ventrolateral medulla/C1 region (RVLM/C1) that innervate the preganglionic sympathetic neurons involved in controlling the heart and the vasculature (Morrison et al., 1991; Jeske et al., 1993, 1995).

Parallel to the inhibitory mechanisms, the RVLM/C1 also receives important excitatory projections (Ito and Sved, 1997; Horiuchi et al., 2002; Moreira et al., 2005). Anatomical and immunohistochemical studies have shown that the NTS sends monosynaptic inputs to the 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 (Colombari et al., 1994). Although L-glutamate injected into the NTS in anesthetized rats usually reduces arterial pressure, similar to baroreflex activation, L-glutamate into the NTS induces pressor responses in anesthetized rats after the inhibition of the CVLM with muscimol (Urbanski and Sapru, 1988). This pressor response to L-glutamate into the NTS in anesthetized rats is abolished by the blockade of excitatory amino acid (EAA) receptors in the RVLM/C1, which suggests the existence of a pressor pathway from the NTS to the RVLM/C1 (Urbanski and Sapru, 1988; Zagon and Spyer, 1996).

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Abbreviations: AoC, abdominal aortic; α2-AR, alpha2-adrenergic receptor; commNTS, commissural nucleus of the solitary tract; CVLM, caudal ventrolateral medulla; EAA, excitatory amino acid; HR, heart rate; I1-R, imidazoline receptor; iSNA, integrated splanchnic nerve activity; MAP, Mean arterial pressure; NTS, nucleus of the solitary tract; PAP, pulsatile arterial pressure; RVLM, rostral ventrolateral medulla; SHR, spontaneously hypertensive rat; SNA, sympathetic nerve activity; sSNA, splanchnic sympathetic nerve activity; TH, tyrosine hydroxylase; TH-ir, tyrosine hydroxylase-immunoreactive.

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Because the brainstem areas involved in the control of sympathetic nervous system plays a key role in the pathophysiology of hypertension and other cardiovascular diseases, more recently a centrally acting sympathetic agent is therefore attractive not only for lowering blood pressure, but also intervening with multiple disease processes. Moxonidine, an alpha2-adrenergic receptor (α2-AR) and imidazoline receptor (I1-R) agonist, are a central acting antihypertensive drug that reduces arterial pressure due to decreasing sympathetic nerve activity (SNA) (Ernsberger et al., 1993, 1994, 1997; Guyenet, 1997). The RVLM/C1 contains the presympathetic neurons involved in cardiovascular regulation (Barman and Gebber, 1989; Guyenet et al., 1989) that has been implicated as one of the most important central sites for the antihypertensive action of moxonidine (Ernsberger et al., 1993, 1994). Evidence indicates that the blockade of α_2-AR and I1-R within the RVLM/C1 region reduces the antihypertensive effects produced by moxonidine or clonidine, suggesting that the antihypertensive effect of these drugs is mainly due to a possible action in the RVLM/C1 region (Ernsberger et al., 1993, 1994, 1997; Guyenet, 1997).

Preliminary results from our laboratory showed that moxonidine can also act in other brainstem regions, as the commissural nucleus of the solitary tract (commNTS), a site that receives mainly the afferents of peripheral chemoreceptors, (Koshiya and Guyenet, 1996; Moreira et al., 2006), to produce hypotension and sympathoinhibition in conscious and anesthetized rats. Given the evidences described above, our hypothesis is that moxonidine can act in a different region of the brainstem, besides RVLM/C1 region, to produce hypotension and sympathoinhibition. Here we show that moxonidine can certainly act in the commNTS to produce its beneficial therapeutics effects, such as hypotension and reduction in SNA in both anesthetized and unrestrained awake rats.

EXPERIMENTAL PROCEDURES

Animals

Experiments were performed in 82 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 and were approved by the Ethics Committee for Animal Research of the Institute of Biomedical Sciences of the University of Sao Paulo (Authorization No. 85/2010).

Surgical procedures

Conscious animals. Rats were anesthetized with intraperitoneal (i.p.) injection of ketamine (80 mg/kg of body wt) combined with xylazine (7 mg/kg of body wt) and placed in a stereotaxic frame (model 900; David Kopf Instruments, Tujunga, CA, USA). In the first group of rats, a partial craniotomy of the occipital bone was performed, and the dorsal surface of the brainstem was exposed. A tungsten electrode (0.1 mm in diameter) bared at the tip (0.5 mm) was inserted into the brain 0.1 and 0.5 mm caudal to the *calamus scriptorius*, in the midline, and 0.4 mm below the dorsal surface of the brainstem. Electrolytic lesions were performed using a cathodal current of 1 mA during 10 s in each one of the two stereotaxic coordinates cited above as previously described (Moreira et al., 2009). A clip attached to the tail was used as the indifferent electrode. Sham-lesioned rats were submitted to the same surgical procedures and had the electrode placed along the same coordinates, except that no current was passed. After the electrolytic or sham lesion, a stainless steel guide-cannula was implanted into the 4th V using the following coordinates: 12.7 mm caudal to bregma, in the midline and 7.0 mm below the duramater.

The next group of rats received stainless steel guide-cannulas implanted into the commNTS using the following coordinates: 15.0 mm caudal to bregma, in the midline and 7.5 mm below dura mater. The cannulas were fixed to the cranium using dental acrylic resin and jeweler screws. Rats received a prophylactic dose of penicillin (30,000 IU) given intramuscularly and a subcutaneous injection of the analgesic Ketoflex (ketoprofen 1%, 0.03 ml/rat) post-surgically. After the surgery, the rats were maintained in individual cages with free access of tap water and food pellets [Guabi rat chow (Paulinia, SP, Brazil)] for at least 5–7 days before the experiments.

To record pulsatile arterial pressure (PAP), mean arterial pressure (MAP) and heart rate (HR) in unanesthetized freely moving rats, one day before the experiments, rats were anesthetized again with i.p. injection of ketamine combined with xylazine to receive a polyethylene tubing (PE-10 connected to PE-50; Clay Adams, Parsippany, NJ, USA) inserted into the abdominal aorta through the femoral artery. Another polyethylene tubing was also inserted into the femoral vein for drug administration. Both cannulas were tunneled subcutaneously to the back of the rats to allow access in unrestrained, freely moving rats.

Anesthetized animals. 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. In a few cases (n = 6), the brachial artery was cannulated to measure arterial pressure and a brachial vein was cannulated to administer fluids and drugs. An inflatable snare was placed around the abdominal aorta just below the diaphragm to permit rapid control of upper body arterial pressure; (c) intracerebral injection by removal of the occipital bone and retracting the underlying duramater 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.