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# Role of the 5-$HT_{1A}$ somatodendritic autoreceptor in the dorsal raphe nucleus on salt satiety signaling in rats

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ABSTRACT

We investigated the possible role of 5-$HT_{1A}$ somatodendritic autoreceptors in the dorsal raphe nucleus (DRN) on salt intake response during basal conditions and following natriorexigenic challenge aroused by sodium depletion in rats. Acute systemic administration (76–1520 nmol/kg s.c.) of 8-OH-DPAT, a selective 5-$HT_{1A}$ somatodendritic autoreceptor agonist, induced a clear and dose-dependent preference for salt intake through free choice between water and 0.3 M NaCl simultaneously offered under basal conditions. Acute intra-DRN microinjection (7.5 nmol/rat) of 8-OH-DPAT significantly mimicked the acute systemic protocol in sodium-replete rats. Interestingly, microinjection of 8-OH-DPAT into the DRN raised an additional long-lasting increase of 0.3 M NaCl intake in sodium-depleted rats despite a high volume ingested 30 min after central injection. Conversely, chronic systemic treatment (1520 nmol/kg s.c.) with 8-OH-DPAT for 2 and 3 weeks or repeated intra-DRN microinjection (7.5 nmol/rat) evoked a significant long-term decrease in 0.3 M NaCl intake in sodium-depleted rats given only water and a sodium-deficient diet over the course of 24 h after furosemide injection. These results show a clear-cut involvement of the DRN 5-$HT_{1A}$ somatodendritic autoreceptors in sodium satiety signaling under basal conditions and during the consummatory phase of salt intake in sodium-depleted rats.



## Introduction

The sodium appetite is a homeostatic behavior that is dependent upon body fluid volume adjustment (Johnson and Thunhorst, 1997; Fitzsimons, 1998; Antunes-Rodrigues et al., 2004; Reis, 2007; Geerling and Loewy, 2008; Morris et al., 2008). Research from our laboratory has shown the involvement of the brain serotonergic system in hydroelectrolytic balance, as demonstrated with the following physiological functions: renal sodium excretion (Reis et al., 1991a; 1991b; 1994), atrial natriuretic peptide release (Reis et al., 1994; Antunes-Rodrigues et al., 2004) and sodium appetite control (Badauê-Passos et al., 2003; Olivares et al., 2003; Lima et al., 2004; Reis, 2007). We have demonstrated that the salt satiety serotonergic mechanism arises from the dorsal raphe nucleus (DRN) (Cavalcante-Lima et al., 2005a,b; Badauê-Passos et al., 2007; Reis, 2007). This structure seems to consist of multiple subpopulations of serotonergic neurons, which convey projections toward *lamina terminalis* circuitries involved in arousal of the sodium appetite as well as body fluid balance (Azmitia and Segal, 1978; Azmitia, 1987; 2001; Reis et al., 1994; Tanaka et al., 2001; Reis, 2007). Using immunoreactive labeling experiments, Badauê-Passos et al. (2007) demonstrated that rats that ingested NaCl had a significantly higher number of cells double-labeled with Fos protein, a marker of neural activity, and Fluorogold (FG), a retrograde tracer, in the subfornical organ, the organum vasculosum of the *lamina terminalis* and the median preoptic nucleus. They concluded that the *lamina terminalis* circuitry may help relay inhibitory messages to the serotonergic neurons in the DRN that, in turn, limit the intake of sodium and prevent excess volume expansion. This theory was originally proposed by Lind (1986) and subsequently supported by research from Franchini et al. (2002), Cavalcante-Lima et al. (2005a,b) and Reis (2007).

Abrams et al. (2004) have proposed an interesting function for the DRN. They demonstrated that topographically organized subpopulations of serotonergic neurons in the DRN comprise exclusive projecting clusters. This functional organization could modulate a myriad of homeostatic behaviors within the basal forebrain, particularly in the *lamina terminalis*.

Studies from our group as well as from the Menani, Vivas and Antunes-Rodrigues groups (Menani et al., 1996; 1998a,b; Franchini et al., 2002; Margatho et al., 2007) have raised the possibility that there is a physiological integration between the lateral parabrachial nucleus (LPBN) and DRN serotonergic neurons. This integration would convey information to the forebrain in order to coordinate adjustments of sodium excretion and the response to salt satiety. Recently, Margatho et al. (2008) demonstrated that blood volume-expanded

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rats showed a significantly greater number of Fos and FG double-labeled cells within the DRN nucleus. These results strengthen our hypothesis (Reis, 2007) that the mechanism responsible for triggering the sodium satiety control in response to salt ingestion and subsequent blood volume enhancement is the result of an interaction between the DRN and the LPBN.

The increased serotonergic activity from specific clusters of DRN neurons, therefore, seems to be clearly connected to sodium balance within the body. Systemic administration of serotonin releasers, selective serotonin re-uptake inhibitors (SSRIs) or 5-$HT_2$ agonists induces sodium appetite inhibition (Rouah-Rosilio et al., 1994; Badauê-Passos et al., 2003; Castro et al., 2003; Reis, 2007). Electrolytic or excitotoxic lesions in the DRN, however, increase both the basal and stimulated sodium appetite (Olivares et al., 2003; Cavalcante-Lima et al., 2005a,b). All of these evidence corroborate the preliminary data of Munaro and Chiaraviglio (1981) which reported that both the levels of serotonin and its metabolite, 5-hydroxyindoleacetic acid increased in sodium-depleted rats allowed to drink sodium.

In contrast to systemic administration of serotonin releasers, SSRIs or 5-$HT_2$ agonists, acute systemic administration of 5-$HT_{1A}$ receptor agonists increases the hypertonic saline intake in water deprived-rats after 30 min (Cooper et al., 1988; Cooper and Ciccocioppo, 1993). Despite this unsuitable protocol the resulting observations raised, for the first time, the possibility that 5-$HT_{1A}$ agonists act at inhibitory autoreceptors and thus diminish central serotonergic activity. Based on this, we decided to focus on 5-$HT_{1A}$ somatodendritic receptors in the DRN, in order to study their relationship with salt satiety signaling after sodium appetite challenge.

In reevaluating this paradigm, we hypothesized that the activation of 5-$HT_{1A}$ somatodendritic autoreceptors within the DRN evokes a reduced ascending transmission, which results in an increased natriorexigenic response. This hypothesis was examined using systemic and intra-DRN administration of 8-OH-DPAT, a selective 5-$HT_{1A}$ somatodendritic autoreceptor agonist, in rats under basal and sodium-depleted conditions. Acute systemic or intra-raphe administration of 5-$HT_{1A}$ receptor agonists decreases the spontaneous firing rate of serotonergic neurons, thereby promoting reduced terminal serotonin synthesis and release (Sprouse and Aghajanian, 1987; Hjorth and Magnusson, 1988; Hutson et al., 1989, Invernizzi et al., 1991).

We examined the natriorexigenic response induced by sodium depletion in rats chronically treated with 8-OH-DPAT. We wanted to determine if the alteration of serotonergic plasticity during chronic exposure to 5-$HT_{1A}$ receptor agonists affects the fluid intake response. In support of our hypothesis, it has been widely reported that chronic treatment with 5-$HT_{1A}$ receptor agonists induces down-regulation of the 5-$HT_{1A}$ somatodendritic autoreceptors, resulting in an increase in ascending transmission and serotonin release (Albert et al., 1996; Hjorth et al., 2000; Assié et al., 2006; Watanabe et al., 2006).

## Materials and methods

### *Animals and maintenance*

Male Wistar rats weighing 280–300 g were maintained in a room with lights on from 7:00 a.m. to 7:00 p.m. and a controlled temperature of 25 °C. The rats also had free access to Purina chow and distilled water. One week before the experiment, rats were randomly assigned to metabolic cages with an *ad libitum* supply of food, water and 0.3 M NaCl in volumetric burettes.

Animal handling and experimental procedures were performed according to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No.85-23, revised 1996) and in compliance with pertinent current Brazilian legislation as well as approval by the institutional committee of ethics and animal welfare.

### *Drugs*

The selective 5-$HT_{1A}$ receptor agonist 8-hydroxy-2-(n-dipropylamino) tetralin-HBr (8-OH-DPAT) (Tocris, St Louis, MO, USA) was diluted in isotonic saline for systemic (S.C.) administration and in artificial cerebrospinal fluid (aCSF) for intra-raphe administration (Arvidsson, et al., 1981; Middlemiss and Fozard, 1983; Pucadyil et al., 2005). To induce sodium depletion, furosemide (Sanofi-aventis, São Paulo, SP, Brazil) was subcutaneously administered 24 h before the initiation of experiments.

### *DRN stereotaxic surgeries and intra-raphe administration*

Rats were placed in a Kopf stereotaxic instrument for the insertion of the intra-DRN cannula implant. Stereotaxic surgeries were performed under anaesthesia induced by ketamine (Vetbrands, Paulinia, Brazil, 60 mg/kg i.p.) combined with xylazine (Bayer, São Paulo, SP, Brazil, 7.5 mg/kg i.p.). After surgery, rats were given a prophylactic dose of Veterinary Pentabiotic (Fort Dodge, Campinas, SP, Brazil). For intra-raphe administrations, stainless-steel guide cannulae (26-gauge, o.d. 0.6 mm, length 12 mm) were stereotaxically implanted into the dorsal–medial region of the DRN (AP = 7.6–7.8 mm; L = 0 mm; V = 5.8 mm) according to the coordinates of Paxinos and Watson (1986). Implantations were performed one week before beginning the experiments. The upper part of the cannula was attached to the skull with dentistry methyl methacrylate and fixed on the bone surface with stainless-steel screws. After surgery, a stainless-steel obturator was inserted into the cannula, and rats were given a prophylactic dose of penicillin (30,000 IU, i.m., Fort Dodge, Campinas, Brazil) and the anti-inflammatory agent, ketoprofen (Merial, Campinas, SP, Brazil). Following surgery, the rats were housed in stainless-steel metabolic cages (roof diameter × floor diameter × height: 310 × 310 × 340 mm) for recovery and were kept in these cages throughout the experiment.

During recovery period, the rats were handled daily and maintained in individual cages, and none of the rats displayed any perceptible motor alterations or unusual behaviors. On the day of the experiment, the obturators were removed. The rats received an intra-DRN injection of solutions in a final volume of 0.2 μl over a period of 30 s using a 2 μl Hamilton syringe connected to a stainless-steel injector with polyethylene tubing. The injector extended an additional 0.4 mm beyond the end of the cannula. This position ensured that microinjections occurred at the dorsal–medial site of the DRN.

After the end of the experiments, the rats were euthanized under profound anaesthesia (sodium thionembutal, 100 mg/kg i.p.) and the brains were removed and fixed in 10% formalin. Coronal sectioning (thickness 30 μm) of the midbrain region was performed, and the slices were subsequently stained with cresyl violet for to determine the injection sites and the location of the tracking cannulae. Only those rats that had cannulae placed into the DRN at the stereotaxic coordinates described above were used in our statistical analyses. A representative histological section showing the site of injection in the midbrain is depicted in Fig. 1.

### *Experimental protocols*

Hypertonic saline (0.3 M NaCl) was used in volumetric burettes for evaluation of the sodium appetite response during both basal and sodium-depleted conditions. This NaCl concentration is normally aversive for gustatory perception in rats. The free choice paradigm with concomitant water and salt offerings was employed under basal conditions, as this situation may occur more frequently in nature than one in which only water or salt is available (Mecawi et al., 2008). For the natriorexigenic response evaluation, sodium depletion was induced by an injection of furosemide (20 mg/kg s.c.) to stimulate natriuresis, which promotes renal sodium loss, which was then followed overnight by free access to only a low sodium diet (corn