Behavioural Brain Research 258 (2014) 27–33

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

The anteroventral third ventricle region is critical for the behavioral desensitization caused by repeated injections of angiotensin II

Peter J. Vento, Derek Daniels*

Behavioral Neuroscience Program, Department of Psychology, The State University of New York at Buffalo, Buffalo, NY 14260, USA

HIGHLIGHTS

- Injection of a low dose of AngII into the AV3V stimulated water intake.
- Repeated AV3V AngII injections reduced drinking after AngII injection into the AV3V.
- Effect was similar when drinking was stimulated by AngII in the lateral ventricle.
- Losartan in the AV3V prevented the effect of repeated central injections of AngII.

ARTICLE INFO

Article history:
Received 10 September 2013
Received in revised form 7 October 2013
Accepted 11 October 2013
Available online 18 October 2013

Keywords:
AV3V
Angiotensin
Desensitization
Thirst

ABSTRACT

A single central injection of angiotensin II (AngII) potently increases water intake; however, a growing body of research suggests that repeated, acute intracerebroventricular injections of AngII cause a reduction in the dipsogenic response to subsequent AngII. This AngII-induced behavioral desensitization is specific to the effects of angiotensin and mediated by the angiotensin type-1 (AT_1) receptor. The neuroanatomical substrate for this phenomenon, however, remains unknown. The anteroventral third ventricle (AV3V) region is an important site for the behavioral and physiological actions of AngII. Therefore, we hypothesized that this region also mediates the effects of repeated central AngII administration. In support of this hypothesis, we found that repeated injections of AngII into the AV3V reduced water intake stimulated by a test injection of AngII given into this region. Moreover, repeated AngII injections in the AV3V reduced water intake after AngII was injected into the lateral ventricle. These studies also demonstrate that activation of the AT_1 receptor within the AV3V is required for AngII-induced behavioral desensitization because direct injection of the AT_1 receptor antagonist, losartan, into the AV3V blocked the desensitizing effect of repeated AngII injections into the lateral ventricle. These findings provide additional support for a role of the AV3V in the dipsogenic actions of AngII, and suggest that this region is critical for the desensitization that occurs after acute repeated central injections of AngII.

1. Introduction

Angiotensin II (AngII) acts at a number of peripheral and central sites to engage coordinated physiological and behavioral responses to hypovolemia. In the periphery, AngII causes retention of water and electrolytes through actions at the kidney, and the peptide also acts directly on the cardiovascular system to increase blood pressure [1–3]. This effect on cardiovascular function has made the angiotensin system a useful target for the treatment of hypertension [4,5]. Within the brain, AngII also engages some of these same cardiovascular responses, but in addition, it causes robust increases in water and salt intake [6–9].

In rats, a single introcerebroventricular (icv) injection of AngII potently increases water intake [8,10], but repeated injections of the peptide, given within a short time frame, reduce this response [11–16]. This AngII-induced behavioral desensitization has been shown to be specific to the angiotensin system, not the result of a broader behavioral impairment, and dependent on activation of the angiotensin type 1 (AT_1) receptor [14,16]. Although the literature on the effects of AngII-induced desensitization in the behaving animal is growing, to date all of the behavioral studies investigating the effects of repeated administration of AngII have given the peptide icv. This route of administration is useful for studying the broad effects of a compound, but it provides little anatomical specificity. As such, a neuroanatomical locus for this phenomenon remains unknown.

* Corresponding author at: Department of Psychology, B74 Park Hall, University at Buffalo, SUNY, Buffalo, NY 14260, USA. Tel.: +1 716 645 0264; fax: +1 716 645 3801.
E-mail address: danielsd@buffalo.edu (D. Daniels).

0166-4328/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbr.2013.10.013

Structures along the *lamina terminalis*, such as the median preoptic nucleus (MnPO) and the *organum vasculosum of the lamina terminalis* (OVLT), play an essential role in the neural control of body fluid homeostasis and are responsive to AngII. The MnPO and OVLT express AT_1 receptors [17–20], and lesions made in the anteroventral third ventricle (AV3V) region, which comprises parts of the MnPO and OVLT, abolish the drinking response to icv AngII [21]. Moreover, c-Fos expression is observed in the AV3V after icv injection of AngII [22,23], and ventricular obstruction that prevents CSF from reaching the AV3V prevents water intake stimulated by AngII injected into the lateral ventricle [21,24–26]. Given this important role for the AV3V in mediating the drinking response to icv AngII, it is reasonable to hypothesize that it is similarly involved in the reduced dipsogenic response observed after repeated icv AngII administration. Accordingly, the present studies tested the role of the AV3V in mediating the reduced water intake observed after repeated central administration of AngII. Consistent with our previous studies [14–16], the present experiments used repeated injections of relatively large doses of AngII to explore receptor-mediated responses that may be unobservable under more physiological parameters. The results provide additional support for a role of the AV3V in the drinking response to icv AngII and suggest that this region is both necessary and sufficient for the reduced water intake observed after repeated central injections of AngII.

2. Materials and methods

2.1. Animals

Adult male Sprague Dawley rats (325–375 g) were obtained from Harlan Laboratories (Indianapolis, IN, USA). Rats were individually housed in stainless steel wire mesh cages in a temperature- and humidity-controlled room on a 12:12 h light:dark cycle, and all experiments were performed early in the lights-on phase of the cycle. Food and water were available *ad libitum*, except where otherwise stated. All experimental protocols were approved by the Institutional Animal Care and Use Committee of the State University of New York at Buffalo, and the handling and care of animals was in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. Subjects were used only once per experiment, unless otherwise stated.

2.2. Cannula implantation

Rats were anesthetized by an intramuscular injection of ketamine (70 mg/kg) and xylazine (5 mg/kg). Their heads were fixed in a stereotaxic apparatus, a small incision was made to expose the skull, and burr holes were drilled. Rats then were implanted with guide cannulae (26 gauge; Plastics One, Roanoke, VA, USA) aimed at the lateral ventricle, the AV3V, or both the lateral ventricle and the AV3V. Stereotaxic coordinates were 0.9 mm posterior to bregma, 1.4 mm lateral to midline, 1.8 mm ventral to the surface of the skull for lateral ventricle injections and 0.11 mm anterior to bregma, on midline, 7.8 mm ventral to the surface of the skull for the AV3V. When the experimental design required implantation of two cannulae, one for injection into the lateral ventricle and one for injection into the AV3V, the lateral ventricle cannula used an angled approach (20°) with the following stereotaxic coordinates: 1.4 mm posterior to bregma, 3.1 mm lateral to midline, 3.1 mm ventral to the surface of the skull. Cannulae were affixed to the skull using bone screws and dental cement. Septocaine (topical) and carprofen (5 mg/kg; sc) were administered during surgery for analgesia, and additional carprofen (5 mg/kg; sc) was given as needed for up to three days after surgery to manage pain and inflammation.

2.3. Drugs and injections

AngII (Biochem Bioscience Inc., King of Prussia, PA, USA) and the AT_1 receptor antagonist losartan (losartan potassium; Sigma–Aldrich, St. Luis, MO, USA) were diluted in tris-buffered saline (TBS). Injections were made by hand using a Hamilton syringe (Hamilton Company, Reno, NV, USA) attached to water-filled PE 50 tubing. Tubing was attached to an injector cannula (33 gauge; Plastics One, Roanoke, VA, USA) that was fabricated to extend either 1.5–2.5 mm (lateral ventricle) or 2.5 mm (AV3V) beyond the guide cannula, and the injector was left in place for approximately 30–40 s after the injection to allow for diffusion of drug. All injections into the AV3V were made using a volume of 0.5 μl, and all icv injections were 1 μl.

2.4. Verification of proper cannula placement and AngII responsiveness

Proper lateral ventricle cannula placement was verified by injection of AngII (10 ng). Only data from rats that drank at least 6 ml of water in the 30 min after AngII injection were included. Proper cannula placement in the AV3V was assessed by histological examination and/or injection of AngII (1 ng). Only rats that drank at least 4 ml of water in the 30 min after AngII injection into the parenchyma were considered to have accurate placement in the AV3V.

2.5. Histological verification

After behavioral testing, a subset of rats were anesthetized by inhaled isoflurane and 0.5 μl of dye (Green Food Color, McCormick & Co. Inc., Hunt Valley, MD) was injected into the cannula aimed at the AV3V. Rats then were sacrificed by decapitation, the brains were removed, and 50 μm coronal brain sections were collected on a cryostat for histological examination. Consistent with previous reports [27], the AV3V was defined as an area ventral to the anterior commissure and dorsal to the roof of the third ventricle, extending rostrally along the course of the optic recess to the anterior extent of the OVLT.

2.6. Intake measures

Water intake at the time of behavioral verification of proper cannula placement and for Experiment 1 was assessed using 100 ml graduated bottles with 1 ml gradations. Water intake was calculated as the starting volume minus the volume remaining at the end of the test. In all other experiments, a custom contact lickometer (designed and constructed by the Psychology Electronics Shop, University of Pennsylvania, Philadelphia, PA, USA) was used to determine the number of times the rats licked the water spout. Electric leads connected the lickometer to an electrically isolated water spout. Rats licked through a slot (3.2 mm wide) in the cage to access a recessed water spout in order to minimize non-tongue contact with the spout. Each time the tongue of the rat contacted the spout, a lick was recorded. The lickometer interfaced with a computer that used an integrated digital I/O device (National Instruments Inc., Austin, TX, USA), and data were processed using a MATLAB (MathWorks, Natick, MA, USA) software environment before being exported for final analysis using Excel (Microsoft Corp., Redmond, WA, USA). Water bottles were weighed before and after each test, and total intake was calculated as the starting bottle weight minus the weight at the end of the test. Total intake was divided by the total number of licks to determine the average volume per lick, and data were broken down into discrete time bins. Binned intake was calculated as the number of licks per bin multiplied by the volume per lick. Lickometer data are represented in 10-min time bins to