Neuroscience 137 (2006) 51–71

DIFFERENTIAL CELLULAR DISTRIBUTION OF TONICITY-INDUCED EXPRESSION OF TRANSCRIPTION FACTOR TonEBP IN THE RAT BRAIN FOLLOWING PROLONGED SYSTEMIC HYPERTONICITY

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Abstract—In a previous work performed on cerebral cortex and hippocampus we reported that tonicity-responsive enhancer binding protein (TonEBP), originally identified as a transactivator of osmoprotective genes involved in osmoadaptation of renal cells, was induced in neurons only, but to varying levels, following acute systemic hypertonicity. Whether or not this cellular specificity reflected a unique ability of neurons or a differential time course among brain cells for tonicity-induction of TonEBP was investigated throughout the brain in this study by subjecting the animals to prolonged systemic hypertonicity. In normal rats, TonEBP immunolabeling and TonEBP-mRNA *in situ* hybridization labeling showed a widespread, uneven and parallel distribution. TonEBP was expressed primarily in the cell nuclei of neurons, where it was heterogeneously distributed in a nucleoplasmic and a granular pool. In rats subjected to prolonged systemic hypertonicity, TonEBP labeling increased in the cell nuclei of neurons only. The tonicity-induced expression of TonEBP for a given cell group of neurons was rather uniform but varied greatly among neuronal cell groups and was positively correlated with the average size of the cell nuclei, as determined by quantitative analysis of digitized images. The detailed distribution of tonicity-induced expression of TonEBP is reported throughout the brain. In normal rats, a very minor proportion of non-neuronal cells, identified as a subset of astrocytes and possibly oligodendrocytes, showed faint nuclear immunolabeling, which however did not increase in hypertonic animals. Ependymocytes, capillary endothelial cells, and microglial cells showed no TonEBP labeling, even in hypertonic animals. Altogether our data indicate that neurons, albeit possibly to a varying extent, are the only brain cells able to use TonEBP-mediated processes for adaptation to a systemic hyperosmotic unbalance.

Key words: tonicity-responsive enhancer binding protein, TonEBP, cell volume regulation, cell osmoregulation, hyperosmolarity, osmoprotective genes.

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Abbreviations: CR3, complement (C3bi) receptor; DAB, 3,3′ diaminobenzidine; DAPI, 4′,6-diamino-2-phenylindol; MDCK, Madin-Darby canine kidney cells; NeuN, neuronal specific nuclear protein; ORE, osmotic responsive element; S100, S100 protein; Tfr, transferrin receptor; TonE, tonicity-responsive enhancer; TonEBP, tonicity-responsive enhancer binding protein.

Tonicity-responsive enhancer binding protein (TonEBP) has been characterized as a transcriptional activator of the osmoprotective genes involved in the cellular adaptive response of renal cells to hypertonicity (for review, see Woo et al., 2002). Cellular osmoadaptation to a hypertonic medium relies upon intracellular accumulation of inorganic (Na^+, K^+, Cl^-) and organic (sorbitol, myo-inositol, betaine, taurine, and some amino-acids) osmolytes that results in osmosis-driven water influx. This allows the cell to recover from the initial shrinkage due to osmosis-driven water efflux and thus to osmotically equilibrate with the surrounding medium while keeping its normal volume (Lang et al., 1998; Wehner et al., 2003; Strange, 2004). Molecular mechanisms for accumulation of osmolytes involve increased activity of ion transporters and exchangers (Hoffmann and Dunham, 1995; O'Neill, 1999) and increased transcription and translation of so-called osmoprotective genes which encode transporters or biosynthetic enzymes of organic osmolytes (Burg et al., 1997; Waldegger and Lang, 1998; Handler and Kwon, 2001). In the promoter of the osmoprotective genes so far investigated a tonicity-responsive enhancer (TonE) has been identified (Takenaka et al., 1994; Rim et al., 1998; Ito et al., 2004) which has also been called an osmotic responsive element (ORE) (Ferraris et al., 1996) and represents a short consensus sequence (Ferraris et al., 1999).

TonEBP was first identified as a protein which binds to TonE (Miyakawa et al., 1999) or ORE and therefore was also called ORE-BP (Ko et al., 2000). By homology screening it was also identified as a new member (NFAT5) of the NFAT family (Lopez-Rodriguez et al., 1999). *In vitro* investigations on a renal epithelial cell line (Madin-Darby canine kidney (MDCK) cells) have established that exposure to hypertonicity results in: a) translocation of TonEBP into the cell nucleus (Woo et al., 2000a), b) phosphorylation of TonEBP (Dahl et al., 2001), which does not modify its binding to DNA (Dahl et al., 2001) but may activate its transactivation domain (Ferraris et al., 2002), and c) induction of TonEBP, shown by increased levels of TonEBP-mRNA, TonEBP and TonEBP neosynthesis (Woo et al., 2000b). The transcriptional activity of TonEBP was also reported to be determined by intracellular ionic strength and cell water content (Neuhofer et al., 2002). TonEBP is thought to be the major transcription factor that activates the osmoprotective genes (Miyakawa et al., 1999; Na et al., 2003; Lopez-Rodriguez et al., 2004).

The brain interstitial and cerebrospinal fluids are in osmotic equilibrium with the blood plasma. Plasma osmolality is maintained in a very narrow range by neurohor-

0306-4522/06$30.00+0.00
doi:10.1016/j.neuroscience.2005.07.037

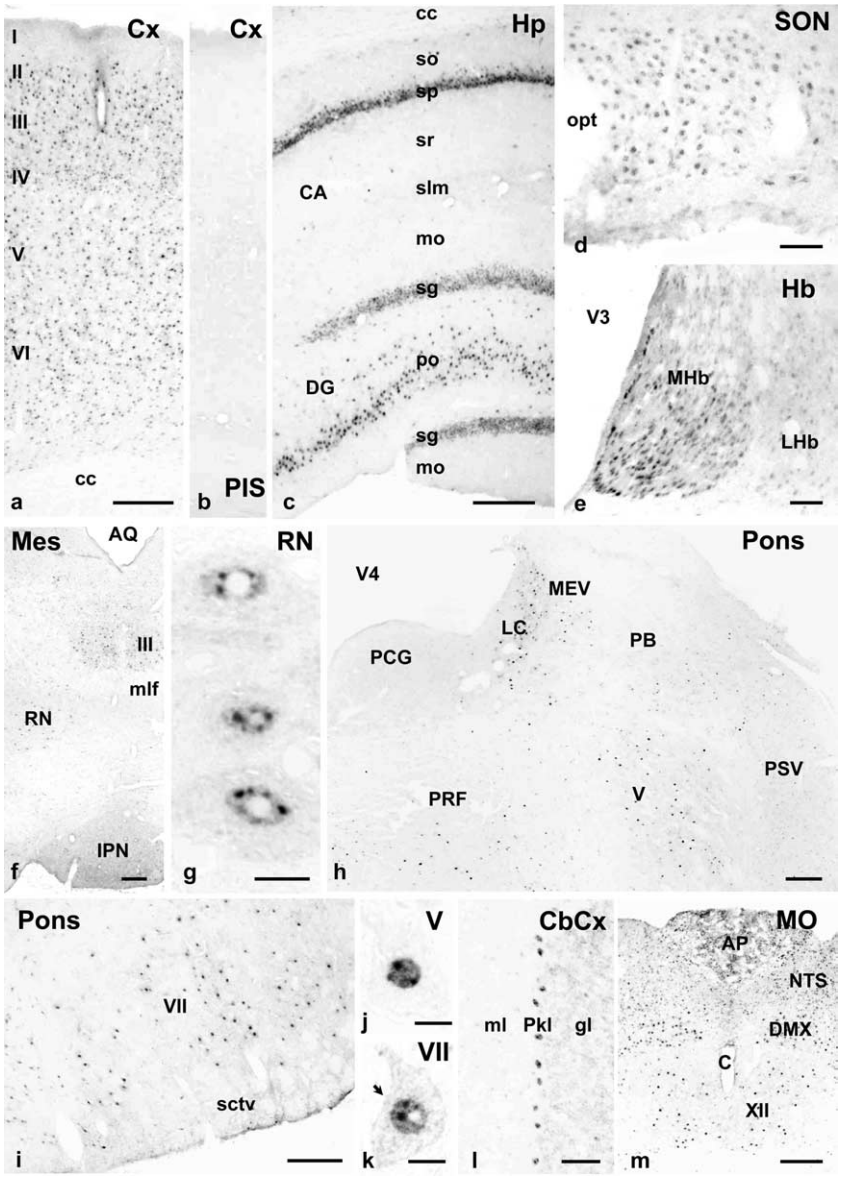

Fig. 1. TonEBP immunolabeling in the brain of a normal rat. Faint, but clear, labeling is seen in layers II–VI of the fronto-parietal cortex (a) and the pyramidal cell layer and granular cell layer of the hippocampus (c). Layer I in the cortex and most other layers of the hippocampus are devoid of labeling except for a few scattered particles. No labeling is seen on tissue sections incubated with the preimmune serum (PIS) (b). Significant labeling is seen in many regions and nuclei of the forebrain, as illustrated here in the supraoptic nucleus (d) and medial habenula (e). In the brain stem, several cell groups show labeling that is stronger than the average labeling of the tissue sections. This is illustrated for the oculomotor nucleus and red nucleus in the mesencephalon (f), the locus coeruleus, the mesencephalic nucleus of the trigeminal nerve, and the motor nucleus of the trigeminal nerve in the dorsal pons (h), the facial nucleus in the ventral pons (i), and the dorsal motor nucleus of the vagus nerve and hypoglossal nucleus in the dorsal medulla oblongata (m). Note that the pontine central gray, the parabrachial nucleus, the principal sensory nucleus of the trigeminal, and the nucleus of the tractus solitarius are devoid of significant labeling (h). In the pontine reticular formation, a few scattered cells are labeled (h). In the cerebellar cortex (l), the Purkinje cells show clear labeling, while the molecular layer and granule cell layer are devoid of labeling. At higher magnification on semi-thin sections, as illustrated in the red nucleus (g), the motor nucleus of the trigeminal nerve (j), and the facial nucleus (k), labeling is located in the cell nucleus, where it is heterogeneously distributed, with a faint labeling of the entire nucleus, except for a spot, presumably corresponding to the nucleolus, and a few scattered more strongly stained granules. In most cases, no cytoplasmic labeling can be detected (g, j). However, in some subsets of cells, such as in the facial nucleus (k), the outline of the cells can be delineated as a result of faint cytoplasmic labeling (arrowhead). Scale bars=200 μm (a, c, f, h, i, m), 50 μm (d, e, l), and 10 μm (g, j, k).

monal regulatory loops which control ingestion and urinary excretion of water and electrolytes (Antunes-Rodrigues et al., 2004; McKinley and Johnson, 2004a,b). Consequently, brain cells are exposed to an extracellular medium that remains isotonic under normal physiological conditions. However there exist pathological states which lead to hypo- or hypertonic blood plasma and neurological disorders (Adrogue and Madias, 2000a,b; Andreoli, 2000; Boulard, 2001; Verbalis, 2003). The neurological disorders result from osmosis-driven water movement between brain fluids