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Research Report

Importance of the central nucleus of the amygdala on sodium intake caused by deactivation of lateral parabrachial nucleus

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ABSTRACT

The lateral parabrachial nucleus (LPBN) and the central nucleus of the amygdala (CeA) are important central areas for the control of sodium appetite. In the present study, we investigated the importance of the facilitatory mechanisms of the CeA on NaCl and water intake produced by the deactivation of LPBN inhibitory mechanisms. Male Holtzman rats ($n=7$–14) with stainless steel cannulas implanted bilaterally in the CeA and LPBN were used. Bilateral injections of moxonidine (α_2-adrenoceptor/imidazoline agonist, 0.5 nmol/0.2 μl) into the LPBN increased furosemide+captopril-induced 0.3 M NaCl (29.7±7.2, vs. vehicle: 4.4±1.6 ml/2 h) and water intake (26.4±6.7, vs. vehicle: 8.2±1.6 ml/2 h). The $GABA_A$ agonist muscimol (0.25 nmol/0.2 μl) injected bilaterally into the CeA abolished the effects of moxonidine into the LPBN on 0.3 M NaCl (2.8±1.6 ml/2 h) and water intake (3.3±2.3 ml/2 h). Euhydrated rats treated with muscimol (0.5 nmol/0.2 μl) into the LPBN also ingested 0.3 M NaCl (19.1±6.4 ml/4 h) and water (8.8±3.2 ml/4 h). Muscimol (0.5 nmol/0.2 μl) into the CeA also abolished 0.3 M NaCl (0.1±0.04 ml/4 h) and water intake (0.1±0.02 ml/4 h) in euhydrated treated with muscimol into the LPBN. The present results show that neuronal deactivation of the CeA abolishes NaCl intake produced by the blockade of LPBN inhibitory mechanisms, suggesting an interaction between facilitatory mechanisms of the CeA and inhibitory mechanisms of the LPBN in the control of NaCl intake.

Abbreviations: ANG II, angiotensin II; CAP, captopril; CeA, central nucleus of amygdala; FURO, furosemide; GABA, γ-aminobutyric acid; LPBN, lateral parabrachial nucleus; NTS, nucleus of the solitary tract; s.c., subcutaneously

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1. Introduction

The lateral parabrachial nucleus (LPBN), a pontine structure that lies dorsolaterally to the superior cerebellar peduncle, is connected with several brain areas that belong to a circuit subserving the control of sodium appetite and thirst like the nucleus of the solitary tract (NTS), area postrema (AP), paraventricular nucleus of the hypothalamus (PVN), central nucleus of amygdala (CeA) and median preoptic nucleus (MnPO) (Ciriello et al., 1984; Fulwiler and Saper, 1984; Herbert et al. 1990; Jhamandas et al., 1992, 1996; Krukoff et al., 1993; Lança and van der Kooy, 1985; Norgren, 1981).

The LPBN strongly inhibits hypertonic NaCl intake, an influence which is hypothesized to prevent excessive solute intake (Andrade-Franze et al., 2010a, 2010b; Andrade et al., 2004, 2011; Callera et al., 2005; De Oliveira et al., 2008; Gasparini et al., 2015a; Menani et al., 2014; Roncari et al., 2014). Signals that influence water and NaCl intake like those from arterial baroreceptors, cardiopulmonary receptors, gustatory receptors and other visceral receptors that reach the NTS ascend to the LPBN (Norgren, 1981; Lança and van der Kooy, 1985; Ciriello et al., 1984; Fulwiler and Saper, 1984; Herbert et al. 1990; Jhamandas et al., 1992, 1996). These signals may modulate the activity of LPBN inhibitory mechanisms by releasing different neurotransmitters like serotonin, cholecystokinin, corticotrophin-releasing factor (CRF) and glutamate which increase the inhibitory action, whereas others like GABA, opioids, ATP and noradrenaline reduce the inhibitory action (Andrade et al., 2004, 2011; Callera et al., 2005; De Gobbi et al., 2009, Gasparini et al., 2009, De Oliveira et al., 2007, 2008, 2011; Menezes et al., 2011, 2014; Roncari et al., 2014). The deactivation of the inhibitory mechanisms by changing the activity of specific neurotransmitters/receptors in the LPBN increases hypertonic NaCl and/or water intake induced by different dipsogenic or natriorexigenic stimuli like angiotensin II (ANG II), sodium depletion, water deprivation, central cholinergic activation or even osmoreceptor activation (Menani and Johnson, 1995, 1998; Menani et al., 1996, 2002, 2014; De Luca et al., 2003; Andrade et al., 2004, 2006; De Gobbi et al., 2009; Gasparini et al., 2015b). In addition, the neuronal deactivation with bilateral injection of the $GABA_A$ agonist muscimol into the LPBN stimulates hypertonic NaCl intake by euhydrated rats (Callera et al., 2005; De Oliveira et al., 2007).

Bilateral electrolytic lesions of the CeA abolish daily 0.5 M NaCl intake and sodium appetite induced by 24 h of sodium depletion, subcutaneous (s.c.) deoxycorticosterone (DOCA) or yohimbine (α_2-adrenoceptor agonist) or by intracerebroventricular (i.c.v.) injections of renin (Covian et al., 1975; Galaverna et al., 1992; Zardetto-Smith et al., 1994), suggesting that contrary to the LPBN, important facilitatory mechanisms for the control of sodium intake are present in the CeA. Damage to the CeA also abolishes the increased water and 0.3 M NaCl intake produced by bilateral injections of the α_2-adrenoceptor agonist moxonidine into the LPBN of rats treated with subcutaneous (s.c.) injections of the diuretic furosemide (FURO) combined with low dose of captopril (CAP) s.c. (Andrade-Franze et al., 2010b). The FURO+CAP is a treatment that induces hypovolemia, mild hypotension and acute NaCl and water intake dependent on central production of angiotensin II (ANG II) (Andrade et al., 2004; Fitts and Masson, 1989; Gasparini et al., 2009; Thunhorst and Johnson, 1994). Furthermore, bilateral electrolytic lesions of the CeA abolish water and 0.3 M NaCl intake produced by bilateral injections of the $GABA_A$ agonist muscimol into the LPBN in normovolemic and euhydrated rats (Andrade-Franze et al., 2010a). These results suggest that the integrity of the CeA is essential for sodium intake that results from the deactivation of the LPBN inhibitory mechanisms (Andrade-Franze et al., 2010a, 2010b).

Although the integrity of the CeA is certainly important for the increase of sodium intake that results from the deactivation of LPBN inhibitory mechanisms (Andrade-Franze et al., 2010a, 2010b), the effects of electrolytic lesions might result from non-specific destruction of fibers of passage and not of neuronal cell bodies. Therefore, the objective of the present study was to find out if the local neuronal activity in the CeA is important for the increased water and NaCl intake that results from deactivation of the LPBN inhibitory mechanisms. For this purpose, the activity of CeA neurons was blocked with injections of muscimol into the CeA. The injections into the CeA were combined with injections of either muscimol or moxonidine into the LPBN to deactivate the inhibitory mechanisms in the LPBN.

2. Results

2.1. *Histological analysis*

Fig. 1A shows the typical bilateral injection sites in the CeA. The CeA injection sites were located lateraly to the tip of the optic tract, above the basomedial amygdaloid nucleus and medial to the basolateral amygdaloid nucleus. The sites of the injections in the present study were similar to those that previous studies showed the effects of lesions of the CeA on NaCl intake (Andrade-Franze et al., 2010a, 2010b).

Fig. 1B shows the typical bilateral injection sites in the LPBN. The LPBN injection sites were centered in the central lateral and dorsal lateral portions of the LPBN (see Fulwiler and Saper, 1984, for definitions of LPBN subnuclei). The sites of the injections in the present study were similar to those that previous studies showed the effects of muscimol or moxonidine injected into the LPBN on NaCl and water intake (Andrade-Franze et al., 2010a, 2010b; Andrade et al., 2004, 2006; Callera et al., 2005).

2.2. *FURO+CAP-induced water and 0.3 M NaCl intake in rats treated with bilateral injections of muscimol into the CeA combined with moxonidine into the LPBN*

ANOVA showed differences between treatments for 0.3 M NaCl [$F(3,18)=10.0$; $p<0.05$] ($n=7$) (Fig. 2A) and water intake, [$F(3,18)=9.1$; $p<0.05$] (Fig. 2B).

In rats treated with saline into the CeA, bilateral injections of moxonidine (0.5 nmol/0.2 μl) into the LPBN increased FURO+CAP-induced 0.3 M NaCl and water intake. Bilateral injections of muscimol (0.25 nmol/0.2 μl) into the CeA abolished the increase in FURO+CAP-induced 0.3 M NaCl and water