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Early oxytocin inhibition of salt intake after furosemide treatment in rats?

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HIGHLIGHTS

- Acute furosemide-induced volume and sodium excretion activate PVN.
- Acute furosemide does not stimulate salt intake.
- PVN activation does not involve parvocellular OT neurons.
- Early inhibition of salt intake may not involve centrally-projecting OT neurons in the PVN.
- Estrogen has no effect on neuronal activation or salt intake after furosemide.

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ABSTRACT

Body fluid homeostasis requires a complex suite of physiological and behavioral processes. Understanding of the role of the central nervous system (CNS) in integrating these processes has been advanced by research employing immunohistochemical techniques to assess responses to a variety of body fluid challenges. Such techniques have revealed sex/estrogen differences in CNS activation in response to hypotension and hypernatremia. In contrast, it has been difficult to conclusively identify specific CNS areas and neurotransmitter systems that are activated by hyponatremia using these techniques. In part, this difficulty is due to the temporal disconnect between the physiological effects of treatments commonly used to deplete body sodium and the behavioral response to such depletion. In some methods, sodium ingestion is delayed in association with increased oxytocin (OT), suggesting an inhibitory role for OT in sodium intake. Urinary sodium loss increases within an hour after treatment with furosemide, a natriuretic-diuretic, but sodium intake is delayed for 18–24 h. Accordingly, we hypothesized that acute furosemide-induced sodium loss activates centrally-projecting OT neurons which provide an initial inhibition of sodium intake, and tested this hypothesis in ovariectomized Sprague-Dawley rats with or without estrogen using immunohistochemical methods. Neuronal activation in the hypothalamic paraventricular nuclei (PVN) after administration of furosemide corresponded to the timing of the physiological effects. The activation was not different in estrogen-treated rats, nor did estrogen alter the initial suppression of sodium intake. However, virtually no fos immunoreactive (fos-IR) neurons in the parvocellular PVN were also immunolabeled for OT. Thus, acute sodium loss after furosemide produces neural activation and an early inhibition of sodium intake that does not appear to involve activation of centrally-projecting OT neurons and is not influenced by estrogen.

1. Introduction

The maintenance of optimal levels of body water and sodium is accomplished by integrated physiological and behavioral mechanisms. Disruptions to body fluid balance elicit compensatory hormonal, neuronal, and behavioral responses that involve the central nervous system (CNS). Understanding of CNS pathways that elicit these compensatory responses has benefitted from studies employing immunohistochemical labeling of the fos protein as a marker of neuronal activation [1] stimulated by body fluid challenges. This approach has revealed sex/estrogen differences in CNS activation in response to body fluid challenges such as hypotension and hypernatremia [2–5] and, when combined with immunolabeling for markers of specific neurotransmitters, has shown stimulus-dependent responses by specific neuronal populations (e.g., to hypotensive vs. non-hypotensive hemorrhage; [6,7]). Importantly, fos immunolabeling has been shown to have functional correlates. For example, both hypovolemia-induced

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fos immunolabeling in the supraoptic nucleus of the hypothalamus (SON; the location of vasopressinergic neurons), and vasopressin release stimulated by hypovolemia are affected by estrogen [5]. Similarly, both hypotension-induced fos immunolabeling in the rostral ventrolateral medulla (RVLM; the hindbrain sympathoexcitatory area), and tachycardic responses to hypotension are influenced by estrogen [3]. In short, a wealth of information about the functional roles of various CNS areas and neurotransmitter systems in rats has facilitated understanding of the relationship between neural activation, as indicated by fos immunolabeling in specific CNS populations, and responses to body fluid challenges such as hypo- or hypertension, hypo- or hypervolemia, and hypernatremia [2–4,6–14]. In contrast, it has been difficult to identify CNS pathways associated with compensatory behavioral and physiological responses to **hypo**natremia.

Identifying CNS pathways involved in responses to hyponatremia is complicated by the temporal disconnect between the physiological effects of experimental methods frequently used to produce hyponatremia and the behavioral responses elicited by body sodium loss. For example, during dietary sodium deprivation, circulating levels of angiotensin and aldosterone increase after as little as 8 h [15], even though 7–10 days typically elapse before substantial ingestion of concentrated sodium solutions occurs (e.g. [16]). Similarly, the loop diuretic, furosemide, increases urine flow and urinary sodium loss within an hour after treatment, but an 18–24 hour delay typically transpires before rats consume concentrated sodium solutions [17]. Most research has focused on excitatory signals that act at the CNS to elicit sodium intake which compensates for hyponatremia and/or the concomitant hypovolemia (for reviews, see [18–20]). However, this behavioral response also may initially be inhibited by the very methods used to produce sodium/volume loss, as suggested by Stricker and colleagues. In a series of studies that used polyethylene glycol to produce iso-osmotic volume loss and stimulate sodium intake, these investigators found that the delay in sodium intake for 6–8 h after the induced hypovolemia was correlated with, though not causally related to, increased circulating oxytocin (OT; [21–25]). Thus, they proposed that parallel activation of centrally-projecting OT neurons inhibits sodium intake, an idea supported by subsequent experiments [26–29].

A logical extension of this idea is that experimental manipulations that stimulate sodium intake have mixed inhibitory and excitatory effects, the 'sum' of which is integrated at the CNS to govern the occurrence of sodium intake (see also, [19]). Indeed, De Luca and colleagues propose that specific neuronal subpopulations in the midbrain parabrachial nucleus (PBN) facilitate or inhibit sodium intake (see [30] for review). We take a broader perspective and postulate that sodium loss produces an initial CNS activation in multiple areas that, on balance, is inhibitory to sodium intake. Thus, ingestion of concentrated sodium solutions is delayed until the inhibition is reduced and/or excitatory signals increase, altering activity in CNS pathways and, ultimately, allowing sodium intake. The delayed sodium intake observed with experimental methods used to produce body sodium loss is consistent with this theory and serves as an opportunity to better understand the role of the CNS in compensatory sodium intake. This opportunity may be especially useful in understanding sex/estrogen-mediated differences in sodium intake [31–41]. Like many mammals, rats precisely regulate body sodium, but differences between males and females in the pattern of sodium and water ingestion suggest the method of achieving sodium balance may differ [31–32]. In this regard, we recently examined CNS activation in male rats and in ovariectomized (OVX) female rats with or without estrogen after furosemide-induced hyponatremia [41], imposing a delay of 18 h after furosemide treatment as typically is done in studies evaluating sodium intake. At that time point, we found elevated numbers of fos-positive neurons in the subfornical organ (SFO) that were greater in male rats; however, neuronal activation was observed in very few additional areas in any of the groups (see also, [9]).

These observations are consistent with our idea that, after an initial CNS activation that is inhibitory to sodium intake, a later-occurring decrement in that activation is necessary for the expression of sodium intake. However, they support our hypothesis only if greater neuronal activation occurs at an earlier time point. Therefore, we used fos immunolabeling to further evaluate this idea, conducting assessments of CNS activation at a time point after furosemide that corresponds with the onset of its physiological effects. In the framework of this concept, previous findings [25,28] suggest central OT as a logical candidate for inhibition at this earlier time. In fact, in our previous study [41], the hypothalamic paraventricular nucleus (PVN), site of centrally-projecting OT neurons, was virtually devoid of fos immunolabeling at the time point that coincides with stimulated sodium intake. Accordingly, we focused on activation in OT neurons of the PVN in these evaluations. Finally, given estrogen-mediated differences in sodium intake after furosemide [36,37,41], we conducted these analyses in OVX rats with and without estrogen to determine whether any differences in earlier activation depend upon the presence of estrogen.

2. Materials and methods

2.1. Animals

Adult female Sprague-Dawley rats weighing 250–325 g were individually housed in plastic cages in a temperature controlled (21–25 °C) room with a 12-h light-dark cycle with lights on at 06:00 and given ad libitum access to rodent chow (Harlan #2018) and water. All procedures were approved by the Oklahoma State University Center for Health Sciences Animal Care and Use Committee in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Ovariectomy and hormone replacement

Rats were bilaterally OVX under sodium pentobarbital anesthesia (50 mg/kg bw, i.p.), treated with Meloxicam (1.5 mg/kg bw, p.o.) for postoperative pain management and allowed to recover. Seven days after surgery, OVX animals were randomly assigned to be given subcutaneous injections of the sesame oil vehicle (OIL; 0.1 ml) or 17-β-estradiol-3-benzoate (EB; Fisher Scientific; 10 μg/0.1 ml sesame oil) to mimic the pattern of estrogen fluctuations [41]. Rats were given EB or OIL injections between 09:00 and 11:00 on days 1 and 2 of a 4-day regimen, with experimental procedures and testing conducted on day four as described below.

2.3. Furosemide treatment

Between 09:00 and 11:00 on the test day, food and water were removed from the cages, rats were weighed, and then randomly assigned to be given two injections of furosemide (FURO; Intervet; 5 mg/kg bw, s.c.) or 0.15 M NaCl (ISO) separated by 1 h. Rats were returned to their home cages after each injection.

2.4. fos and oxytocin immunolabeling

2.4.1. Perfusion, tissue collection, immunohistochemistry

One hour after the second injection of FURO or ISO, rats (OIL-FURO: n = 7, OIL-ISO: n = 4; EB-FURO: n = 6, EB-ISO: n = 5) were weighed and then anesthetized with sodium pentobarbital (30 mg, i.p). Blood samples were collected from the heart into heparinized tubes for later analysis of plasma protein concentration using a refractometer (Leica) and plasma sodium concentration using an ion-sensitive electrode system (EasyLyte). Rats then were perfused transcardially with 0.15 M NaCl followed by 4% paraformaldehyde. Uteri were removed and placed in 0.15 M NaCl prior to dissecting out and weighing a 10-mm section near the bifurcation. Brains were removed, post-fixed in 4%