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Research Report

Anteroposterior distribution of AT_1 angiotensin receptors in caudal brainstem cardiovascular regulatory centers of the rat

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ABSTRACT

Angiotensin II acts on Ang II type 1 (AT_1) receptors in areas of the caudal brainstem involved in cardiovascular regulation. In particular, activation of AT_1 receptors in the rostral ventrolateral medulla (RVLM) has been suggested to contribute to hypertension. However, the characteristics of AT_1 receptors in the RVLM of rat, the species in which the most experimental work has been done, are not well documented. This study evaluated AT_1 receptor binding along a 2.7-mm length of rat medulla, which included the full extent of the RVLM and the caudal ventrolateral medulla (CVLM). Sections of medulla from female rats cut on a cryostat were incubated with five concentrations of ^{125}I-sarcosine^1, isoleucine^8 angiotensin II to assess the density (B_{max}) and dissociation constant (K_D) of the receptors for the radioligand. The dorsomedial medulla (DMM) displayed a high density of AT_1 binding (1207±100 fmol/g), which peaked at 0.4 mm rostral to the calamus scriptorius (approximately 14 mm caudal to Bregma). The RVLM and CVLM displayed significantly lower ($p<0.01$) densities of AT_1 binding, 278±38 and 379±64 fmol/g, respectively. However, the dissociation constants were significantly lower (i.e., higher affinity) in RVLM and CVLM (164±38 and 178±27 pM, respectively,) than in DMM (328±12 pM, $p<0.01$ and $p<0.05$, respectively). These results provide an anatomical and pharmacological framework for future studies on the role in cardiovascular regulation of AT_1 receptors in the caudal brainstem.

1. Introduction

Angiotensin II (Ang II), long known for its peripheral cardiovascular effects (Kaschina and Unger, 2003; Laragh et al., 1975), is now recognized as having significant effects on the cardiovascular system via its actions in the brain (Bourassa et al., 2008; Carlson and Wyss, 2008; McKinley et al., 2003; Phillips and de Oliveira, 2008; Sved et al., 2003; Veerasingham

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Abbreviations: 125I-SI Ang II, 125I-sarcosine1,isoleucine8-Angiotensin II; Ang II, Angiotensin II; AT1, Angiotensin II type 1; Bmax, Receptor concentration; CVLM, Caudal ventrolateral medulla; DMM, Dorsomedial medulla; KD, Dissociation constant; MnA, Median accessory nucleus of the medulla; NTS, Solitary tract nucleus; RVLM, Rostral ventrolateral medulla; SHR, Spontaneously hypertensive rat; VLM, Ventrolateral medulla; WKY, Wistar–Kyoto

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and Raizada, 2003). Within the caudal brainstem, there is evidence that Ang II acts in the dorsomedial medulla (DMM, including the nucleus of the solitary tract (NTS) and area postrema), rostral ventrolateral medulla (RVLM), and caudal ventrolateral medulla (CVLM) to regulate the cardiovascular system. The area postrema is a circumventricular organ which is reported to be sensitive to blood-borne Ang II (Bishop and Hay, 1993; Joy and Lowe, 1970). *In vitro* studies have shown that area postrema neurons are responsive to Ang II (Consolimcolombo et al., 1996; Hay and Lindsley, 1995; Sun and Ferguson, 1996). Injection of Ang II into the NTS region of the DMM (Diz et al., 1997; Fow et al., 1994; Katsunuma et al., 2003; Tan et al., 2005) or the CVLM (Alzamora et al., 2006; Muratani et al., 1991) decreases blood pressure whereas injection of Ang II into the RVLM increases it (Allen et al., 1988; Andreatta et al., 1988). In each instance, this action of Ang II appears to be mediated by an action on the AT_1 subtype of angiotensin receptors as AT_1 selective blockade abolishes these responses (Averill et al., 1994; Fontes et al., 1997; Ito et al., 2002; Tagawa and Dampney, 1999).

The actions of Ang II on caudal brainstem AT_1 receptors are receiving increasing attention because of the possibility that they are major contributors to hypertension. For example, in the spontaneously hypertensive rat (SHR) model of hypertension, Ang II injection into the RVLM increases blood pressure significantly more than in the Wistar–Kyoto (WKY) strain. Also, AT_1 receptor blockade in the RVLM produces a significant depressor response in the SHR, but fails to produce any significant effect in the WKY (Ito et al., 2002; Muratani et al., 1991). Similar responses are also seen in the Dahl-salt sensitive rat model of hypertension (Ito et al., 2003). This suggests that activation of ventrolateral brainstem AT_1 receptors contributes to hypertension in these animal models, but in normotensive animals these AT_1 receptors are not tonically activated under resting conditions. In line with this, it has been shown that expression of a constitutively active AT_{1a} receptor in the RVLM of normotensive WKY rats increased blood pressure significantly, whereas overexpression of the wild-type AT_{1a} receptor did not (Allen et al., 2006). Heightened responsiveness of the RVLM to Ang II can even be induced in normotensive animals with an elevated salt intake (Adams et al., 2008).

Despite this increasing interest in AT_1 receptors in the ventrolateral medulla (VLM), these receptors have not been adequately characterized in this region of the rat brain. Indeed, whereas autoradiographic studies of Ang II binding have provided evidence of AT_1 binding sites in the VLM, the specific localization to RVLM or CVLM has not been discriminated. Two studies have attempted to quantitate the AT_1 receptor binding found in the VLM; one of these studies presents data in the VLM without clearly distinguishing between RVLM and CVLM, and in fact the figure presented appears to be in a transition zone between the two (Allen et al., 1987), while the other (Song et al., 1992) describes sampling as far dorsal as the NTS.

The existing studies of Ang II binding sites in the medulla have focused on the distribution of these sites, assuming similar binding characteristics among the different regions. However, this has left open the question of whether binding affinities are the same in these different regions. The purpose of this study was to determine the density of the AT_1 receptors in the RVLM, CVLM, and DMM regions of the rat using quantitative densitometric receptor autoradiography with saturation isotherm analysis to determine receptor concentration (B_{max}) and dissociation constant (K_D) of the receptors.

2. Results

2.1. *Saturation receptor autoradiography*

A major goal of the present study was to determine B_{max} and K_D of the AT_1 receptor in the CVLM and RVLM of the rat caudal brainstem at different anterior–posterior coordinates with a concurrent comparison to the DMM (Fig. 1). Specific AT_1 binding was noted as a rostrocaudal column in the DMM region and in the VLM. AT_1 binding was also noted stretching between these two regions in the reticular formation as well as in the spinal trigeminal nucleus. For the DMM and VLM regions, at each coordinate noted, a saturation isotherm was constructed and B_{max} and K_D were derived. Fig. 2 shows autoradiographic images of the ^{125}I-SI Ang II saturation binding in the DMM and CVLM. Fig. 3 shows autoradiographic images of ^{125}I-SI Ang II saturation binding in the RVLM. Demonstrative saturation isotherms for the DMM, CVLM, and RVLM are shown in Fig. 4. Fig. 5 shows B_{max} for each brain region along the anteroposterior axis.

In the DMM, the B_{max} peaks approximately 0.4 mm rostral to calamus scriptorius (>1800 fmol/g wet weight) before leveling off at approximately 1.4 mm rostral to calamus scriptorius to ~750 fmol/g wet weight. In the VLM (both caudal and rostral), the B_{max} is significantly lower ($p<0.001$) than in the DMM averaging around 330 fmol/g wet weight. The K_D did not vary along the anteroposterior axis in any of the brain regions studied (data not shown), however the K_Ds of the CVLM and RVLM were significantly less than that of the DMM ($p<0.01$), but did not significantly differ from each other. Also, the B_{max} did not differ significantly between the CVLM and the RVLM. Table 1 summarizes the B_{max} and K_D data for the three brain regions.

There was a substantial amount of ^{125}I-SI Ang II binding in the cerebellum (Fig. 1). However, most of this binding is not displaceable by 3 μM Ang II and it was not evaluated in this study.

3. Discussion

This is the first study utilizing receptor binding autoradiography to fully characterize AT_1 receptor binding in the regions of the rat medulla that are critically involved in cardiovascular regulation. The key findings of this study are: (1) in addition to the previously characterized AT_1 receptor binding in the DMM, AT_1 receptor binding is clearly present in the rat ventrolateral medulla, extending throughout the CVLM and RVLM in the rostral–caudal plane; (2) the density of AT_1 receptor binding is lower in CVLM and RVLM than in DMM; (3) the K_D of AT_1 binding sites is lower (i.e., higher affinity) in the ventrolateral medulla than in the DMM. Each of these issues, along with