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BRAIN RESEARCH

Research Report

AT_1 receptor blockade in the lateral parabrachial nucleus reduces the effects of muscimol on sodium intake

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ABSTRACT

The blockade of the lateral parabrachial nucleus (LPBN) with $GABA_A$ receptor agonist muscimol induces robust hypertonic NaCl and water intake by rats. In the present study we investigated the effects of previous injections of losartan (AT_1 angiotensin receptor antagonist) into the LPBN on 0.3 M NaCl and water intake induced by muscimol injected bilaterally in the same area in fluid replete rats and in rats treated with the diuretic furosemide combined with a low dose of the angiotensin-converting enzyme inhibitor captopril injected subcutaneously. Male Wistar rats with stainless steel cannulas implanted bilaterally into the LPBN were used. Bilateral injections of muscimol (0.5 nmol/0.2 μl, n=8) into the LPBN in fluid replete rats induced 0.3 M NaCl intake (23.4±4.1 vs. saline: 0.4±0.4 ml/3 h) and water intake (9.3±1.9 vs. saline: 0.7±0.4 ml/3 h) and pre-treatment of the LPBN with losartan (50 μg/0.2 μl) reduced 0.3 M NaCl intake (3.3±2.5 ml/3 h) and water intake (4.0±2.9 ml/3 h) induced by muscimol. In rats treated with furosemide+captopril, pre-treatment with losartan into the LPBN attenuated the increase of 0.3 M NaCl intake produced by muscimol (12.8±5.3, vs. saline+muscimol: 36.7±6.7 ml/3 h) without changing water intake. Therefore, the results suggest that deactivation of LPBN inhibitory mechanisms by muscimol injections into the LPBN is facilitated by endogenous angiotensin II acting on AT_1 receptors in the LPBN, which drives rats to ingest large amounts of hypertonic NaCl.

1. Introduction

Important mechanisms for the control of sodium and water intake are present in the lateral parabrachial nucleus (LPBN), a pontine structure located dorsolaterally to the superior cerebellar peduncle (Andrade et al., 2006; Callera et al., 2005; De Luca et al., 2003; De Oliveira et al., 2007; Menani et al., 2002; Menani and Johnson, 1995). The LPBN is reciprocally connected to forebrain areas, such as the paraventricular nucleus of the hypothalamus (PVN), the central nucleus of the amygdala (CeA) and the median preoptic nucleus (MnPO), and to medullary regions, like the area postrema (AP) and the medial portion of the nucleus of the solitary tract (mNTS) (Ciriello et al., 1984; Fulwiler and Saper, 1984; Herbert et al., 1990; Jhamandas et al., 1992, 1996; Norgren, 1981). Therefore, the LPBN may convey signals that ascend from AP/mNTS to

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the forebrain areas that regulate fluid and electrolyte balance and related behaviors like water and sodium intake.

Numerous neurotransmitter systems have implicated the LPBN in the control of sodium intake. For example, bilateral LPBN injections of methysergide, a serotonergic receptor antagonist, increase hypertonic NaCl intake induced by angiotensin II (ANG II) administered intracerebroventricularly (i.c.v.) or into the subfornical organ (SFO), by 24 h of water deprivation, by 24 h of sodium depletion or by deoxycorticosterone acetate (DOCA) (De Gobbi et al., 2001; Menani et al., 1996, 1998a, 2000; Menani and Johnson, 1995). Blockade of cholecystokinin (CCK) or serotonin receptors or activation of α_2-adrenergic receptors in the LPBN enhances NaCl intake by rats injected subcutaneously with the diuretic furosemide (FURO) combined with the angiotensin converting enzyme inhibitor captopril (CAP) (Andrade et al., 2004; De Gobbi et al., 2001; Menani et al., 1996, 1998b). The blockade of LPBN neurons with bilateral injections of the $GABA_A$ agonist muscimol induces robust ingestion of hypertonic NaCl and slight ingestion of water in fluid replete rats and increases FURO+CAP- and 24 h sodium depletion-induced sodium intake, suggesting that a GABAergic mechanism present in LPBN is involved in the control of sodium intake (Callera et al., 2005; De Oliveira et al., 2007).

The cardiovascular, neuroendocrine and ingestive effects of ANG II acting centrally are mediated mainly by angiotensin type 1 (AT_1) receptors located in different areas of the central nervous system, such as the LPBN, anterior hypothalamic area (AHA), amygdala, SFO, rostral and caudal ventrolateral medulla and NTS (Fitzsimons, 1998; Fregly and Rowland, 1991; Mckinley et al., 1996; Rowland et al., 1992; Thunhorst and Fitts, 1994). The nonpeptide antagonist losartan selectively binds on AT_1 receptors (Chiu et al., 1989).

Studies using whole cell voltage-clamp techniques have suggested that ANG II acting on AT_1 receptors may modulate GABAergic synaptic transmission and produce opposite effects, depending on whether pre- or post-synaptic AT_1 receptors are activated (Henry et al., 2009; Li et al., 2003; Li and Pan, 2005; Xing et al., 2009). It has been suggested that ANG II acting on pre-synaptic AT_1 receptors reduces GABA release and decreases the amplitude of evoked GABAergic inhibitory post-synaptic currents (IPSCs) (Li et al., 2003; Li and Pan, 2005; Xing et al., 2009). In contrast, it was shown that endogenous ANG II acting on post-synaptic AT_1 receptors increases IPSCs in sodium-sensitive neurons in the median preoptic nucleus (MnPO) (Henry et al., 2009). According to these studies, treatment with ANG II increased the firing of PVN neurons that project to the rostroventrolateral medulla (RVLM) and decreased the amplitude of evoked GABAergic IPSCs and the frequency of miniature IPSCs, effects blocked by the AT_1 receptor antagonist losartan (Li et al., 2003; Li and Pan, 2005). Treatment with ANG II also decreased the amplitude of evoked IPSCs and the frequency of miniature IPSCs in neurons of the dorsolateral periaqueductal gray, an effect blocked by losartan, but not by the AT_2 antagonist PD123319 (Xing et al., 2009). Treatment with ANG II 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). In contrast, another study showed that the amplitude of the IPSCs in the MnPO was reduced by the treatment with losartan, suggesting a post synaptic action of endogenous ANG II that facilitated the effect of the GABAergic input to the MnPO (Henry et al., 2009).

Considering the effects of the activation of $GABA_A$ receptors in the LPBN on hypertonic NaCl and water intake (Callera et al., 2005; De Oliveira et al., 2007) and the results of previous studies showing that AT_1 receptor activation may modulate the action of the GABAergic mechanisms (Henry et al., 2009; Li et al., 2003; Li and Pan, 2005; Xing et al., 2009), in the present study we investigated the effects of injections of the specific AT_1 receptor antagonist, losartan, into the LPBN on water and hypertonic NaCl intake induced by the activation of $GABA_A$ receptors by muscimol injections into the LPBN in fluid replete or FURO+CAP-treated rats.

2. Results

2.1. *Histological analysis*

Fig. 1 is a photomicrograph of a transverse section of the brainstem of one rat, representative of the groups tested, showing the typical bilateral injection sites in the LPBN. The injections were centered in the central lateral and dorsal lateral portions of the LPBN (see Fulwiler and Saper, 1984 for definitions of LPBN subnuclei). In some rats, LPBN injections reached the ventral lateral and external lateral portions, as well as the Kölliker–Fuse nucleus. The sites of injections were similar to those in previous studies that showed the effects of LPBN injections of methysergide, proglumide, moxonidine or muscimol on water and 0.3 M NaCl intake (Andrade et al., 2006; Callera et al., 2005; De Gobbi et al., 2001; De Luca et al., 2003; De Oliveira et al., 2007). In some rats, injections spread to the brachium (superior cerebellar peduncle), or slightly ventral to this structure, reaching the dorsal portions of the medial parabrachial nucleus (MPBN) uni- or bilaterally. There was no difference in the effects if the injections were restricted to the LPBN or if they spread to the ventral structures described above.

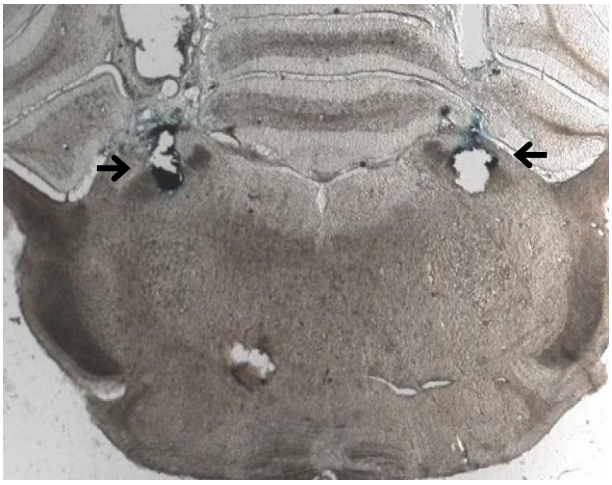

Fig. 1 – Photomicrograph of a brain slice from one rat representative of the groups studied showing the sites of injections into the LPBN (arrows).