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NEUROCHEMICAL BRAIN GROUPS ACTIVATED AFTER AN ISOTONIC BLOOD VOLUME EXPANSION IN RATS

A. GODINO,[a] A. GIUSTI-PAIVA,[b] J. ANTUNES-RODRIGUES[b] AND L. VIVAS[a]*

[a]*Instituto de Investigación Médica M. y M. Ferreyra (INIMEC-CONICET), Casilla de Correo 389-5000, Córdoba, Argentina*

[b]*Dept. Fisiología, Faculdade de Medicina, Ribeirao-Preto, Universidade de São Paulo, Brazil*

Abstract—In order to establish the involvement of particular neurochemical brain groups in the response to blood volume expansion, we analyzed Fos-labeling in combination with immunolabeling for serotonin, tyrosine hydroxylase, vasopressin and oxytocin, 90 min after a sham or i.v. isotonic blood volume expansion (BVE) in unanesthetized, unrestrained rats. We also examined the changes in concentration of oxytocin, atrial natriuretic peptide and vasopressin plasma, induced by blood volume load, to confirm our previous studies. The results demonstrate the participation of specific paraventricular and supraoptic nucleus groups of cells (oxytocinergic–vasopressinergic), serotoninergic dorsal raphé nucleus cells and catecholaminergic A1/A2/A6 groups (in the caudal ventrolateral medulla, nucleus of the solitary tract and locus coeruleus respectively), in the regulatory response to BVE. They provide detailed neuroanatomical evidence to support previous observations showing the contribution of these neurochemical systems in the neural, behavioral and endocrine response to isotonic BVE.

Key words: Fos-oxytocin immunoreactivity, Fos-vasopressin immunoreactivity, Fos-serotonin immunoreactivity, Fos-tyrosine hydroxylase immunoreactivity, central cardiovascular pathways.

*Corresponding author. Tel: +54-351-468-1465/66/67x108 or 120; fax: +54-351-469-5163.
E-mail address: lvivas@immf.uncor.edu, URL:http://www.immf.uncor.edu (L. Vivas).

Abbreviations: ANP, atrial natriuretic peptide; AP, area postrema; AVP, vasopressin; BNST, bed nucleus of the stria terminalis; BVE, blood volume expansion; CeL, central amygdaloid nucleus, lateral division; CVLM, caudal ventrolateral medulla; DAB, diaminobenzidine hydrochloride; DBB, diagonal band of Broca; DRD, dorsal subdivision of the dorsal raphé nucleus; DRN, dorsal raphé nucleus; EDTA, ethylenediaminotetraacetic acid; ir, immunoreactivity; LC, locus coeruleus; LPBN, lateral parabrachial nucleus; MAP, mean arterial pressure; ME, median eminence; MnPO, median preoptic nucleus; mNTS, medial subnucleus of the nucleus of the solitary tract; NHS, normal horse serum; NTS, nucleus of the solitary tract; NTS/AP, nucleus of the solitary tract adjacent to the area postrema; OT, oxytocin; OVLT, organum vasculosum of the lamina terminalis; PaAP, anterior parvocellular subdivision of the paraventricular nucleus of the hypothalamus; PaDC, dorsomedial subdivision of the paraventricular nucleus of the hypothalamus; PaLM, lateral magnocellular subdivision of the paraventricular nucleus of the hypothalamus; PaMM, medial magnocellular subdivision of the paraventricular nucleus of the hypothalamus; PaMP, medial parvocellular subdivision of the paraventricular nucleus of the hypothalamus; PaV, ventral subnucleus of the paraventricular nucleus of the hypothalamus; PB, phosphate buffer; PVN, paraventricular nucleus of the hypothalamus; RVLM, rostral ventrolateral medulla; SFO, subfornical organ; SON, supraoptic nucleus; TH, tyrosine hydroxylase; TH-ir, tyrosine hydroxylase immunoreactivity; 5HT, serotonin; 5HT-ir, serotonin immunoreactivity.

After blood volume expansion (BVE), several neural, behavioral and hormonal mechanisms work in coordination to inhibit water and salt ingestion and to increase natriuresis and urine flow. In order to restore body fluid balance, thirst and salt appetite are inhibited, renal sympathetic activity and vasopressin (AVP) secretion decrease, and the secretion of oxytocin (OT) and atrial natriuretic peptide (ANP) is stimulated. Although the secretion of OT can induce natriuresis directly in the kidney, it is mainly induced indirectly by atrial ANP release (Antunes-Rodrigues et al., 2004).

There have been various studies that use the expression of the immediately-early c-fos gene to identify the central pathways of both medulla and hypothalamus activated by volume load (Narváez et al., 1993; Badoer et al., 1997; Randolph et al., 1998; Potts et al., 2000). However, they do not determine systematically or quantitatively the extent to which serotonergic, catecholamine, AVP and OT neurons are involved in these regions. Furthermore, evidence for the participation of some neurochemical groups is contradictory, as in the case of the oxytocinergic cells of the paraventricular nucleus of the hypothalamus (PVN) (Randolph et al., 1998), or even ignored, as the serotonergic dorsal raphé nucleus (DRN) cells.

Hypervolaemia does not always cause a significant increase of Fos expression in the PVN (Badoer et al., 1997) and when it does, the fos-positive nuclei of the PVN do not co-localize with OT cells (Randolph et al., 1998). These results contradict a number of recent studies showing that this nucleus plays a major role in the compensatory autonomic response to a volume load (Deering and Coote, 2000), and that oxytocinergic projections to the solitary vagal complex are involved in the baroreceptor reflex control of the heart rate (HR) (Higa et al., 2002). However, the pattern of c-fos expression depends on the characteristics of the BVE model under analysis, such as the intensity of the stimulus and the post-stimulation period studied.

As regards serotonergic DRN cells, previous evidence indicates that serotonergic neurons in the raphé system modulate ANP release induced by BVE. Animals with electrolytic lesions of the DRN or with depletion of serotonin (5HT) (from all serotonergic neurons) by central administration of *p*-chlorophenylalanine, display attenuated ANP release both under resting conditions and after BVE (Reis et al., 1994).

In order to investigate the involvement of specific neurochemical brain groups, including the oxytocinergic, va-

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sopressinergic, serotoninergic and catecholaminergic neuronal clusters, in the regulatory response to BVE, we analyzed the brain pattern of Fos-labeling in combination with immunohistochemical labeling for 5HT, tyrosine hydroxylase (TH), AVP and OT, 90 min after acute isotonic volume loading in unanesthetized, unrestrained rats. This approach was selected because the increased formation of Fos protein, 1 to 2 h post-stimulation, correlates with increased neural activity in a wide range of neural systems (Morgan and Curran, 1989).

Plasma levels of ANP, OT and AVP were also measured following BVE, to confirm our previous studies (Antunes-Rodrigues et al., 1992; Haanwinckel et al., 1995).

EXPERIMENTAL PROCEDURES

The experiments used 40 adult, Wistar-derived, male rats, born and reared in the breeding colony at the Instituto Ferreyra (INIMEC, Córdoba, Argentina). Animals weighing 250–300 g were housed in groups of six, in a temperature-controlled environment, with a 12-h light/dark cycle, with food and water *ad libitum*. After surgery, they were kept in individual cages for at least 3 days before the experiments began. All experimental protocols were approved by the appropriate animal care and use committee of our institute, following the guidelines of the International Public Health Service Guide for the Care and Use of Laboratory Animals, and both the suffering and the number of animals used was minimized.

BVE

Three days before the experiment, a catheter was inserted into the right external jugular vein and advanced to the right atrium as described by Harms and Ojeda (1974). On the day of the experiment, BVE was performed in the conscious, freely moving rats by an i.v. injection of 0.15 M NaCl (2 ml/100 g body weight), for over 60 s. A group of rats that underwent the same procedures but did not receive the i.v. injection was used as controls.

Ninety minutes after the sham or BVE, the animals were deeply anesthetized with thiopentone (100 mg/kg i.p.) and perfused transcardially with 200 ml of normal saline, followed by 400 ml of paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.2. The brains were removed, fixed overnight in the perfusion solution, and stored at 4 °C in PB containing 30% sucrose. Three series of 40 μm coronal sections were placed in a mixture of 10% H_2O_2 and 10% methanol until oxygen bubbles ceased appearing. They were then incubated in 10% normal horse serum (NHS) in PB for 1 h to block non-specific binding sites.

Blood pressure measurements

Animals separate from those used in the immunohistochemical studies were fitted with two catheters; one was inserted into the right external jugular vein and advanced to the right atrium for BVE procedure and the other polyethylene catheter (PE-10 fused to PE-50, Clay-Adams, Parsipany, NJ, USA) was inserted in the abdominal aorta via the femoral artery, for direct arterial blood pressure measurements. The mean arterial pressure (MAP) of conscious, freely moving rats (n=7 per group) was continuously recorded with an HP-7754A recorder and an HP-1280C transducer (Hewlett Packard, Palo Alto, CA, USA). On the day of experiment, MAP was allowed to stabilize for 30 min. After baseline recording, BVE was performed by i.v. injection of 0.15 M NaCl (2 ml/100 g body weight; during 1 min) and the MAP monitored for 60 min.

Determination of plasma ANP, OT and AVP concentrations

For the assay of plasma ANP, OT or AVP concentrations, other animals were subjected to a sham or BVE and then decapitated and bled 5 min later. Trunk blood was collected in chilled plastic tubes containing heparin for the measurement of AVP and OT, or EDTA (2 mg/ml) and proteolytic-enzyme inhibitors (20 μl of 1 mM phenylmethylsulfonyl fluoride and 20 μl of 500 μM pepstatin) for ANP.

Plasma levels of ANP, OT and AVP were measured by radioimmunoassay as described by Gutkowska et al. (1984), Morris and Alexander (1989), and Elias et al. (1997), respectively. For the assays, AVP and OT were extracted from 1 ml of plasma with acetone and petroleum ether, and ANP was extracted from 1 ml of plasma in Sep-Pak C-18 cartridges (Waters Corporation, Milford, MA, USA). The percentages of recovery after extraction were 83%, 85% and 90% for AVP, OT and ANP, respectively. The assay sensitivity and intra- and inter-assay coefficients of variation were 0.9 pg/ml, 7.7% and 11.9% for AVP, 0.9 pg/ml, 7% and 12.6% for OT, and 7.0 pg/ml, 6.0% and 10.0% for ANP.

Staining procedure for Fos and TH, 5HT, AVP or OT immunoreactivity

All the series of free-floating sections from each brain were first processed for Fos immunoreactivity using an avidin–biotin–peroxidase procedure. The sections from the medulla and pons

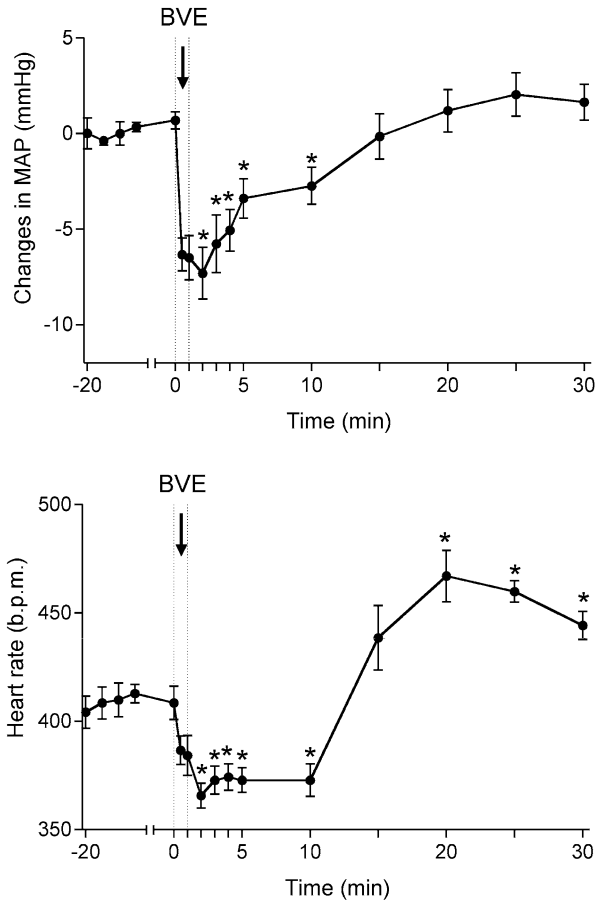

Fig. 1. Change from resting levels in MAP and HR in rats that were volume loaded. Values are means±S.E.; n=7. * P<0.05 versus basal levels.