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Research report

Serine/threonine-kinase 33 (Stk33) – Component of the neuroendocrine network?

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ABSTRACT

The present study was conducted to investigate the expression of serine/threonine-kinase 33 (Stk33) in neuronal structures of the central nervous system in rat and hamster as well as the presence of the protein in the brain of higher mammals, using a polyclonal antibody on cryosections of fixed brains. We found a distinct immunostaining pattern that included intense fluorescence of the ependymal lining of cerebral ventricles, and of hypothalamic tanycytes and their processes. We further observed intense staining of magnocellular neurons in the hypothalamic paraventricular, supraoptic and accessory neurosecretory nuclei, in particular the circular nuclei, and less intense stained neurons in other diencephalic regions. Double-immunostaining experiments showed a partial colocalization of Stk33 with arginine-vasopressin, oxytocin or neuronal nitric oxide-synthase in a large number of neurons of the hypothalamic nuclear regions. Colocalization of Stk33 with substance P or the catecholamine-synthesizing enzyme tyrosine-hydroxylase was not observed. Immunofluorescence was not found in autonomic regions of the lateral horn, suggesting that Stk33 does not contribute to hypothalamo-spinal connections. However, large Stk33-immunoreactive axonal projections from magnocellular hypothalamus to the neurohypophysis were evident. These functionally important connections provide the bridge from neuronal to humoral regulation of the endocrine system. Additionally, Western blots from mouse brain showed two distinct bands representing two Stk33 isoforms. We also present first evidence for the presence of Stk33/STK33 in neuronal structures, ependymal cells and tanycytes in tree shrew, baboon, and human brain.

1. Introduction

Serine/threonine kinase 33 is a member of the calcium/calmodulin-dependent kinases (CaMK) (Manning et al., 2002; Mujica et al., 2001, 2005). Co-immunoprecipitation experiments revealed that Stk33 and the intermediate filament protein vimentin are, in vivo, associated proteins and that Stk33 phosphorylates vimentin in its head domain. Recombinant Stk33 undergoes obligatory autophosphorylation, which might be a requirement for its kinase function, suggesting that Stk33 is involved in intermediate filament assembly/disassembly through the specific and regulated phosphorylation of vimentin (Brauksiepe et al., 2008). This clue to Stk33-function in the regulation of cytoskeleton dynamics by phosphorylation fits to the differential expression pattern of Stk33 (Mujica et al., 2005) that resembles those of some related members of CaMK.

In a recent study (Brauksiepe et al., 2014), we observed the expression of Stk33 and its colocalization to vimentin in ventricular ependymal cells and in tanycytes of rat and hamster as well as its regulation by photoperiod in the Djungarian hamster Phodopus sungorus. The protein was also present in circumventricular organs such as area postrema, subfornical organ, pineal gland and anterior and posterior lobes of the pituitary gland, as well as in magnocellular neurons of the neuroendocrine hypothalamus. As vimentin is present in neurons only in early development (Yabe et al., 2003), other functions of neuronal Stk33 have to be assumed. Since these are unknown as yet, and detailed knowledge of Stk33-expression is an essential prerequisite for the understanding of its function, the present study aimed at characterizing Stk33-neurons with regard to location and co-expression of selected neuroactive substances in rodents. For this purpose, we chose neuroactive substances found in many regions

Abbreviations: ANS, accessory neurosecretory nuclei; AVP, arginine-vasopressin; CiN, circular nucleus; CSF, cerebrospinal fluid; IR, immunoreactive; MCN, magnocellular neurons; nNOS, neuronal nitric oxide-synthase; OT, oxytocin; PVN, paraventricular nucleus; SON, supraoptic nucleus; Stk33, serine/threonine-kinase 33; SP, substance P; TH, tyrosine-hydroxylase

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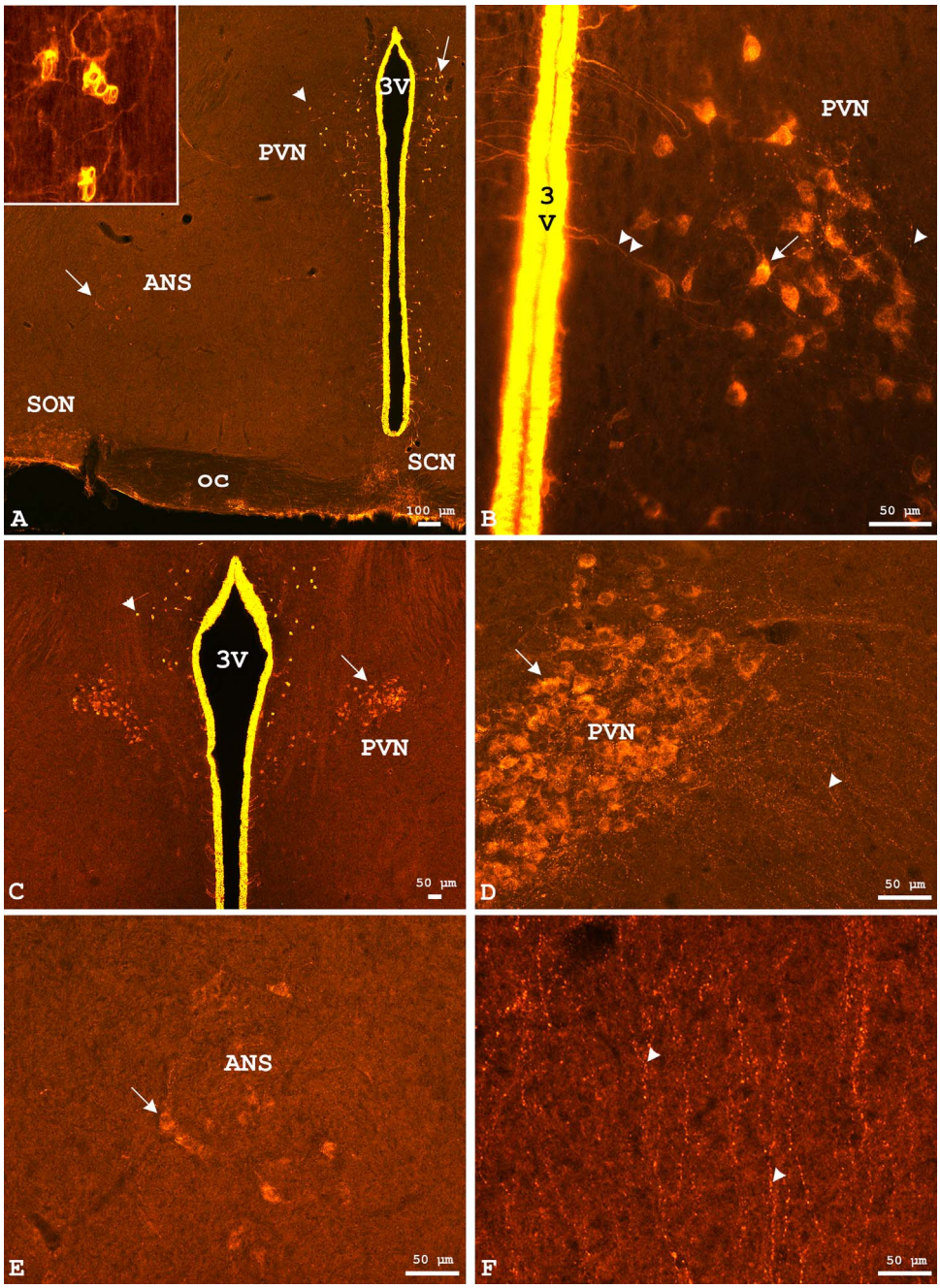

Fig. 1. Stk33-immunoreactivity in frontal sections of the rat brain. Strong staining was found in ependymal cells of the third ventricle (3 V in A–C). Less intense staining was observed in neurons and their processes. At the level of the optic chiasm (oc in A; approximately interaural +7.9 mm corresponding to Fig. 42 of the rat brain atlas), and in the PVN region at an intermediate level (approximately interaural +7.6 mm corresponding to Fig. 45 of the rat brain atlas), further IR structures are tanycyte processes wrapped around prospective blood capillaries (*arrowheads*, dorsal periventricular region in A,C; insert in A) and, more ventrally, IR neurons (*arrows)* of the PVN (A–D). Some of these were located close to the ventricular ependyma and apparently sent processes (*arrowheads*) into ventricular space (B). Others were located in the lateral PVN aspects (D) and provided beaded fibers advancing to median eminence and neurohypophysis (F). A group of IR neurons (*arrows)* of the accessory neurosecretory nuclei (ANS) is seen in E. No immunofluorescent neuronal somata were observed in the suprachiasmatic nucleus (SCN in A). All images were taken from the same animal.

of the rodent hypothalamus, i.e., arginine-vasopressin, oxytocin, neuronal nitric oxide-synthase, substance P, and tyrosine-hydroxylase (cf. Armstrong, 2015; Harding et al., 2004; van den Pol et al., 1984; Woodside and Amir, 2000). We also studied, more cursory, the presence of Stk33-protein in brain of higher mammals including man.

By means of immunofluorescence, we now provide the first overview of the expression of neuronal Stk33-protein in the rodent brain, and demonstrate that the protein is present in the CNS of higher mammals.

2. Results

2.1. Neuronal expression of Stk33 in rat and hamster

Immunostaining of brain sections from adult rats showed a distinct and cell type-specific distribution of Stk33-immunoreactivity. Strong immunostaining was seen in ependymal cells of the ventricular system (Figs. 1A–C, 2E and 3AB) and in tanycytes of the basolateral walls of the third ventricle. The latter cell type exhibited basal, unbeaded processes that were often seen to extend over a long distance and appeared to be wrapped around blood vessels (insert in Fig. 1A and