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AT1 RECEPTOR BLOCKADE IN THE CENTRAL NUCLEUS OF THE AMYGDALA ATTENUATES THE EFFECTS OF MUSCIMOL ON SODIUM AND WATER INTAKE

B. HU,[a,b†] H. QIAO,[a†] B. SUN,[a] R. JIA,[a] Y. FAN,[a] N. WANG,[a] B. LU[a*] AND J. Q. YAN[a*]

[a] *Department of Physiology and Pathophysiology, Xi'an Jiaotong University, Health Science Center, 76# West Yanta Road, Xi'an, Shaanxi 710061, PR China*

[b] *Department of Prosthodontics, Xi'an Jiaotong University, College of Stomatology, 98# Xiwu Road, Xi'an, Shaanxi 710004, PR China*

Abstract—The blockade of the central nucleus of the amygdala (CeA) with the $GABA_A$ receptor agonist muscimol significantly reduces hypertonic NaCl and water intake by sodium-depleted rats. In the present study we investigated the effects of previous injection of losartan, an angiotensin II type-1 (AT1) receptor antagonist, into the CeA on 0.3 M NaCl and water intake reduced by muscimol bilaterally injected into the same areas in rats submitted to water deprivation–partial rehydration (WD–PR) and in rats treated with the diuretic furosemide (FURO). Male Sprague–Dawley rats with stainless steel cannulas bilaterally implanted into the CeA were used. Bilateral injections of muscimol (0.2 nmol/0.5 μl, *n* = 8 rats/group) into the CeA in WD–PR-treated rats reduced 0.3 M NaCl intake and water intake, and pre-treatment of the CeA with losartan (50 μg/0.5 μl) reversed the inhibitory effect of muscimol. The negative effect of muscimol on sodium and water intake could also be blocked by pretreatment with losartan microinjected into the CeA in rats given FURO (*n* = 8 rats/group). However, bilateral injections of losartan (50 μg/0.5 μl) alone into the CeA did not affect the NaCl or water intake. These results suggest that the deactivation of CeA facilitatory mechanisms by muscimol injection into the CeA is promoted by endogenous angiotensin II acting on AT1 receptors in the CeA, which prevents rats from ingesting large amounts of hypertonic NaCl and water.

Key words: sodium appetite, $GABA_A$ receptor, angiotensin type-1 receptor, central nucleus of the amygdala.

INTRODUCTION

The central nucleus of the amygdala (CeA) in the forebrain is a critical brain site for the integration of gustatory and visceral signals (McDonald, 1998). The CeA is reciprocally connected to brainstem areas such as the lateral parabrachial nucleus (LPBN) and the nucleus of the solitary tract (NTS), and to other forebrain regions, such as the paraventricular nucleus (PVN) of the hypothalamus and median preoptic nucleus (MnPO) (Jhamandas et al., 1996; Jolkkonen and Pitkanen, 1998; Geerling and Loewy, 2006). Therefore, the CeA may receive and regulate signals involved in the control of hydromineral homeostasis and related behaviors like sodium and water intake.

Bilateral electrolytic lesions of the CeA produce deficits in spontaneous sodium intake, as well as a sodium appetite induced by subcutaneous injections of the mineralocorticoid deoxycorticosterone, the α_2-adrenoceptor antagonist yohimbine, angiotensin II (ANG II), intracerebral ventricular injections of renin or by 24 h of sodium depletion in rats treated with furosemide (FURO) (Galaverna et al., 1992; Zardetto-Smith et al., 1994). However, water intake induced by subcutaneous injections of ANG II or by cellular dehydration is not affected by similar lesions of the CeA, revealing that lesions of the CeA particularly reduce sodium appetite (Zardetto-Smith et al., 1994).

Connections among the amygdala, LPBN and NTS are suggested to be part of the neural circuitry related to the modulation of salt intake. Retrograde and anterograde neural marker and immunocytochemistry techniques have demonstrated the existence of GABAergic monosynaptic connections between the CeA and the LPBN (Jia et al., 2005). Lesions in the CeA impair sodium intake induced by the blockade of the LPBN (Andrade-Franze et al., 2010b), suggesting an important facilitatory mechanism present in the CeA, which is essential for water and hypertonic NaCl intake that arises after the blockade of the inhibitory mechanisms of the LPBN. Lately, our laboratory has also demonstrated that the blockade of CeA neurons with bilateral injections of the $GABA_A$ receptor agonist muscimol inhibits the ingestion of hypertonic NaCl and water in sodium-depleted rats. This suggests that GABAergic mechanisms present in the CeA are involved in the control of sodium intake (Wang et al., 2012).

*Corresponding authors. Tel: +86-29-82655199; fax: +86-29-82655199.
E-mail address: jqyan810@163.com (J. Q. Yan).
† These authors contributed equally to this work.
Abbreviations: ANG II, angiotensin II; AT1, angiotensin II type-1; CeA, central nucleus of the amygdala; FURO, furosemide; IPSCs, inhibitory post-synaptic currents; LPBN, lateral parabrachial nucleus; MnPO, median preoptic nucleus; NTS, nucleus of the solitary tract; PVN, paraventricular nucleus; WD–PR, water deprivation–partial rehydration.

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The cardiovascular, neuroendocrine and ingestive effects of ANG II are mainly mediated by angiotensin II type-1 (AT1) receptors (Kirby et al., 1992; Thunhorst and Fitts, 1994; McKinley et al., 1996; Fitzsimons, 1998). The CeA contains AT1 receptors (Thunhorst and Fitts, 1994; McKinley et al., 1996) and has been proposed as a possible site of interaction between ANG II and mineralocorticoids to stimulate sodium appetite (Galaverna et al., 1992; McKinley et al., 2003). Previous studies using whole-cell voltage-clamp techniques have suggested that ANG II acting on AT1 receptors may modulate GABAergic synaptic transmission and produce opposite effects, depending on whether pre- or post-synaptic AT1 receptors are activated (Li et al., 2003; Li and Pan, 2005; Henry et al., 2009; Xing et al., 2009). The pharmacological activation of pre-synaptic AT1 receptors reduces GABA release and decreases the amplitude of evoked GABAergic inhibitory post-synaptic currents (IPSCs), which can be blocked by the AT1 receptor antagonist losartan, but not by the AT2 receptor antagonist PD123319 (Li et al., 2003; Li and Pan, 2005; Xing et al., 2009). In contrast, endogenous ANG II acting on post-synaptic AT1 receptors increases IPSCs in sodium-sensitive neurons in the MnPO, revealing a post-synaptic action of endogenous ANG II, which facilitates the effects of the GABAergic input to the MnPO (Henry et al., 2009). In addition, ANG II failed to excite PVN neurons in the presence of bicuculline and had no effect on excitatory post synaptic currents in the PVN neurons or in the dorsolateral periaqueductal gray (Li et al., 2003; Li and Pan, 2005; Xing et al., 2009).

Considering the effects of the activation of $GABA_A$ receptors in the CeA on hypertonic NaCl and water intake (Kang et al., 2004; Wang et al., 2012), and the already-reported role of AT1 receptor activation in the modulation of GABAergic mechanisms (Li et al., 2003; Li and Pan, 2005; Henry et al., 2009; Xing et al., 2009), in the present study we investigated the effects of previous injection of the specific AT1 receptor antagonist, losartan, into the CeA on water and hypertonic NaCl intake reduced by muscimol injections into the CeA in water deprivation–partial rehydration (WD–PR) or FURO-treated rats.

EXPERIMENTAL PROCEDURES

Animals

The experimental design is shown in Fig. 1. A total of 108 adult male Sprague–Dawley rats (provided by Medical Experimental Animal Center of Xi'an Jiaotong University, Shaanxi Province, China) weighing 250 ± 20 g were housed individually in stainless-steel cages with free access to food pellets and distilled water. The rats were maintained at a room temperature of 23 ± 2 °C, humidity of 55 ± 10% and on a 12-h light/dark cycle with light onset at 8:00 am. The experimental protocols followed the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication No. 80-23, 1996).

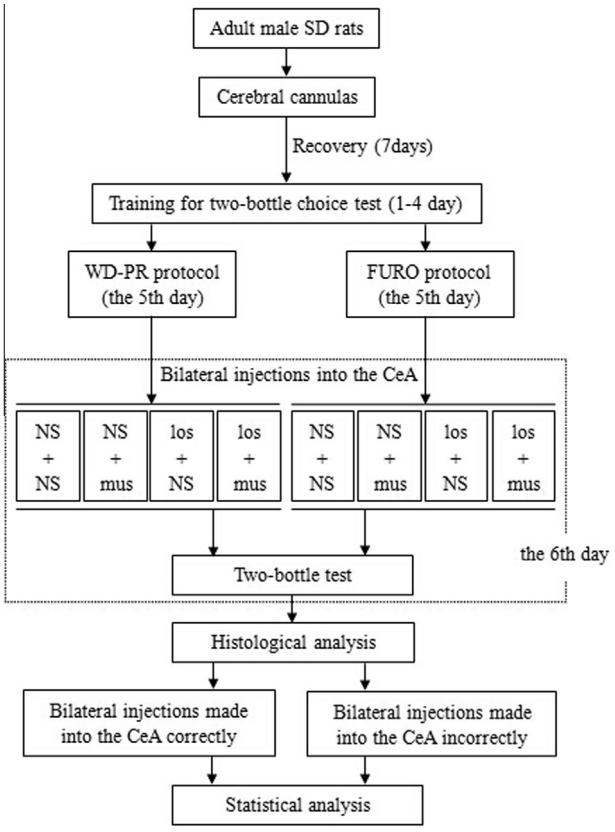

Fig. 1. Schema of experimental design. WD–PR, water deprivation–partial rehydration; FURO, furosemide; CeA, central nucleus of the amygdala; NS, normal saline; los, losartan; mus, muscimol.

Cerebral cannulas

Rats were anesthetized with chloral hydrate (300 mg/kg intraperitoneally, i.p.) and secured in a stereotaxic apparatus (SN-2N, Narishige Group, Tokyo, Japan). The skull was leveled between the bregma and lambda. Two stainless steel 23-gauge cannulas were bilaterally implanted into the CeA using the following coordinates: 2.5 mm caudal to the bregma, 4.0 mm lateral to the midline suture, and 7.0 mm below the skull surface. The tips of the cannulas were positioned 1 mm above the CeA. The cannulas were fixed to the cranium using dental acrylic resin and jeweler screws. 30-Gauge metal obturators filled the cannulas between tests. All rats were injected with penicillin (20,000 units, i.p.) during the first three post-operative days to prevent infection and were allowed to recover for at least 7 days before starting the ingestion tests.

Injections into the CeA

Bilateral injections into the CeA were administered using 1-μl Hamilton syringes (Hamilton, Reno, NV, USA) connected by PE-10 polyethylene tubing to 30-gauge injection cannulas. At the time of testing, obturators were removed and the injection cannula (1 mm longer than the guide cannula) was carefully inserted into the