available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Water deprivation increases angiotensin-converting enzyme but not AT_1 receptor expression in brainstem and paraventricular nucleus of the hypothalamus of the rat

Erick A. Bourassa[a,1], *Robert C. Speth*[b,*]

[a]*Department of Pharmacology, School of Pharmacy, University of Mississippi, University, MS 38677, USA*
[b]*Department of Pharmaceutical Sciences, College of Pharmacy, Nova Southeastern University, Fort Lauderdale, FL 33328*

ARTICLE INFO

Article history:
Accepted 24 December 2009
Available online 4 January 2010

Keywords:
Angiotensin II receptors
RVLM
^{125}I-351A
Receptor autoradiography
Dehydration
Subfornical organ

ABSTRACT

The rostral ventrolateral medulla (RVLM) is critical to the maintenance of blood pressure. It has been proposed that blood-borne Ang II can influence the RVLM via a neural connection between the circumventricular organs and paraventricular nucleus of the hypothalamus (PVH) and that a component of this pathway is angiotensinergic. A period of water deprivation leads to increased ability of angiotensin type 1 (AT_1) receptor antagonists to reduce blood pressure when administered into the RVLM and PVH. We studied the differences in AT_1 receptor and angiotensin-converting enzyme (ACE) expression in these and other brain regions involved in blood pressure regulation and water intake following dehydration. AT_1 receptor and ACE expression in brains of rats deprived of water for 48 h were compared to that of water-replete rats by quantitative receptor autoradiography. AT_1 receptor expression was increased in the subfornical organ and periventricular nucleus of the hypothalamus, but not in other brain regions measured. ACE expression was increased in the RVLM, PVH, choroid plexus, median preoptic nucleus, and organosum vasculosum of the lamina terminalis. These findings suggest that increased Ang II production but not increased receptor expression in the PVH and RVLM is the mechanism by which Ang II in the brain helps to sustain systemic blood pressure during periods of water deprivation.

1. Introduction

The rostral ventrolateral medulla (RVLM) is a critical brainstem region that provides excitatory efferents to the intermediolateral cell column (IML) of the thoracic spinal cord. These pre-sympathetic efferents provide a major excitatory stimulus to the cardiovascular system (Guyenet, 2006). The RVLM receives afferents from multiple sources, most notably

* *Corresponding author*. Department of Pharmaceutical Sciences, College of Pharmacy, Nova Southeastern University, Fort Lauderdale, FL 33328.
E-mail address: Robert.Speth@nova.edu (R.C. Speth).

Abbreviations: ACE, angiotensin-converting enzyme; Ang II, angiotensin II; AT_1, type 1 receptor for angiotensin II; CHP, choroid plexus; CPu, caudate putamen; CVLM, caudal ventrolateral medulla; CVOs, circumventricular organs; IML, intermediolateral cell column; LHA, lateral hypothalamic area; MnPO, medial preoptic area; NTS, solitary tract nucleus; OVLT, organosum vasculosum of the lamina terminalis; PEH, periventricular hypothalamus; Pir, piriform cortex; PVH, paraventricular nucleus of the hypothalamus; RVLM, rostral ventrolateral medulla; SCN, suprachiasmatic nucleus; SHR, spontaneously hypertensive rat; SFO, subfornical organ; SNS, sympathetic nervous system; WKY, Wistar-Kyoto

[1] Current address: Department of Biological Sciences, Northwest Missouri State University, Maryville, MO 64468, USA.

0006-8993/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2009.12.079

the caudal ventrolateral medulla (CVLM) and nucleus of the solitary tract (NTS), but also the paraventricular nucleus of the hypothalamus (PVH) (DiBona and Jones, 2001; Guyenet, 2006; Tagawa and Dampney, 1999).

Angiotensin II (Ang II), long known for its direct vasoconstrictor and aldosterone-releasing actions, is now known to also act in the brain to regulate thirst, sodium appetite, and blood pressure (Dampney et al., 2002; DiBona, 2003; Diz et al., 2002; Mayorov and Head, 2003; McKinley et al., 2004; Morris et al., 2002; Speth and Grove, 1991; Wong et al., 2002). All of the abovementioned brain regions contain Ang II type 1 receptors (AT_1 receptor) (Bourassa et al., 2010; Gehlert et al., 1990; Rowe et al., 1990; Song et al., 1992; Tsutsumi and Saavedra, 1991). They are responsive to exogenously applied Ang II and, in certain conditions, to endogenous Ang II (Allen et al., 1988; Alzamora et al., 2006; Bourassa et al., 2009; Ito et al., 2002; Ito et al., 2003; Tan et al., 2005).

Microinjection of Ang II into the RVLM of multiple species (including the rat) elicits a pressor response that can be blocked by application of AT_1 receptor antagonists (Averill et al., 1994; Hirooka et al., 1997; Ito et al., 2002; Ito et al., 2003). However, because application of receptor antagonists alone fails to reduce blood pressure in normotensive rats, it is generally believed that endogenous Ang II plays little, if any, role in the maintenance of normal blood pressure (Averill et al., 1994; Fontes et al., 1997; Tagawa and Dampney, 1999). On the other hand, it seems that Ang II does play a tonic role in the maintenance of blood pressure in animal models of hypertension, as well as in animals that are dehydrated. Microinjection of AT_1 receptor antagonists into the RVLM of the spontaneously hypertensive rat (SHR) produces a depressor response, which contrasts to the lack of a depressor response in normotensive Wistar-Kyoto (WKY) rats (Ito et al., 2002). Recently it has been shown that rats deprived of water for 48 h showed significant depressor responses to microinjection of an AT_1 receptor antagonist in the RVLM (Freeman and Brooks, 2007). In this same study, it was reported that water deprivation also increases the ability of an AT_1 receptor antagonist to lower blood pressure when administered into the PVH. It is known that the PVH sends efferents to the RVLM (Badoer, 2001), that the neurons involved in this relay are activated by water deprivation (Stocker et al., 2004), and that activation of the RVLM caused by disinhibition of the PVH can be attenuated with AT_1 receptor antagonists (Tagawa and Dampney, 1999). Based upon these observations, Freeman and Brooks (2007) suggested that a relay exists between the circumventricular organs such as the subfornical organ (SFO) and organosum vasculosum of the lamina terminalis (OVLT), the PVH, and the RVLM to regulate sympathetic tone in response to those changes in peripheral osmolarity or Ang II.

The purpose of the present study was to test the hypothesis that a 48-h water deprivation period in normotensive rats produces an upregulation of the central renin–angiotensin system in areas of the brain involved in the proposed pathway described by Freeman and Brooks (2007) compared to water-replete controls. AT_1 receptor expression was measured in the dorsomedial medulla (encompassing the NTS, area postrema, and dorsal motor nucleus of the vagus), CVLM, RVLM, lateral hypothalamic area (LHA), PVH (parvocellular division), periventricular nucleus of the hypothalamus (PEH), medial preoptic area (MnPO), suprachiasmatic nucleus (SCN), SFO, and piriform cortex (Pir) using quantitative AT_1 receptor autoradiography. Also, angiotensin-converting enzyme (ACE) expression was measured in the choroid plexus (CHP), RVLM, PVH, MnPO, OVLT, and the caudate putamen (CPu) using quantitative ACE autoradiography.

Table 1 – Effects of water deprivation on measured blood values.

Measure	Water-replete rats	Dehydrated rats	*p* Value
Hematocrit (%)	44.9±0.67	47.6±0.85	0.018
Osmolality (mOsm/kg)	311.0±2.61	314.5±0.43	0.140
Urea nitrogen (mg/dl)	25.3±1.93	27.7±0.49	0.151
Creatinine (mg/dl)	0.43±0.06	0.42±0.02	0.453
Sodium (mmol/l)	144.5±0.65	148.3±0.42	0.002
Chloride (mmol/l)	105.8±0.25	108.2±0.6	0.004
Total protein (g/dl)	6.2±0.02	6.5±0.06	0.003
Weight change (g)	2.5±1.32	−35.5±1.26	<0.0001

Blood values and weight change between water-replete rats and dehydrated rats. Values are expressed as mean±SEM.

2. Results

2.1. Blood values

Hematocrit and plasma sodium, chloride, and total protein values were significantly higher in dehydrated rats compared to water-replete controls, as expected. However, there were no significant differences in urea nitrogen, creatinine, or osmolality between the two groups. See Table 1 for details.

2.2. AT_1 receptor density

AT_1 receptor density was not significantly different between dehydrated rats or water-replete rats except in the SFO, as has been previously reported (Barth and Gerstberger, 1999; Mendelsohn et al., 1983) and PEH. A summary of AT_1 receptor density values are given in Table 2. Figs. 1 and 2 show

Table 2 – Effect of water deprivation on AT_1 receptor binding in the brain.

Brain region	Water-replete rats	Dehydrated rats	*p* Value
NTS	936.1±24.53	906.5±33.23	0.247
CVLM	175.4±18.7	181.1±18.52	0.417
RVLM	140.4±9.31	149.6±13.92	0.298
MnPO	528.1±29.26	589.0±48.14	0.159
SFO	762.9±25.33	864.1±24.71	0.011
LHA	328.3±13.05	358.7±19.20	0.114
PVH	938.0±56.45	985.17±46.13	0.270
PEH	634.9±31.12	744.8±44.56	0.039
SCN	824.5±22.9	806.1±16.95	0.271
Pir	501.2±33.0	619.8±67.33	0.084

AT_1 receptor densities (fmol/g) between water-replete rats and dehydrated rats in different brain regions measured. Values are expressed as mean±SEM.