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NATRIOREXIGENIC EFFECT OF DAMGO IS DECREASED BY BLOCKING AT1 RECEPTORS IN THE CENTRAL NUCLEUS OF THE AMYGDALA

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Abstract—μ-Opioid receptor (μ-OR) activation with agonist [D-Ala^2, N-Me-Phe^4, Gly^5-ol]-enkephalin (DAMGO) in the central nucleus of the amygdala (CeA) induces sodium (0.3 M NaCl) intake in rats. The purpose of this study was to examine the effects of pre-injections of losartan (AT1 angiotensin receptor antagonist) into the CeA on 0.3 M NaCl and water intake induced by DAMGO injected bilaterally in the same area in rats submitted to water deprivation–partial rehydration (WD–PR) and in rats treated with the diuretic furosemide (FURO) combined with a low dose of the angiotensin-converting enzyme inhibitor captopril (CAP) injected subcutaneously (FURO/CAP). Male Sprague–Dawley rats with stainless steel cannulas implanted bilaterally into the CeA were used. In WD–PR rats, bilateral injections of DAMGO (2 nmol in 0.5 μL) into the CeA induced 0.3 M NaCl and water intake, and pre-treatment with losartan (108 nmol in 0.5 μL) injected into the CeA reduced 0.3 M NaCl and water intake induced by DAMGO. In FURO/CAP rats, pre-treatment with losartan (108 nmol in 0.5 μL) injected into the CeA attenuated the increase in 0.3 M NaCl and water intake induced by DAMGO (2 nmol in 0.5 μL) injected into the same site. The results suggest that the natriorexigenic effect of DAMGO injected into the CeA is facilitated by endogenous angiotensin II acting on AT1 receptors in the CeA, which drives rats to ingest large amounts of hypertonic NaCl.

Key words: sodium appetite, the central nucleus of the amygdale, μ-opioid receptors, angiotensin II, losartan.

INTRODUCTION

The central nucleus of the amygdala (CeA), a critical coordinator of emotion and behavior (Lang and Davis, 2006), hosts important facilitatory mechanisms for the control of sodium intake. Previous studies showed that bilateral electrolytic lesions of the CeA abolished spontaneous sodium intake and sodium appetite induced by subcutaneous injections of the mineralocorticoid deoxycorticosterone, the α_2-adrenoceptor antagonist yohimbine, or angiotensin II (ANG II), intracerebral ventricle injections of renin or by 24 h of sodium depletion in rats treated with furosemide (Galaverna et al., 1992; Zardetto-Smith et al., 1994), suggesting that induction of sodium appetite by different excitatory stimuli like ANG II or mineralocorticoids depends on CeA facilitatory mechanisms for the control of sodium intake. Lately, we have also shown that CeA μ-opioid receptor (μ-OR) activation enhances 0.3 M NaCl intake in rats submitted to water deprivation–partial rehydration (WD–PR) or in rats treated with the diuretic furosemide (FURO) (10 mg/kg b.w.) combined with a low dose of the angiotensin-converting enzyme inhibitor captopril (CAP) (5 mg/kg b.w.) injected subcutaneously (FURO/CAP) (Yan et al., 2013).

The CeA produces its actions through extensive efferent projections to the basal forebrain, hypothalamus, midbrain, and brainstem nuclei that mediate fear response, reward behavior, and environmental analgesia (Pitkanen et al., 1997; Swanson and Petrovich, 1998; Davis, 2000). μ-ORs are present in the CeA (Mansour et al., 1995; Poulin et al., 2006; Glass et al., 2009) and the CeA contains intrinsic neurons and axon terminals that contain opioid peptides (Fallon and Leslie, 1986; Cassell and Gray, 1989; Poulin et al., 2006). Activation of μ-ORs in the CeA has shown to inhibit most of CeA neurons (Zhu and Pan, 2004; Chieng et al., 2006). Given the anatomical evidence that CeA cells and their efferent projections are predominantly GABAergic (Swanson and Petrovich, 1998; Sah et al., 2003), it is possible that CeA neurons hyperpolarized by the activation of CeA μ-ORs are mostly GABAergic neurons and at least some of them send GABAergic output projections (Zhu and Pan, 2004). Taken together, opioid could negatively modulate CeA GABAergic projection neurons.

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Abbreviations: μ-ORs, μ-opioid receptors; AHA, anterior hypothalamic area; ANOVA, analysis of variance; ANG II, angiotensin II; AT1, ANG II type 1; CAP, angiotensin-converting enzyme inhibitor captopril; CeA, central nucleus of the amygdala; DAMGO, [D-Ala^2, N-Me-Phe^4, Gly^5-ol]-enkephalin; FURO, diuretic furosemide; IPSCs, inhibitory post-synaptic currents; LPBN, lateral parabrachial nucleus; MnPO, median preoptic nucleus; NTS, nucleus of the solitary tract; WD–PR, water deprivation–partial rehydration.

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The effects of ANG II acting centrally on regulation of fluid and electrolyte balance and related behaviors are mediated mainly by ANG II type 1 (AT1) receptors located in different regions of the brain, such as the lateral parabrachial nucleus (LPBN), anterior hypothalamic area (AHA), amygdala, subfornical organ (SFO) and organum vasculosum of the lamina terminalis (OVLT) (Fregly and Rowland, 1991; Rowland et al., 1992; Mckinley et al., 1996; Morris et al., 2002; Krause et al., 2008). The CeA contains AT1 receptors 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., 2002). Different investigations using whole-cell voltage-clamp techniques have shown that ANG II acting on AT1 receptors may modulate GABAergic synaptic transmission and the effects of ANG II acting on pre- and post-synaptic AT1 receptors are just opposite (Li et al., 2003; Li and Pan, 2005; Henry et al., 2009; Xing et al., 2009). It has been suggested that ANG II acting on pre-synaptic AT1 receptors decreases GABA release and reduces 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 AT1 receptors increases IPSCs in sodium-sensitive neurons in the median preoptic nucleus (MnPO), 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 possibility of opioid negatively modulating CeA GABAergic projection neurons, the effect of the activation of CeA μ-ORs on sodium intake (Yan et al., 2013), and the results of previous studies showing that AT1 receptor activation may modulate GABAergic synaptic transmission (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 injections of losartan, the nonpeptide antagonist that selectively binds on AT1 receptors (Chiu et al., 1989), into the CeA on 0.3 M NaCl and water intake induced by the activation of μ-ORs with [D-Ala^2, N-Me-Phe^4, Gly^5-ol]-enkephalin (DAMGO) injections into the CeA in WD–PR rats or in FURO/CAP rats.

EXPERIMENTAL PROCEDURES

Animals

A total of 32 adult male Sprague–Dawley rats weighing 290 ± 20 g were used in the present study. The rats were housed in individual stainless steel cages (before cerebral cannulas) and metabolism cages (after cerebral cannulas) with free access to pelleted laboratory rodent chow, distilled water and 0.3 M NaCl solution. Rats were maintained at a colony room temperature of 23 ± 2 °C, humidity of 55 ± 10% and on a 12-h light/dark cycle with light onset at 7:00 AM. The experimental protocols followed the US National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication No. 80-23, 1996). All efforts were made to reduce animal discomfort and the number of animals used.

Cerebral cannulas

Following anesthesia with an intraperitoneal dose of chloral hydrate (300 mg/kg b.w.), the rats were secured in a stereotaxic apparatus (SN-2N, Narishige Group, Tokyo, Japan) for bilateral implantation of stainless steel cannulas (23 gauge) into the CeA. The stereotaxic coordinates of the CeA were determined according to the brain atlas of the rat (Paxinos and Watson, 1997) and were: 2.3 mm posterior to bregma, 4.0 mm lateral to the midline suture, and 7.0 mm below the skull surface. The tips of the cannulas were placed 1 mm above the CeA. The cannulas were cemented to the skull bone with dental acrylic resin and jeweler screws and filled with obstructors (30 gauge). After the cerebral surgery, the rats were allowed to recover for 7 days in individual metabolism cages, a part of Feeding-Drinking-Activity Analyser (Cat. No. 41800111213) (UGO Basline Biological Research Apparatus, COMERIO-Varese, Italy), with free access to pelleted laboratory rodent chow, distilled water and 0.3 M NaCl solution before starting 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 guide cannula, and manual injection was initiated 15 s later. The injection volume into the CeA was 0.5 μL in each site and the injection was delivered at a flow rate of 0.5 μL/min. The injection cannulas were maintained in place for 30 s after delivery of the drugs or vehicle to minimize the backflow. The obturators were replaced after the injections, and the rats were placed back into their individual metabolism cages.

Drugs

The drugs including the selective μ-OR agonist DAMGO, the specific AT1 receptor antagonist losartan potassium, the diuretic FURO, and the CAP, were purchased from Sigma–Aldrich (Sigma–Aldrich, Saint Louis, MO, USA). All the drugs were dissolved in sterile 0.9% (w/v) saline solution. Accordingly, the 0.9% saline solution was used as vehicle. The drugs and vehicle solutions were made just before the infusion. DAMGO was administered in the CeA at the dose of 2 nmol in 0.5 μL which has previously been shown to be effective in dose–response study on the effects of CeA injections of DAMGO on sodium and water intake (Yan et al., 2013). Losartan was injected into the CeA at the dose of 108 nmol in 0.5 μL based on previous studies that have tested the effects of central injections of losartan on sodium and water intake and on the pressor response to ANG II (Grippo et al., 2002; Menani et al., 2004; Da Silva et al., 2011a,b; Roncari et al., 2011). FURO and CAP were administered subcutaneously at 10 mg/kg and 5 mg/kg of body weight respectively as described previously