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Activation of paraventricular nucleus of hypothalamus 5-HT$_{1A}$ receptor on sodium intake

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Abstract

Hypothalamic paraventricular nucleus (PVN) has an important role in the regulation of water and sodium intake. Several researches described the presence of 5-HT$_1$ receptors in the central nervous system. 5-HT$_{1A}$ was one of the prime receptors identified and it is found in the somatodendritic and post-synaptic forms. Therefore, the aim of this study was to investigate the participation of serotonergic 5-HT$_{1A}$ receptors in the PVN on the sodium intake induced by sodium depletion followed by 24 h of deprivation (injection of the diuretic furosemide plus 24 h of sodium-deficient diet). Rats (280–320 g) were submitted to the implant of cannulas bilaterally in the PVN. 5-HT injections (10 and 20 μg/0.2 μl) in the PVN reduced NaCl 1.8% intake. 8-OH-DPAT injections (2.5 and 5.0 μg/0.2 μl) in the PVN also reduced NaCl 1.8% intake. pMPPF bilateral injections (5-HT$_{1A}$ antagonist) previously to 8-OH-DPAT injections have completely blocked the inhibitory effect over NaCl 1.8% intake. 5-HT$_{1A}$ antagonists partially reduced the inhibitory effect of 5-HT on NaCl 1.8% intake induced by sodium depletion. In contrast, the intake of palatable solution (2% sucrose) under body fluid-replete conditions was not changed after bilateral PVN 8-OH-DPTA injections. The results show that 5-HT$_{1A}$ serotonergic mechanisms in the PVN modulate sodium intake induced by sodium loss. The finding that sucrose intake was not affected by PVN 5-HT$_{1A}$ activation suggests that the effects of the 5-HT$_{1A}$ treatments on the intake of NaCl are not due to mechanisms producing a nonspecific decrease of all ingestive behaviors.

Keywords: PVN; Sodium appetite; 5-HT; 5-HT$_{1A}$; pMPPV; Sucrose

1. Introduction

Several studies have demonstrated that hypothalamic paraventricular nucleus (PVN) is an extremely important central area in the regulation of water and sodium intake [1–4].

Evidences in autoradiographics studies demonstrate the existence of ascendant projections from raphe mesencephalic nucleus (RMN) to the PVN. The mesencephalic cell groups send serotonergic projections particularly to the parvocellular portion of PVN. These fibers are usually placed in the medial part of the parvocellular division (generally periventricular), where cells are projected to the medium eminence, and they contain hypophysiotrophic substances as somatostatin, CRH and dopamine [5].

Sawchenko et al.'s [5] experiments demonstrated serotonergic fibers immunoreactive in PVN, which indicates they are more abundant in periventricular, dorsal and ventromedial regions of the PVN parvocellular portion. The concentration of serotonergic fibers in the magnocellular portion is slightly smaller and it is located preferentially where there is a great concentration of ocytocinergic cells.

There are seven families and at least 14 subtypes of serotonin receptors identified in the mammalian central system [6,7]. Autoradiographics studies show a regional difference of these subtypes

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inside the brain. Subtype 5-HT_{1A} is particularly concentrated in several central sites including hypothalamus [8].

Most of the serotonin receptors belong to the large family of seven transmembrane domains G-protein coupled receptors, that couple to and transduce signals via guanine nucleotide binding regulatory proteins (G-proteins). The specific agonist 8-OH-DPTA has preferential affinity for and stabilizes the active receptors state (R∗) and subsequently promotes G-protein coupling to the receptor to form the complex, i.e., the agonist-activated receptor *n*-G-protein complex. The selective antagonist for the 5-HT_{1A}-receptor pMPPF and their binding does not require G-proteins to interact with the receptors [9].

Considering that the PVN serotonergic pathways, especially 5-HT_{1A} receptors, are important in several physiological mechanisms, the following studies investigated the effects of bilateral PVN injections of 5-HT, 8-OH-DPTA (a specific 5-HT_{1A} agonist) and pMPPF (a selective 5-HT_{1A} antagonist) on the ingestion of hypertonic NaCl in rats depleted of sodium with furosemide–sodium deficient diet.

2. Methods

2.1. Animals

Male Holtzman rats weighting 300 g were kept in individual cages, an acclimated room (temperature 23 ± 2 °C and humidity 60 ± 10%) with 12–12 h light–dark cycles. They were fed with rat pellet chow (Purine Rat chow, sodium control 0.5%) water and NaCl 1.8% *ad libitum*. Experimental protocols were approved by Animal Experimentation Ethics Committee from Faculdade de Odontologia de Araraquara.

2.2. Brain surgery

Rats were anesthetize with a mixture of ketamine (80 mg/kg body-weight) and xylazine (7 mg/kg body-weight) and restrained in a stereotaxic apparatus (Kopf 900 model). Lambda and bregma were used as a reference to the rats' heads level. Using bregma, it was identified stainless steel cannula introduction points in rats' heads. In these points cranial bone trepanation with a spherical drill opening holes with an approximate diameter of 1.5 mm was performed. Stainless steel cannulas (12 × 0.5 mm d.i.) were placed bilaterally in the brain according to the coordinates: 1.8 mm caudal to bregma, 0.6 mm lateral to midline and 5.0 mm below dura-mater. Cannulas were fixed to the skull with screws and acrylic resin. An intramuscular injection of prophylactic dose of penicillin (60.000 UI) and topical Betadine were administered after surgery to control infection. Experimentation began at least 10 days after surgery.

2.3. Drugs

Furosemide was dissolved in water with adjusted pH in 9.0 using NaOH 1 M and it was administered subcutaneously with 10 mg/kg body-weight.

The other drugs were dissolved in physiologic saline solution and injected into rats' brains using Hamilton micro syringe (10 μl) connected to a PE-10 polyethylene tube and an injector needle introduced into the brain through guide cannulas previously fixed to the animals' head. Injector needle (diameter 0.3 mm) was 2 mm larger than guide cannulas. The volume of PVN injection was 0.2 μl. Drugs used in central injections: 5-hydroxytryptamine, 5-HT (Sigma Chemical, St Louis, MO, USA), 8-hydroxy-2-(di-*n*-propylamino) tetralin HBr, 8-OH-DPAT, 4-(2′-methoxyphenyl)-1-[2′-[*N*-(2″-pyridyl)-*p*-fluorobenzamide] etyl] piperazine, pMPPF (RBI, Natick, MA, USA).

2.4. Induction and measure of NaCl 1.8% intake

The amount of NaCl 1.8% ingested in different experimental protocols was quantified with glass burettes 0.1 ml, graduated and adapted with a metal spout. The animals had no access to food during the ingestion experiments. Rats had water and NaCl 1.8% tubes 24 h a day since five days before the surgery.

NaCl 1.8% ingestion was induced by furosemide treatment (10 mg/kg) sc. The cages were washed immediately after furosemide treatment so that rats could be confined in a depleted sodium environment followed by access to sodium deficient diet (rat modified chow with 0.03%NaCl, ICN Biochemical) and the rats were kept without NaCl 1.8% for 24 h (water burettes were not removed).

Bilateral injections with 5-HT (10 and 20 μg), 8-OH-DPAT (1, 2.5 and 5 μg) or saline were made just before offering water and NaCl 1.8% burettes to the animals.

pMPPF and the vehicle (NaCl 0.9%) were administered isolated or combined with 5-HT and 8-OH-DPAT injections. When combined, pMPPF (3.8 μg) or vehicle injections were made 10 min before 5-HT (20 μg) or 8-OH-DPAT (2.5 μg) injections. Water and NaCl 1.8% burettes were offered immediately after brain injections. In the experiments with two associated injections the burettes were offered right after the second injections.

In each experimental session, one-half of the rats received bilateral PVN injections of vehicle and the remaining animal received drug injection into this structure in a counter-balanced design. Rats received no more than three tests. A recovery period of at least 5 days was allowed between tests.

The measure of NaCl 1.8% ingestion was made at 15, 30, 60, 90 and 120 min of experiment.

2.5. Brain histology

After the experiments rats were anesthetized with sodium thiopental (80 mg/kg) intraperitoneal, received dye injections (Evans blue, 0.2 μl) in the PVN and had a brain perfusion through heart injection with saline solution (30 ml) followed by formalin 10% solution (30 ml). Brains were removed and fixed in formalin 10% for a few days. Transversal cuts (thickness 30 μm) of the injection points were made with a freezing microtome (Leica). Histological cuts were placed in a glass slide, dyed with Giemsa and analyzed to identify injection points according to Paxinos and Watson atlas [10]. The presence of the dye in the third ventricle was observed at this time. Only animals in which the dye was restricted bilaterally in the PVN were used in this study. The PVN parameters studied were observed in subjects that had injection