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Angiotensin 1-7 in the rostro-ventrolateral medulla increases blood pressure and splanchnic sympathetic nerve activity in anesthetized rats

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ABSTRACT

Angiotensin 1-7 (ANG-(1-7)), a derivative of angiotensin I or II, is involved in the propagation of sympathetic output to the heart and vasculature, and the receptor for ANG-(1-7), the Mas receptor, is expressed on astrocytes in the rostral ventrolateral medulla (RVLM). We recorded blood pressure (BP) and splanchnic sympathetic nerve activity (SSNA) before and after focal injection of ANG-(1-7) into the RVLM of rats. Unilateral injection of ANG-(1-7) into the RVLM, acting through the Mas receptor, increased SSNA and BP, and glutamate receptor antagonists, CNQX and D-AP5, partially reduced the ANG-(1-7) effect. ATP is often co-released with glutamate, and blocking ATP with PPADS also reduced the pressor response to microinjection of ANG-(1-7) within the RVLM. The effects of ANG-(1-7) were blocked by the MAS receptor antagonist, A-779 (which had no consistent effect on blood pressure or sympathetic nerve activity when injected on its own). We conclude that astrocytes in the RVLM participate in central, angiotensin-dependent regulation of blood pressure and sympathetic nerve activity, and the Mas receptor, when activated by ANG-(1-7), elicits the release of the gliotransmitters, glutamate and ATP. These gliotransmitters then cause an increase in sympathetic nerve activity and blood pressure by interacting with AMPA/kainate and P2X receptors in the RVLM.

1. Introduction

Central processing of cardiovascular afferent nerve signaling is organized in the nucleus tractus solitarius (NTS), which then regulates sympathetic tone by stimulating the caudal portion of the ventrolateral medulla (CVLM) to inhibit excitatory neurons in the RVLM. Excitatory neurons of the RVLM project to the intermediolateral nucleus (IML) and consist of the C1 cell group and non-C1 neurons (Granata et al., 1983; Hokfelt et al., 1974; Jeske and McKenna, 1992; Kapoor et al., 1992). The C1 adrenergic cell group is thought to be the primary source of innervation of the IML from the RVLM, but this is an area of contention (Lipski et al., 1995; Ruggiero et al., 1994; Schreihofer and Guyenet, 1997). There are catecholamines released at the spine (Head and Howe, 1987; Sevigny et al., 2008); however the principle excitatory neurotransmitter secreted by all RVLM vasomotor cells at the IML is glutamate (Bazil and Gordon, 1993; Llewellyn-Smith et al., 1998; Verberne et al., 1990). Since the 1970s, there has been increasing interest in the role of angiotensin peptides in the central nervous system (CNS) and central regulation of blood pressure and sympathetic nerve activity. The octapeptide angiotensin II (ANG II) acts in many parts of the CNS and plays a critical role in the regulation of cardiovascular function, particularly in the RVLM (Allen et al., 1988; Casto and Phillips, 1984; Rettig et al., 1986). ANG II is derived from angiotensin I, and both of these peptides can be cleaved into a variety of smaller peptides, some of which are biologically active (Newman, 2003; Parri et al., 2001; Ralevic et al., 1999). One of these smaller peptides, angiotensin-(1-7) (ANG-(1-7)), also has a role in the CNS control of cardiovascular function, though the physiological actions of ANG-(1-7) are less well studied.

Increased levels of ANG II in the RVLM are implicated in both hypertension (Gao et al., 2004) and the enhanced sympathetic tone present in chronic heart failure (CHF) (Liu et al., 2006). The effects of ANG-(1-7) in these diseases are unknown. The production of ANG-(1-7) is dependent on the enzyme ACE-2, and when ACE-2 was overexpressed in hypertensive rats, arterial blood pressure was reduced. This is likely due at least in part to loss of direct effects of ANG II (Wang et al., 2014; Yamazato et al., 2007), but is also consistent with the hypothesis that the ANG-(1-7) produced by ACE-2 mediated ANG II degradation may directly depress blood pressure (BP). However, microinjection of ANG-(1-7) into the RVLM actually increased BP (Fontes et al., 1994; Guo et al., 2010; Potts et al., 2000)}. ACE-2 can generate ANG-(1-7) by cleaving a phenylalanine from the carboxy terminal end of ANG II (Donoghue et al., 2000; Lazartigues et al., 2007) or by cleaving angiotensin-1 into ANG-(1-9), which can then be transformed by ACE into ANG-(1-7) (Donoghue et al., 2000; Lazartigues et al., 2007). If the

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pressor effect of ANG II is more robust than that of ANG-(1-7) (Fontes et al., 1994), then the net effect of generating ANG-(1-7) by increased ACE-2 activity would be to lower BP, since the pressor effect of ANG II would decrease more than the pressor effect of ANG-(1-7) would increase (Wang et al., 2014; Yamazato et al., 2007). Thus, ANG-(1-7) may exert a net pressor effect in the RVLM while still being associated with hypotension when its production is induced by increased ACE-2 activity.

There is evidence that astrocytes interact with neurons in the RVLM (Guo et al., 2010) and are likely involved in the enhanced excitatory response from these neurons in the pathological states of hypertension and CHF. Astrocytes release several different substances that may affect neuronal function including neurotransmitters such as glutamate, ATP, and D-serine and proteins such as TNFα (Perea and Araque, 2005). The receptor for ANG-(1-7), the Mas receptor, is a G-protein coupled receptor (GPCR) that is functional on astrocytes, but not neurons in the RVLM (Guo et al., 2010), even though the Mas receptor may be expressed on neurons (Becker et al., 2007). Therefore, we sought to determine the specific involvement of ANG-(1-7) and glutamate in the interaction between astrocytes and neurons of the RVLM. Our hypothesis was that ANG-(1-7) causes release of glutamate from the astrocytes of the RVLM, which in turn excites neurons within the RVLM (presumably the C1 neurons) and increases sympathetic output. If this were the case, the glutamate receptor antagonists CNQX and D-AP5 should block any pressor effect caused by the microinjection of ANG-(1-7). Conversely, injecting the Mas receptor antagonist D-Ala^7-ANG-(1-7) (A-779) should attenuate the pressor effect of ANG-(1-7), but should have no effect on the pressor response to injected glutamate. To test these hypotheses, we examined the blood pressure and sympathetic nervous system responses to unilateral injection of ANG-(1-7) directly into the RVLM of normotensive rats. We confirmed that microinjection of ANG-(1-7) increased both BP and sympathetic output. When we injected ANG-(1-7) into the RVLM while blocking the AMPA and NMDA glutamate receptors with their respective antagonists this increase was partially blocked, which confirmed that ANG-(1-7) acted through a glutamatergic mechanism. However, glutamate antagonists incompletely blocked the ANG-(1-7) pressor response, and we tested the hypothesis that ATP, co-released with glutamate from astrocytes, has a pressor and sympatho-excitatory effect. Finally, we investigated the involvement of the Mas receptor in this cascade of events by blocking it with its antagonist, A-779. A-779 blocked ANG-(1-7) pressor effects, but had no consistent effect on blood pressure or sympathetic nerve activity on its own.

2. Materials and methods

2.1. Animals

All experiments were carried out on male Sprague-Dawley rats weighing between 280 and 320 g. Rats were obtained from Harlan Laboratories and housed in the Center for Comparative Medicine and Research facilities at Dartmouth College. The experimental protocol was approved by the Institutional Animal Care and Use Committee at Dartmouth. The rats were kept in a temperature controlled room on a 12 h–12 h light-dark cycle with free access to standard chow and tap water.

2.2. Surgical procedure

The rats were anesthetized with an IP injection of chloralose (40 mg/kg) and urethane (1200 mg/kg). Once the rat was unresponsive, we exposed the left femoral artery and inserted a cannula (26G PTFE tubing), which we advanced into the aorta until the tip was approximately 1 cm above the aortic bifurcation. The cannula was attached to a pressure transducer and amplifier (BP-1, World Precision Instruments, Sarasota, FL) to measure arterial blood pressure. We also placed a cannula in the left femoral vein to administer fluids and drugs. We performed a tracheotomy and placed the rat on a ventilator (Model 683, Harvard Apparatus, Holliston, MA). We set the respiratory rate at 100 bpm and a tidal volume of 2.5 ml and used an equal mixture of room air and O_2. We next fixed the rat's head in a stereotaxic device (Kopf Instruments, Tujunga, CA) with the bite bar set at −3.9 mm. Last, we created a pneumothorax to minimize movement artifact in our nerve recording.

2.3. Splanchnic nerve recordings

We made a retroperitoneal incision to expose the splanchnic nerve, and isolated a 0.5 cm section of the nerve by inserting a strip of parafilm beneath it just distal to the celiac ganglion. We placed silver bipolar recording electrodes under the nerve and insulated the connection with silicone elastomer (KwikSil, WPI). Once the nerve-electrode connection was firmly set, we cut the nerve immediately distal to the electrodes to eliminate afferent traffic. The electrophysiological signals were amplified (DAM 80, WPI) and filtered with the high pass set at 10 Hz and the low pass set at 3 KHz. In addition, the signal was filtered through a Hum Bug device (Quest Scientific, North Vancouver, BC, Canada). The signal was digitized at 6 kHz, integrated and smoothed (PowerLab, ADInstruments, Australia) before being stored on a computer for subsequent analysis.

2.4. RVLM microinjections

We made injections into the RVLM with a 10 µl syringe fitted with a 33 gauge needle using the co-ordinates (from Lambda) 2.1 mm lateral, 3.2 mm caudal and 10.2 mm ventral. We gave the injections at a rate of 10 nl/sec with an UMP 3 microinjection pump (WPI). All the injections included fluorescent latex microbeads (0.40 µm, Polysciences, Inc., Warrington, PA). After euthanizing the rats with an IV bolus of urethane, we removed the brain. We froze the brains at −80 °C and later cut forty-five micron coronal sections using a Leica cryostat. We mounted the slices on gelatin-coated glass slides and imaged them using fluorescent microscopy, and recorded the location of injection sites using a rat stereotaxic brain atlas (Paxinos and Watson, 2005). We segregated microinjections into those in which the fluorescent beads were in the RVLM ('hits') and those in which the fluorescent beads were outside the RVLM ('misses'). We defined the RVLM as the region between rostro-caudal distances −11.88 mm to −12.84 mm from bregma, lateral distances 1.80 mm to 2.60 mm and dorso-ventral distances 9.50 mm to 10.80 mm. Since the drugs we tested are likely to diffuse greater distances than the fluorescent beads, any beads that were on the border of this RVLM region were considered hits; we erred on the side of RVLM inclusion rather than exclusion.

2.5. Reagents

We dissolved all the drugs in saline with ~1% micro-beads and titrated the vehicle to pH 7.4 ± 0.05. We purchased the Angiotensin-(1–7), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), (2*R*)-amino-5-phosphonopentanoate (D-AP5), and pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid (PPADS) from Tocris bioscience, Bristol, UK, the A-779 from Abcam Inc., Cambridge, MA., and the glutamate and sodium nitroprusside from Sigma-Aldrich, St. Louis, MO.

2.6. Experimental protocol

Once the animal was instrumented and the microinjection needle was inserted into the RVLM, we waited until the BP and splanchnic sympathetic nerve activity (SSNA) signals were stable and began recording. We recorded two minutes of stable BP and SSNA before starting injections.

For the initial ANG-(1-7) experiments, we injected 50 nl of either