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The effects of osmotic stimulation and water availability on c-Fos and FosB staining in the supraoptic and paraventricular nuclei of the hypothalamus

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Abstract

We studied the effects of osmotic stimulation on the expression of FosB and c-Fos in the supraoptic nucleus (SON) and paraventricular nucleus (PVN). Adult male rats were divided into two groups that were injected with lidocaine (0.1–0.2 ml sc) followed by either 0.9% or 6% NaCl (1 ml/100 g bw sc). After the NaCl injections, the rats were anesthetized and perfused 2, 6, or 8 h after the injections. Their brains were prepared for immunocytochemistry and stained with FosB and c-Fos antibodies. The number of c-Fos-positive cells was significantly increased only at 2 h in the SON and PVN. In contrast, the number of FosB-positive cells was significantly increased at 6, and 8 h in both the SON and PVN. In a second experiment, the effect of water availability on FosB staining 8 h after injections of 6% NaCl was tested in 3 groups of rats: water ad libitum, rats that had no access to water, and rats that were given water 2 h prior to perfusion. FosB staining was significantly reduced in both the SON and the PVN of rats that had ad libitum water compared to the two water-restricted groups. In the third experiment, rats were injected with either 0.9% NaCl or 6% NaCl and were either given ad libitum access to water or water restricted for 6 h after the injections and perfused 24 h after the saline injections. FosB staining was not increased when water was available ad libitum. FosB staining was significantly increased at 24 h in the rats injected with 6% NaCl when water was restricted. Thus, FosB may continue to influence protein expression in the SON and PVN for at least 24 h following acute osmotic stimulation.

Keywords: Vasopressin; Oxytocin; AP-1; c-Fos; FosB; Drinking behavior

Introduction

Neurons in the supraoptic nucleus (SON) and the magnocellular neurons of the paraventricular nucleus of the hypothalamus (PVN) are part of the neurohypophyseal system. These neurons project to the posterior pituitary where their axon terminals release vasopressin and oxytocin, two peptide hormones, into peripheral circulation (Armstrong, 1995). Vasopressin participates in body fluid homeostatis by influencing water re-absorption in the kidney (Cunningham and Sawchenko, 1991). In addition, it may act as a vasoconstrictor and alter cardiovascular reflex function (Hasser et al., 1997). Oxytocin, on the other hand, is involved in lactation and parturition (Cunningham and Sawchenko, 1991) and may be a natriuretic (Cunningham and Sawchenko, 1991; Forsling et al., 1994).

The activity of neurons in the SON and PVN is correlated with hormone release from the neurohypophysis

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(Dyball, 1971). Therefore, the afferent regulation of SON and PVN neurons determines the endocrine output of the posterior pituitary. One factor that influences the activity of both vasopressin- and oxytocin-releasing neurons in the rat is plasma osmolality. The osmoregulation of vasopressin and oxytocin release has been the subject of a great deal of debate. The magnocellular neurons of the SON are intrinsically osmosensitive and, therefore, capable of responding to a change in osmotic pressure without synaptic input (Bourque and Oliet, 1997). However, lesion studies and electrophysiological data indicate that input from the organum vasculosum of the lamina terminalis, a forebrain circumventricular organ, and other regions provides excitatory input to the SON that mediates the increase in vasopressin release associated with increased osmolality (Bourque and Oliet, 1997; McKinley et al., 1992).

Inducible transcription factors (ITFs) are activity-dependant proteins that regulate protein production in a variety of cell types including neurons (Herdegen and Leah, 1998). One member of the activator protein-1 (AP-1) family of ITFs, c-Fos, has been extensively used as an indicator of synaptic activation in the nervous system (Curran and Morgan, 1995; Dampney et al., 1995, 2003; Herdegen and Leah, 1998). Hyperosmotic stimulation has been shown to be a potent stimulus for ITF activation in the SON (Luckman et al., 1996; Miyata et al., 2001a,b; Moellenhoff et al., 1998; Oldfield et al., 1991; Xiong and Hatton, 1996; Ying et al., 1996). Changes in ITF expression may be an important aspect of the cellular response to osmotic stimulation. It has been hypothesized that ITFs may mediate changes in gene expression necessary to support alterations in neural function that are associated with increased physiological demand (Miyata et al., 2001a,b). Since different ITFs have different levels of constitutive expression and different time courses of expression (Herdegen and Leah, 1998; Herdegen et al., 1993; Luckman et al., 1996; Moellenhoff et al., 1998; Nestler et al., 2001), determining the time course of ITF expression following osmotic stimulation could be instrumental in understanding the role of various ITFs in the SON and PVN neurohypophyseal neurons.

Previous work demonstrates that FosB expression is maintained in the SON for 8 h following osmotic stimulation (Miyata et al., 2001a,b). The goal of this study was to extend the time course of c-Fos and FosB expression in the SON and PVN following acute osmotic stimulation. In addition, water intake inhibits osmotically stimulated vasopressin release in the rat (Huang et al., 2000) as well as water deprivation and angiotensin-induced c-Fos staining in the SON (De Luca et al., 2002; Xu and Herbert, 1994) and PVN (Xu and Herbert, 1994). Therefore, we conducted additional experiments to determine whether water intake would also inhibit the expression of FosB in the SON and PVN.

Experimental procedures

Animals

Experiments were conducted on male Sprague–Dawley rats, weighing 250–350 g (Harlan; Indianapolis, IN, USA). Rats were maintained in a temperature-controlled environment in a 12:12-h light:dark cycle. All surgical procedures and experimental protocols were approved by the IACUC at the University of Missouri-Columbia in accordance with the guidelines of the Public Health Service, American Physiological Society and the Society for Neuroscience.

Hypertonic saline injections

Adult male rats were injected with lidocaine (0.1–0.2 ml sc), followed by either 0.9% saline or 6% NaCl (1 ml/100 g bw sc). The lidocaine was used to anesthetize the injection site, since hypertonic saline can irritate the skin. In order to test the time course of FosB and c-Fos expression, rats were perfused at different time points following the sc injections. Rats were sacrificed 2 (0.9% n= 8; 6% n = 8), 6 (0.9% n = 8; 6% n = 8), or 8 h (0.9% n = 8; 6% n = 9) after sc injections. Injections were given between 8:30 and 9:30 A.M., and the rats did not have access to water following the injections unless indicated otherwise.

At the conclusion of the specified time, each rat was injected with pentobarbital sodium (50 mg/kg ip) and transcardially perfused with phosphate buffered saline (PBS) followed by 4% formaldehyde in PBS. Brains were removed from the cranium and placed in vials filled with 30% sucrose solution for cryoprotection.

Additional experiments tested the effects of water availability on FosB and c-Fos staining 8 h and 24 h after 6% NaCl injections. Three different treatment conditions were tested at the 8-h time point: no water available, water available ad libitum, or water available 6 h following NaCl injections (n = 10 for each group). For the 24-h study, two different treatment conditions were tested: water available ad libitum (0.9% n = 9; 6% n = 10) or water available 6 h after the NaCl injections (0.9% NaCl n = 9; 6% NaCl n = 11). The water intake of the ad libitum group and the water-restricted group in the 8-h study was measured with drinking tubes calibrated to the nearest 1 ml.

Plasma measurements

Separate groups of rats were used to measure the effects of the hypertonic saline injections on plasma osmolality, vasopressin, hematocrit, and specific gravity. Groups were prepared as described above. In the first study, rats were sacrificed at 0.5, 2, and 6 h after injections with either 0.9% or 6% NaCl sc (n = 7 for each of 6 groups). Groups for the second and third experiments were sacrificed 8 h (n = 21) and 24 h (n = 28) after receiving the sc injections. All rats were lightly anaesthetized (inactin 100 mg/kg ip), immedi-