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BRAIN RESEARCH

Research Report

Forebrain regions affected by lateral parabrachial nucleus serotonergic mechanisms that influence sodium appetite

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ARTICLE INFO

Article history:
Accepted 9 April 2010
Available online 24 April 2010

Keywords:
Lateral parabrachial nucleus
Serotonin receptor
Salt appetite
c-fos-immunohistochemistry

ABSTRACT

Blockade of serotonergic receptors in the lateral parabrachial nucleus (LPBN), via bilateral injections of nonselective 5-hydroxytryptamine (5-HT)$_{1/2}$-receptor antagonist, methysergide causes a robust sodium appetite. Our aim was to elucidate which brain regions are activated when serotonergic pathways to the LPBN are blocked and combined with subcutaneous injection of isoproterenol causing a salt appetite. In the experimental group, conscious rats were administered methysergide (4 μg/0.2 μl) injected bilaterally into the LPBN. Control groups included rats administered with injections of vehicle bilaterally into the LPBN, rats administered methysergide into injection sites outside the LPB region, and rats that did not undergo surgery. Each group was treated with a subcutaneous injection of isoproterenol (30 μg/kg), a β-adrenergic agonist, and NaCl and water intakes were measured over 2 h. Bilateral injections of methysergide into the LPBN followed by subcutaneous isoproterenol induced a strong intake of 0.3 M NaCl ($p<0.01$) compared with all controls. Greater numbers of c-Fos-positive stained nuclei were observed in all brain regions assessed. The extended amygdala is rich in AT_1 receptors and ablation of these regions has been shown to reduce sodium appetite; therefore, neurons in these sites, and to a lesser extent the lamina terminalis, are likely primary targets of an inhibitory mechanism arising from the LPBN that acts to modulate sodium appetite.

1. Introduction

The LPBN has been described as receiving a strong serotonergic projection from the area postrema and nucleus of the solitary tract (NTS) (Lanca and van der Kooy, 1985) and sending efferent projections to other brain regions known to play a role in fluid and electrolyte balance (Fulwiler and Saper, 1984; Herbert et al., 1990). Previous investigations demonstrated that serotonergic mechanisms in the LPBN play an important inhibitory role in controlling water intake and sodium appetite. Blockade of serotonergic receptors in the LPBN via bilateral injections of methysergide resulted in a robust sodium appetite following administration of a variety of dipsogenic and or natriorexigenic stimuli compared with vehicle infused controls. Sodium

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Abbreviations: AP, area postrema; BST, bed nucleus of the stria terminalis; CeAm, central nucleus of the amygdala; MnPO, median preoptic nucleus; PVN, paraventricular nucleus of the hypothalamus; NTS, nucleus of the solitary tract; OVLT, organum vasculosum of the lamina terminalis; SFO, subfornical organ; SON, supraoptic nucleus

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doi:10.1016/j.brainres.2010.04.022

appetite was markedly increased when rats injected bilaterally with methysergide into the LPBN were also administered angiotensin II (Ang II) either ICV (Menani and Johnson, 1995) or directly into the SFO (Colombari et al., 1996). NaCl intake in response to several other stimuli was also increased by LPBN injection of serotonergic antagonists including administration of: systemic furosemide with captopril (Menani et al., 1998a), furosemide and a 24-h sodium-deficient diet (Menani et al., 1998b), 24 h water deprivation, intragastric load of hypertonic solution (De Luca et al., 2003) and SC isoproterenol (Menani et al., 2000). Conversely, rats injected with a serotonin receptor agonist, DOI bilaterally into the LPBN followed by both systemic furosemide and captopril were reported to consume significantly less hypertonic solution (Menani et al., 1996). These data suggest that a serotonergic pathway in the LPBN plays an important inhibitory role in the control of sodium appetite. Additionally, normohydrated rats administered injections of methysergide bilaterally into the LPBN and systemic relaxin resulted in a significant increase in NaCl intake with only a slight increase in water intake compared with controls. By contrast, the effects of this treatment were abolished when ICV losartan blocked central AT_1 receptors (Menani et al., 2004). Therefore, it is likely that the natrioexigenic effect of relaxin in methysergide treated rats is mediated by the activation of brain Ang II acting via AT_1 receptors.

Experiments have also focused on the effect of treatments that typically elicit a water drinking response. However, when administered in combination with serotonergic blockade of the LPBN, these rats also ingest significant volumes of hypertonic saline. Therefore, treatments that typically increase water intake can become natriorexigenic when the inhibitory serotonergic mechanism in the LPBN of rats is blocked. These treatments include administration of: systemic isoproterenol and furosemide (Menani et al., 2000) and either central Ang II (Colombari et al., 1996), carbachol (Menani et al., 2002) or relaxin (Menani et al., 2004). The purpose of the present study is to elucidate neural regions that drive thirst mechanisms and sodium consumption. We aimed to confirm that a thirst stimulus (isoproterenol), typically associated only with water intake, can induce sodium appetite following blockade of serotonergic mechanisms in the LPBN; and determine which brain regions are activated when serotonergic pathways to the LPBN are blocked and SC isoproterenol causes a salt appetite.

2. Results

2.1. *Effect of combining methysergide injected bilaterally into the LPBN and sc isoproterenol on water and 0.3 M NaCl intake*

Rats administered SC isotonic saline only drank an average of 0.6 ml of 0.3 M NaCl and less than 0.6 ml of water during the 2-h experimental phase. Rats injected with SC isoproterenol (30 μg/kg) drank an average of 3.0±0.5 ml of water; but no significant intake in 0.3 M NaCl was observed. Bilateral injections of the nonselective 5-$HT_{1/2}$-receptor antagonist, methysergide into the LPBN immediately followed by SC isoproterenol induced a strong 0.3 M NaCl intake ($p<0.01$) and an average of 3.6±1.6 ml of water. Rats administered bilateral injections of methysergide into the LPBN followed by SC isoproterenol were observed to consume more than 7.4±1.9 ml of 0.3 M NaCl ($p<0.01$), on average, 45 min following treatment. The combination of methysergide into the LPBN and SC isoproterenol induced a maximum intake of 10.0±1.8 ml of 0.3 M NaCl ($p<0.01$), 75 min after treatment, this volume remained unchanged for up to 2 h. Rats in each of the control groups in which isoproterenol was also administered, but no injection of methysergide was made into the LPBN, drank less than 1 ml of 0.3 M NaCl throughout the 2-h experimental phase. Water intake was not increased and indeed only slightly increased in one group of rats. This distinction was only

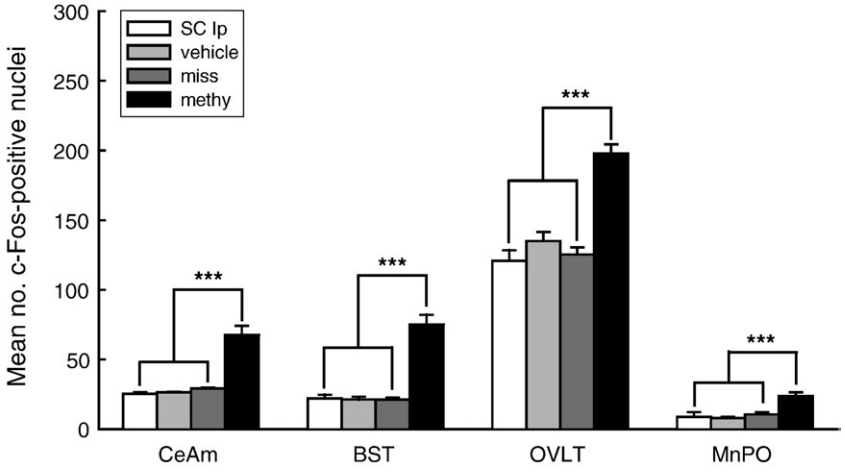

Fig. 1 – Mean number of nuclei detected as c-Fos-positive in rat brain. Control groups were injected with SC Ip and include: SC Ip, indicating rats administered SC Ip only; vehicle, indicating rats with vehicle injected bilaterally into the LPBN; miss, rats administered methysergide outside the LPBN region. The experimental group was administered with methysergide injected bilaterally into the LPBN combined with a SC injection of Ip (methy). Abbreviations: SC Ip, subcutaneous injection of isoproterenol; CeAm, central nucleus of the amygdala; BST, bed nucleus of the stria terminalis; OVLT, vascular organ of the lamina terminalis; MnPO, median preoptic nucleus. ***$p<0.001$ compared with all controls.