Experiments were done in conscious rats to investigate the effect of i.c.v. infusions of hypertonic NaCl solutions on the induction of the protein Fos in the arcuate nucleus (Arc). Neurons containing Fos-like immunoreactivity were observed throughout the rostrocaudal extent of Arc after i.c.v. infusions of hypertonic saline solutions (337-744 mM). However, most of the labelled neurons were confined to the middle third of the nucleus, in the region of the dorsomedial and ventromedial subnuclei. Few, if any Fos-labelled neurons were observed in Arc of animals that received i.c.v. infusions of isotonic (142 mM) or mild hypertonic (173 mM) saline solutions or a hyperosmotic (660 mOsm/kg) saline solution of mannitol. No Fos-labelled neurons were found in the subfornical organ, although a few were observed scattered throughout the organum vasculosum laminae terminalis (OVLT) in all the animals studied. The density nor the distribution pattern of Fos-labelled neurons in OVLT was altered in animals receiving i.c.v. infusions of hypertonic saline or hyperosmotic solutions. These data demonstrate that Arc neurons are activated during a hypertonic saline challenge and suggest that Arc may function as a sodium-sensitive structure that is involved in body-fluid and circulatory homeostasis.
similarly infused i.c.v. The osmolality of the hyperosmotic mannitol solution and the 337 mM NaCl solution was similar. All the infusions were made at ~12:00. During the infusions, water bottles were removed and the animals were carefully monitored. No signs of pain or discomfort were detected. To determine background Fos activity in Arc, one animal was similarly prepared and handled but no infusion was made.

The animals were anesthetized with pentobarbital sodium (60 mg/kg i.p.) 90 min after the completion of the infusion and perfused transcardially as previously described [14]. The brains were removed and stored in 10% sucrose in PBS at 4°C overnight. Serial, frozen transverse sections (50 μm) of the forebrain were cut and placed in rabbit polyclonal c-fos antisera (lot H172, Santa Cruz Biotechnology, Santa Cruz, CA; raised against the N-terminal epitope) and processed for c-fos immunoreactivity as previously described [14]. Forebrain sections were analyzed using bright-field microscopy. The total number of Fos-labelled nuclei in Arc were counted on every section (38-48) through the nucleus of each animal and an average value/section was calculated for each group (control group, 136-142 mM saline; group 2, 173 mM saline; group 3, 337-744 mM saline; and group 4, hyperosmotic mannitol-saline solution). Means ± S.E. were calculated and compared using an ANOVA followed by Bonferroni posthoc test. A P value of < 0.05 was considered statistically significant.

Controls for Fos immunoreactivity were processed as previously described [14]. In these control sections of the forebrain, no Fos-immunoreactive neurons were observed.

In all animals, the guide cannula tracts were histologically verified. In 12 animals, the tips of the infusion cannulas were located in the lumen of the 3V at the level of the rostral anterior commissure. In the animal infused with 136 mM and in the one infused with 744 mM solutions, the cannula tracts were located in the lumen of the 3V at the level of the middle third of Arc.

Fos-immunoreactive neurons (304 ± 50/section) were observed throughout the rostrocaudal extent of Arc (Fig. 1a) in all animals that received i.c.v. infusions of hypertonic saline (337-744 mM) solutions. However, it was evident that in animals that received infusions of 173 mM saline solutions, the number of Fos-labelled neurons (25 ± 8/section) was significantly smaller (Fig. 1b) compared with that from animals that received infusions of ≥ 337 mM saline solutions (Fig. 1a). Most of the Fos-labelled neurons in Arc were found within the middle third of the nucleus, in the dorsomedial and ventromedial subnuclei (Fig. 1a). The ventrolateral subnucleus of Arc had a few Fos-labelled neurons that were primarily located along its ventral aspect.

In control animals that received infusions of hypotonic (136 mM) or isotonic (142 mM) saline solutions, the number of Fos-labelled neurons (21 ± 12/section) was not statistically different from animals that received the 173 mM saline solutions (Fig. 1c). Similarly, in animals that received i.c.v. infusions of a hyperosmolar mannitol (660 mOsm/kg)-saline (142 mM) solution Arc was not distinguishable from that in animals that received infusions of isotonic saline with regards to the number of Fos-labelled neurons (13 ± 0.9/section) (Fig. 1d). In the one animal that did not receive an i.c.v. infusion of saline, Arc contained <1 cell/section.

A small and inconsistent number of Fos-labelled neurons was observed scattered throughout organum vasculosum laminae terminalis (OVLT) in all animals studied. On the other hand, no Fos-labelling was observed in SFO. Infusions of hypertonic saline did not alter these patterns in OVLT or SFO (Fig. 1e,f). However, the ependymal layer covering the ventricular surface of both structures contained numerous Fos-labelled cells (Fig. 1e,f).

This study has provided immunohistochemical evidence of a forebrain structure that may participate in the pathway involved in the regulation of sodium concentration of the cerebrospinal fluid (CSF). I.c.v. infusions of hypertonic saline solutions resulted in c-fos induction in Arc neurons. This c-fos induction was likely not due to a nonspecific activation of the neurons as a result of local distortion of neuronal tissue from the small volumes infused i.c.v., as infusions of similar volumes of isotonic saline did not increased Fos-labelling in Arc. Furthermore, the possibility that a change in osmolality was responsible for the c-fos induction is unlikely as the infusion of a hyperosmotic solution of mannitol that was as osmotically active as the 337 mM NaCl solution, did not alter c-fos activity in Arc. Finally, it is interesting to note that Fos-labelling in the two circumventricular organs classically recognized as central osmosensitive sites, OVLT and SFO [20], was not altered during the i.c.v. infusions of hypertonic saline solutions. This suggests that these two structures were likely not responsible for the activation of Arc neurons. Therefore, taken together, these data suggest that Arc neurons were activated as a result in the change in the sodium concentration of the CSF. The suggestion that the sodium concentration of the CSF may be detected by a sensor in the forebrain has previously been made [1, 2, 6, 22, 25]. It has been shown that vasopressin release, antidiuresis and drinking behaviour can be induced after i.c.v. infusions of hypertonic saline solutions, but not with equiosmolar solutions of mannitol, d-glucose or fructose [2, 9, 16]. Although the mechanism by which sodium exerts its stimulatory effect on the cells is not known, a prominent ana-
Fig. 1. Bright-field photomicrographs of transverse sections of rat forebrain taken through region of Arc (a–d), OVLT (e) and SFO (f) after i.c.v. infusions of 337 (a,e,f), 173 (b) and 142 mM (c) saline solutions and a hyperosmotic (660 mOsm/kg) solution of mannitol in 142 mM saline (d). Note dense pattern of Fos-immunoreactive neurons in dorsomedial (dm) and ventromedial (vm) subnuclei of Arc after 337 mM NaCl infusions (a). In addition, note very few Fos-labelled neurons after 173 (b) and 142 mM (c) saline infusions and in OVLT and SFO in hypertonic saline-infused animals (e,f). Arrows in (e) and (f) point to Fos-labelled cells in ependymal layer of 3V surrounding OVLT and SFO. LPO, lateral preoptic area; ME, median eminence; vhc, ventral hippocampal commissure; oc, optic chiasm; vl, ventrolateral subnucleus of Arc. Calibration mark of 100 μm in (f) applies to all photomicrographs.

tomical feature of Arc is that it is invaginated by tanyocytes [29]. It has previously been suggested that the tanyocyte may act as an interface that aids in the diffusion of substances from the ventricular lumen [4, 29].

In summary, it has been demonstrated that hypertonic saline solutions infused i.c.v. into the anterior 3V induces c-fos activity in Arc neurons. These data suggest that Arc may function as detector of sodium concentration in the CSF and are consistent with the idea that forebrain sodium sensors exit, in addition to osmoreceptors, both of which are involved in osmoregulation.

The technical assistance of Z.M. Zhang is gratefully acknowledged. This work was supported by the Heart and Stroke Foundation of Ontario. J. Ciriello is a Career Investigator of the Heart and Stroke Foundation of Ontario. L.P. Solano-Flores and M.P. Rosas-Arellano are visiting scientists from the Departamento de Fisiología, Facultad de Medicina, Universidad Nacional Autónoma de México and holders of DGAPA awards.

5 Chronwall, B.M., Anatomy and physiology of the neuroendocrine arcuate nucleus, Peptides, 6 (1985) 1–11.


