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Parabrachial lesions in rats disrupt sodium appetite induced by furosemide but not by calcium deprivation

P.S. Grigson [a,*], E.M. Colechio [a], M.L. Power [b], J. Schulkin [c], R. Norgren [a]

[a] Department of Neural and Behavioral Sciences, College of Medicine, The Pennsylvania State University, 500 University Drive, Hershey, PA 17033, USA
[b] Nutrition Laboratory and Conservation Ecology Center, Smithsonian Conservation Biology Institute, National Zoological Park, P. O. Box 37012, MRC 5503, Washington, DC 20013-7012, USA
[c] Department of Neuroscience, Basic Sciences Building, Georgetown University, 3900 Reservoir Road, N.W., Washington, DC 20057, USA

HIGHLIGHTS

- Controls and rats with parabrachial lesions (PBNx) failed to ingest 0.5 M CaCl2 after extended dietary calcium depletion.
- Both groups did ingest significantly more 0.5 M NaCl after the same calcium depletion.
- Following sodium depletion with a diuretic, the PBNx rats ingested only one-third as much 0.5 M NaCl as the Controls.
- Damage to the second central gustatory relay in the parabrachial nuclei disrupts an appetite for 0.5 M NaCl in sodium but not calcium depleted rats.

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ABSTRACT

An appetite for $CaCl_2$ and NaCl occurs in young rats after they are fed a diet lacking Ca or Na, respectively. Bilateral lesions of the parabrachial nuclei (PBN) disrupt normal taste aversion learning and essentially eliminate the expression of sodium appetite. Here we tested whether similar lesions of the PBN would disrupt the calcium-deprivation-induced appetite for $CaCl_2$ or NaCl. Controls and rats with PBN lesions failed to exhibit a calcium-deprivation-induced appetite for $CaCl_2$. Nevertheless, both groups did exhibit a significant calcium-deprivation-induced appetite for 0.5 M NaCl. Thus, while damage to the second central gustatory relay in the PBN disrupts the appetite for 0.5 M NaCl induced by furosemide, deoxycorticosterone acetate, and polyethylene glycol, the sodium appetite induced by dietary $CaCl_2$ depletion remains intact.

1. Introduction

The gustatory system helps regulate the nutrient and mineral selection required for homeostasis [1,2]. The central gustatory system plays an important role in this adaptive process [3]. In rodents, the second central gustatory relay in the pontine parabrachial nuclei (PBN) is critical for the acquisition of a conditioned taste aversion (CTA) and for the expression of a sodium appetite [4–6]. Specifically, when assessed using a 0.5 M concentration of NaCl, rats with bilateral lesions of the PBN failed to demonstrate a sodium appetite following treatment with the diuretic, furosemide, with the mineralocorticoid, deoxycorticosterone acetate (DOCA), or with 30% polyethylene glycol (PEG). These same rats also failed to exhibit a LiCl-induced conditioned taste aversion, now the behavioral index of well-placed bilateral PBN lesions [7].

We sought to further examine the role of this region with regard to another phenomenon, the ingestion of calcium and sodium salts after dietary calcium deprivation. Rats on a calcium deficient diet [8–11], or that are parathyroidectomized, will ingest both calcium [12–14] and sodium salts [10,11,13] at concentrations they normally avoid. Calcium deprivation also raises both $CaCl_2$ and NaCl intake during sham drinking [15], and increases the palatability of calcium salts [16]. One suggestion for why rats that are calcium deprived also ingest NaCl is that short-term ingestion of NaCl may raise ionized calcium and, therefore, may confer relief of the deficit [17]. The question addressed here is whether rats with lesions of the PBN also will be able to express either or both of these appetites.

Calcium deprivation changes gustatory afferent neural activity. There are known differences in calcium solution preferences and the activity of the chorda tympani in calcium-deprived rats [18,19] and in the gustatory region of the nucleus of the solitary tract [20]. In addition,

* Corresponding author.
E-mail addresses: psg6@psu.edu (P.S. Grigson), emc231@psu.edu (E.M. Colechio), mpower@acog.org (M.L. Power), Jschulkin@acog.org (J. Schulkin), rxn5@psu.edu (R. Norgren).

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large lesions of the medial region of the thalamus that include the gustatory relay, compromise calcium intake after parathyroidectomy [21]. The pontine parabrachial nuclei receive gustatory projections from the nucleus of the solitary tract and, in turn, project to the gustatory thalamus [22,23] (for a review see [3]). Thus, in the following experiments we compared PBN-intact Controls with rats that had taste-guided bilateral PBN lesions (PBNx) for differences in the following three phenomena: 1) expression of a calcium and sodium appetite following calcium deprivation, 2) expression of a furosemide-induced sodium appetite, and 3) the ability to learn a LiCl-induced conditioned taste aversion. The latter two tests serve as functional indexes for PBN lesions. Thus, if the lesions are well placed, both the furosemide-induced sodium appetite and the LiCl-induced conditioned taste aversion should be disrupted.

2. Methods

2.1. Subjects

Sprague–Dawley rats (n = 36, Charles River Laboratories, Wilmington, MA) weighed between 302 and 456 g at the time of surgery. Between surgery and the current tests these rats were used in another (unpublished) experiment that involved testing their ability to learn a LiCl-induced conditioned taste aversion with corn oil as the conditioned stimulus (CS). The rats were housed in individual, hanging, wire mesh cages. The room was temperature controlled, with an automatic light: dark cycle (12 h, lights on at 7 AM). All experimental manipulations were performed during the lights on period. Rats were maintained on ad libitum food and water except where noted below.

2.2. Surgery

Seven rats were non-surgical Controls. The remaining 29 animals consisted of 7 full surgical Controls that were subjected to all procedures except that their intracerebral infusions were saline rather than ibotenic acid. The remaining 22 rats had bilateral ibotenic acid lesions centered on electrophysiologically-identified gustatory responses in the pontine parabrachial nuclei (PBN; 0.2 μl per side, 20 μg/μl in phosphate buffered saline, pH = 7.4). The procedures are essentially identical to those used previously and will be only summarized here [see 24,25]. All procedures conformed to NIH guidelines and were approved by the Institutional Animal Care and Use Committee of the Penn State College of Medicine.

The rats were pretreated with atropine sulfate (0.1 mg, ip) and then anesthetized with pentobarbital sodium (50 mg/kg, ip). Anesthesia was supplemented every 45–60 min as needed (5–8 mg, ip). The rats were mounted in a Kopf stereotaxic apparatus with the skull flat between β and λ, the skull bared, and holes burred over the intended recording targets. First, gustatory responses were located bilaterally in the dorsal pons using standard recording techniques and glass insulated tungsten electrodes (Z = 1.0–2.5 MΩ at 1 KH). The initial penetration ranged between −11.0 and −12.0 mm posterior to β and 1.8–2.0 mm lateral to the midline with the electrode inclined caudally 20° off vertical. Testing began ~5.5 mm deep to the dura and consisted of noting neural activity on an audio monitor and an oscilloscope while washing the anterior tongue with distilled water and 0.1 M NaCl, both at room temperature. When a multiunit taste response was encountered, typically after 1–5 penetrations, the electrode was removed and the process repeated on the contralateral side. With the taste responses located, the recording electrode was replaced with a glass micropipette that had been glued to the shaft of a Hamilton 1.0 μl syringe loaded with ibotenic acid. The pipette was positioned using the coordinates established previously. Typically, a gustatory response also was recorded through the pipette. The ibotenic acid (0.2 μl) was then infused over a period of 10 min. The pipette remained in place for an additional 10 min before being withdrawn. After both infusions, the wound was closed with clips and the rat given gentamicin prophylactically (0.15 ml, im).

2.3. Test conditions

Three experiments were performed on these rats. In Experiment 1, the ingestion of water, calcium salt (0.5 M $CaCl_2$), and sodium salt (0.5 M NaCl) was measured under baseline conditions and then after Ca deprivation. The 0.5 M $CaCl_2$ was chosen to match the concentration of the 0.5 M NaCl solution, a concentration that is consistently rejected by intact, Na^+ replete Controls. In Experiment 2, ingestion of water, $CaCl_2$, and NaCl was measured after furosemide-induced sodium depletion. For each of these experiments, Control and PBNx rats were separated into three groups — one was given a choice between water and 0.5 M $CaCl_2$ (group WC), another had water and 0.5 M NaCl (group WN), and the third, all three fluids (group WCN). Experiment 3 examined the ability of the rats to learn a LiCl-induced conditioned taste-aversion to a sweet CS. Neither $CaCl_2$ nor NaCl was provided.

2.3.1. Experiment 1: calcium deficient diet

For 10 days all rats were placed on a calcium deficient diet (AIN 76A, Dyets Inc., Bethlehem, PA) with the calcium added back. As mentioned above, in addition to distilled water (dH_2O), one group had access to a 0.5 M $CaCl_2$ solution, the second to 0.5 M NaCl, and the third to both 0.5 M NaCl and 0.5 M $CaCl_2$. Water and the tastants were presented in graduated cylinders affixed to the front of the cages with springs and intake was recorded daily. For the two groups with only 2 tubes, the left/right positions were switched daily. For the group with 3 choices, the tubes were repositioned randomly among the 6 possible orders. Following this 10 day baseline period, the salt solutions were removed, and the rats were fed only the calcium deficient diet for 30 days. Subsequently, the respective salts were replaced on the front of the cage for each group and 24 h intake was measured daily for 5 days.

2.3.2. Experiment 2: sodium depletion test conditions

At the end of the Ca-appetite test period, the diet was changed to the base diet (AIN 76, ICN Biochemicals #902903, Irvine, CA), which had adequate Ca^{++} included, but was without added NaCl. During this baseline period (11 days), the appropriate amount of NaCl was added back to this diet. The rats were maintained in the same groups and offered the same fluid choices described above. Intake stabilized over the last 7 days of this period. On day 8, the rats received an injection of furosemide (Furo, 10.0 mg in two equal doses 2 h apart, sc; see [26]). Furosemide promotes sodium excretion and a subsequent sodium appetite. Between the injection and the test the following morning, the rats had access only to water and the sodium deficient diet without added NaCl. The fluids were then returned to the cages and intake of the fluids was measured at 15, 30, 60, and 120 min, and again at 24 h. Normal chow (with NaCl) was returned 120 min into the test period. After a week on the normal chow, the entire regimen was replicated but, rather than Furo, the rats were injected with an equivalent volume of saline (sc).

2.3.3. Experiment 3: conditioned taste aversion (CTA)

All rats were water deprived and adapted to a regimen in which they had access to a water bottle at the front of the cage every morning for 15 min and every afternoon for 1 h. Water intake stabilized over 10 days. On the morning of day 11, all rats were presented with 0.15% Na-saccharin for 15 min instead of the water. Five minutes later, the rats were injected with lithium chloride (0.15 M; 1.5 mEq/kg body weight, ip). In the afternoon they were again offered water for 1 h. This was repeated twice with 2 water days (15 min a.m., 1 h p.m.) intervening between each cycle. On the fourth trial the rats were offered saccharin again but without the subsequent lithium injection.