



Immunohistochemical Localization of Translationally Controlled Tumor Protein in Axon Terminals of Mouse Hippocampal Neurons

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Translationally controlled tumor protein (TCTP) is a cytosolic protein with microtubule stabilization and calcium-binding activities. TCTP is expressed in most organs including the nervous system. However, detailed distribution and functional significance of TCTP in the brain remain unexplored. In this study, we investigated the global and subcellular distributions of TCTP in the mouse brain. Immunohistochemical analyses with anti-TCTP revealed that TCTP was widely distributed in almost all regions of the brain including the cerebral cortex, thalamus, hypothalamus, hippocampus, and amygdala, wherein it was localized in axon tracts and axon terminals. In the hippocampus, TCTP was prominently localized to axon terminals of the perforant path in the dentate gyrus, the mossy fibers in the cornu ammonis (CA)3 region, and the Schaffer collaterals in the CA1 field, but not in cell bodies of granule cells and pyramidal neurons, and in their dendritic processes. Widespread distribution of TCTP in axon tracts and axon terminals throughout the brain suggests that TCTP is likely involved in neurotransmitter release and/or maintaining synaptic structures in the brain, and that it might have a role in maintaining synaptic functions and synaptic configurations important for normal cognitive, stress and emotional functions.

Key words: Cognition, Immunohistochemistry, Mossy fiber, Mouse hippocampus, Translationally Controlled Tumor Protein (TCTP)

INTRODUCTION

TCTP, also known as histamine releasing factor (HRF), is a

highly conserved multifunctional protein among eukaryotes across animal and plant kingdoms. In mammalian cells, TCTP is known to play a role in cell division, regeneration, microtubule stabilization, apoptosis, carcinogenesis, tumor reversion, and ion homeostasis [1]. In rodents, TCTP was expressed during neural differentiation of embryonic stem cells [2], and in the cultured hippocampal neurons [3]. In the human nervous system, TCTP and its mRNA were distributed in the cerebral cortex, thalamus, caudate nucleus, and cerebellum [4, 5].

TCTP was also expressed in a higher level in human glioma cells [6]. Overexpression of TCTP in glioma cell lines led to

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the progression of glioma [7]. TCTP/HRF immunoreactivity was higher in the cytoplasm of intact epithelial cells than that of lipofuscin-laden cells in choroid plexus from patients with paraneoplastic cerebellar degeneration, suggesting a potential role of TCTP in the cerebrospinal fluid barrier [8]. Oxidative modification of TCTP plays an important role in the neurodegeneration in amyotrophic lateral sclerosis [9]. TCTP/HRF levels were decreased in the hippocampus of schizophrenia patients [10] and in the temporal cortex, thalamus, and caudate nucleus in patients with Down's syndrome and Alzheimer's disease [5]. However, detailed distribution and functional significance of TCTP in the brain are not clearly understood.

In the present study, we investigated global and subcellular distributions of TCTP in brain sections using a previously well-characterized anti-TCTP antibody [11, 12]. Our immunohistochemical analyses indicated that TCTP was distributed globally throughout the brain, where-in it was localized in axon tracts and mostly in axon terminals in the brain.

MATERIALS AND METHODS

Animals and tissue sampling

Eight week-old male C57BL/6 mice (n=8) were purchased from Orient Bio. Inc. (Seongnam; Gyeonggi, Korea). Mice were maintained on a 12-h light/dark cycle and fed standard rodent chow and water ad libitum. All animal experiments were performed according to the guidelines provided in National Institutes of Health (Publication No. 8523: Guide for the Care and Use of Laboratory Animals), and were approved by Ewha Womans University's Institutional Animal Care and Use Committee (Approval ID: IACUC 2012-01-029).

For tissue preparation, mice were deeply anesthetized with ethyl ether, and perfused transcardially with phosphate buffered saline (PBS), followed by 10% formalin. Brains were then excised, fixed in 10% formalin for 24 h, and embedded in paraffin. Brains were coronally cut at 5~8 μ m of thickness and sections were placed on glass slides, which resulted in 1~2 stained slides per tissue of each animal.

Immunohistochemistry

After deparaffinization and hydration, tissue sections were boiled for 15 min in sodium citrate buffer, pH 6.0 to retrieve the epitope. Endogenous peroxidase was blocked by incubation of brain sections with 1% hydrogen peroxide for 15 min. Immunostaining was performed with ImmPress Reagent kit (Vector laboratories) according to manufacturer's instructions. Following primary antibodies were used: anti-TCTP (Abcam, ab37506, 1:1000),

anti-MAP2a+2b+2c (Abcam, ab68852, 1:100), anti-NMDAR2B (Abcam, ab93610, 1:1000), and anti-synapsin II (Sigma Aldrich, S2822, 1:1000). Signals reacted with primary antibodies were substituted with normal rabbit IgG (Abcam, ab27478). Secondary antibody was reacted with ABC kit and 0.05% DAB solution (Sigma-Aldrich) was used as a chromogen. After staining with cell nuclei using Harris hematoxylin (Sigma-Aldrich), the sections were mounted with "Permount" (Fisher Scientific). Stained sections were analyzed under the microscope (Axio Scope. A1, Zeiss), and representative photographs were made with a digital camera.

RESULTS

Localization of TCTP in the brain

To understand the localization of TCTP in the brain, we performed immunohistochemical staining of coronal sections of mouse brains using anti-TCTP and anti-synapsin II antibodies, and analyzed the stained immunoreactivity by light microscopy. Immunohistochemical analyses indicated that TCTP was widely expressed in the brain with specific localizations in axons of neurons in almost all regions of the brain including the cerebral cortex, thalamus, hypothalamus, hippocampus, and amygdala (Fig. 1a). TCTP was localized in the lateral habenula whose expression was relatively higher than that in the medial habenula (Fig. 1e). TCTP immunoreactivity was observed in all subregions of the hippocampus, but not in the granule cells and pyramidal cells (Fig. 1f). TCTP was also distributed in all layers of the cerebral cortex, where its expression was localized in the inter-neuronal space, but TCTP immunoreactivity was barely detected in cell bodies of neurons and in dendritic processes (Fig. 1g), as interpreted from specific staining patterns by anti-TCTP and anti-synapsin II, and the latter was used as an axon fiber and axon terminal-labeling marker (Fig. 1h). TCTP immunoreactivity also was detected in the subplate, the area containing afferent fibers from the thalamus and various cortical regions (Fig. 1g).

Localization of TCTP in the dentate gyrus (DG) region of the hippocampus

Distinct subcellular localization of TCTP particularly to axon tracts and/or axon terminals broadly in the brain led us to examine its detailed distribution in hippocampal neurons, since the hippocampus is well-characterized for cell types, axonal tracts and synaptic circuits. The hippocampus, an important area for memory and learning processes, is comprised by distinctive subregions such as dentate gyrus (DG), cornu ammonis 3 (CA3) and CA1 regions, which have differential composition and molecular profiles. Based on the information flow through the

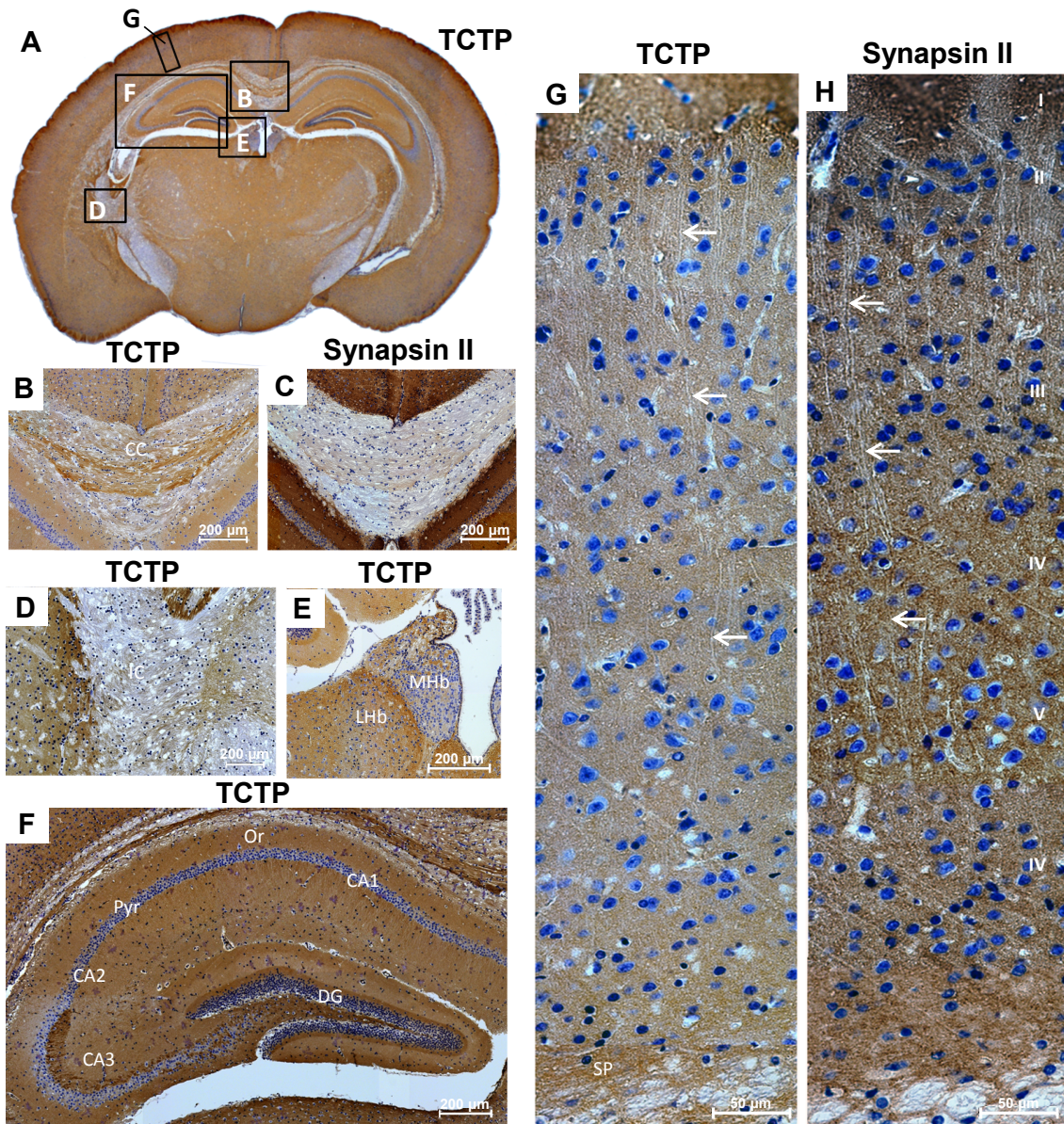


Fig. 1. Immunohistochemical localization of TCTP in coronal sections of the mouse brain. Sections were stained by anti-TCTP antibody (a, b, d, e, f, and g) or anti-synapsin II antibody (c, h). Immunohistochemical signals were visualized using DAB and cell nuclei were stained with hematoxylin. Anti-synapsin II staining was used for staining of axonal tracts (c, h). Anti-TCTP staining was widely distributed in the brain, along with distinct localizations in axon tracts and axon terminals. A subset of axon fibers in the corpus callosum (CC) were moderately stained by anti-TCTP, while the internal capsule (IC) was absent from TCTP (d). Axonal fibers running in the subplate (SP) underneath the parietal cortex were stained by anti-TCTP (g, h). Red arrows in the parietal cortex indicate dendritic shafts arising from pyramidal neurons in the layers 3-5 heading towards the layer 1 (g, h). CC, corpus callosum; MHb, medial habenula; LHb, lateral habenula; Or, stratum oriens; Pyr, stratum pyramidale; DG, the dentate gyrus; SP, subplate; I-VI, cortical layers 1-VI. Scale bars: 200 μm (B, C, D, E, F), and 50 μm (G, H).

hippocampal regions, we described the localization of TCTP from the DG to the CA3 to the CA1.

The DG is constituted with a series of strata, including polymorphic layer, stratum granulosum, and stratum moleculare. In the DG, TCTP was highly expressed in the molecular layer, but not in cell bodies of granule cells (Fig. 2a and 2b). This

subcellular distribution of TCTP in the DG area was quite close to that of synapsin II (Fig. 2c and 2d). In contrast, anti-Microtubule associated protein 2 (MAP2) stained the internal side of dendritic processes of granule cells (Fig. 2e and 2f). Anti-N-methyl D-aspartate receptor subtype 2B (NMDAR2B) appeared to label the cell membrane of the dendritic processes of granule

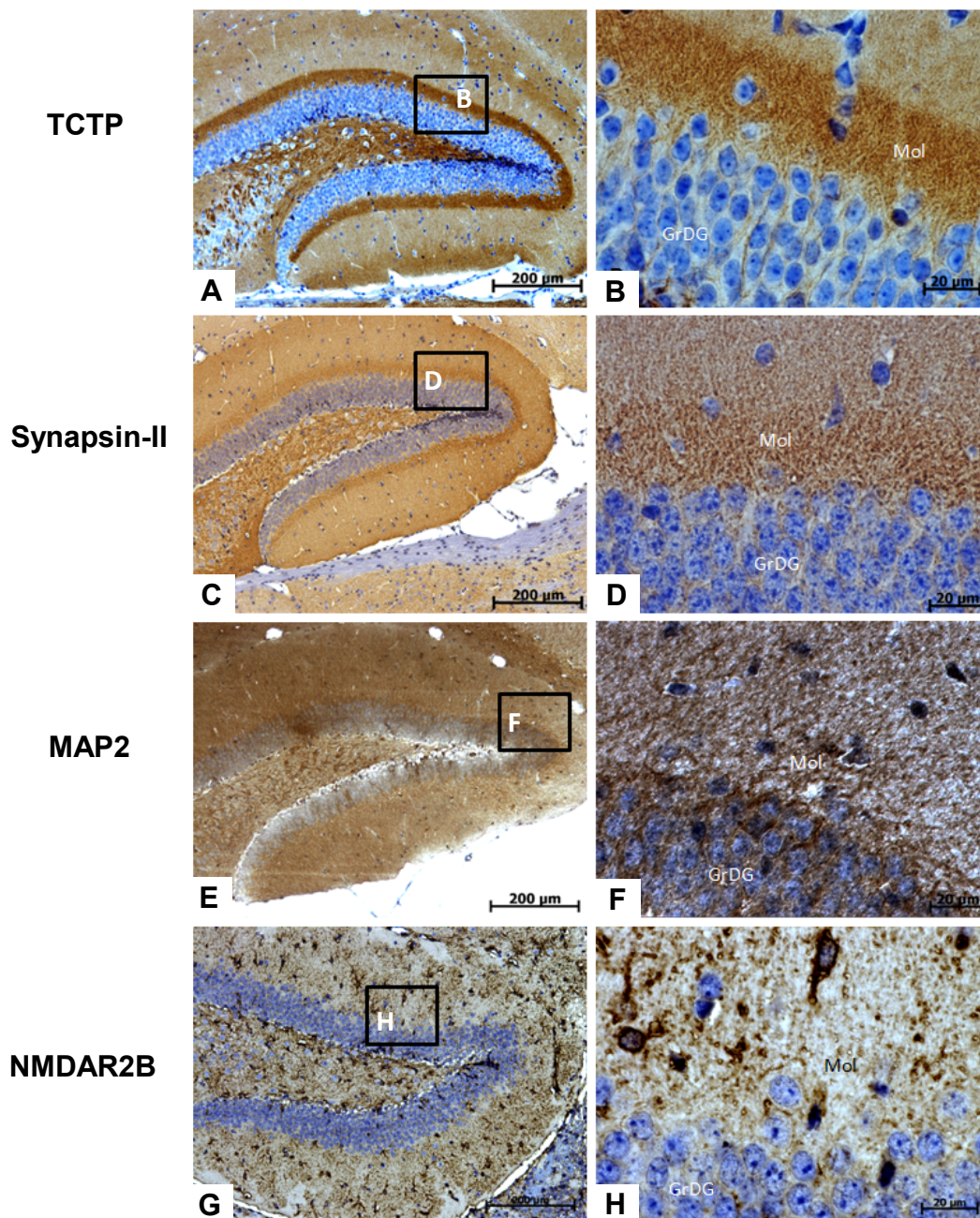


Fig. 2. Photomicrographs showing the localization of TCTP in the dentate gyrus. Sections were stained by anti-TCTP antibody (a, b), anti-synapsin II antibody (c, d), anti-MAP2 antibody (e, f), or anti-NMDAR2B (g, h). Immunohistochemical signals were visualized using DAB and cell nuclei were stained with hematoxylin. b, d, f, and h: high magnification of the boxed areas in a, c, e and f. Mol, molecular layer; GrDG, granule cell layer of the dentate gyrus. Scale bars: 200 μm (A, C, E, G), and 20 μm (B, D, F, H).

cells and cell body of astrocytes in the DG area (Fig. 2g and 2h). Thus, subcellular distributions of MAP2 and NMDAR2B were indifferent from that of TCTP. TCTP is shown to be predominantly expressed in axonal region in DG rather than in the dendrites.

Localization of TCTP in the CA3 region of the hippocampus

The CA regions are constituted with defined layers including stratum oriens, stratum pyramidale, stratum lucidum, stratum radiacum, stratum lacunosum, and stratum moleculare. TCTP was also found in the CA3 subregion of the hippocampus, preferentially in the stratum lucidum (Fig. 3a). At higher

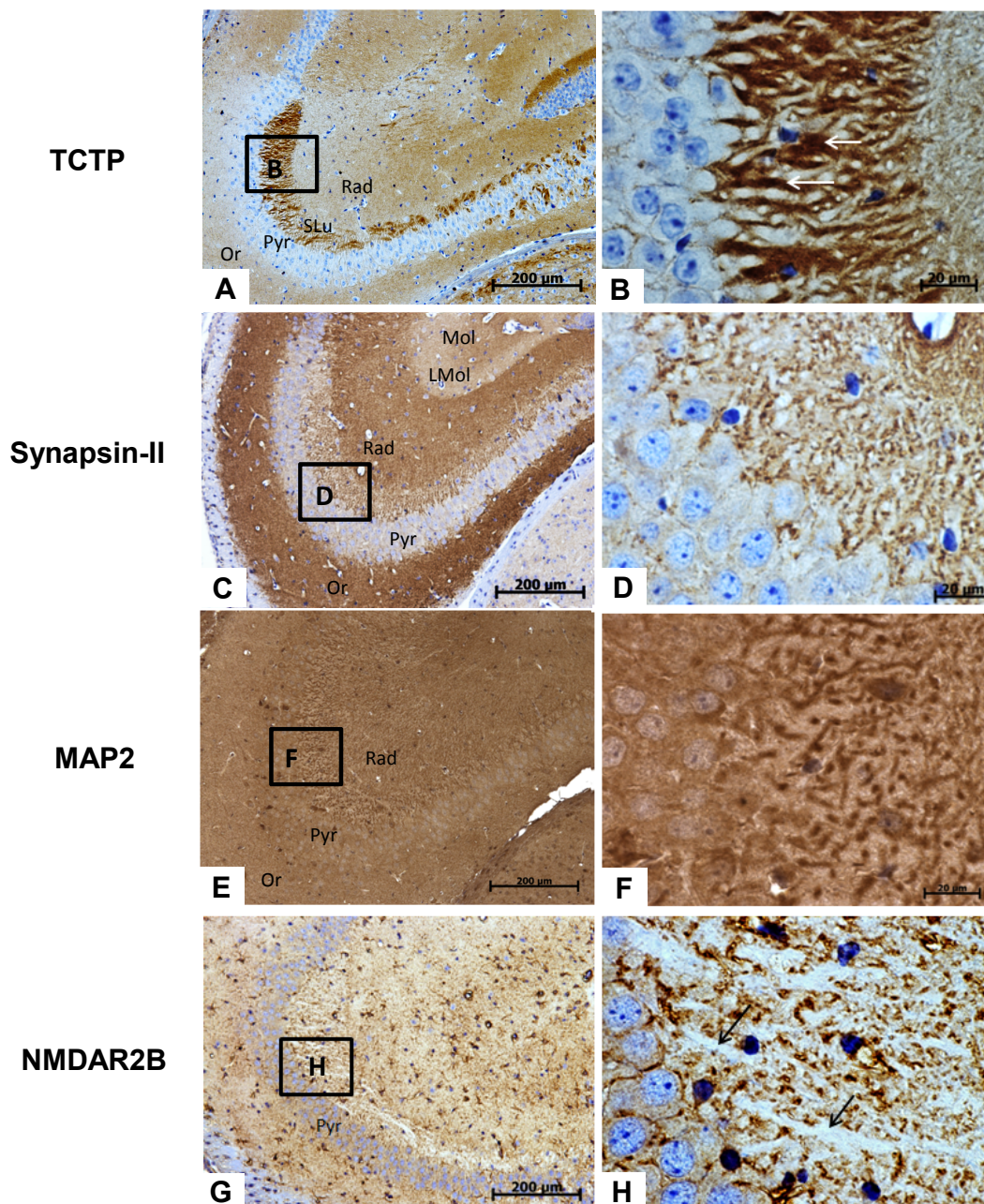


Fig. 3. Photomicrographs showing the localization of TCTP in the CA3 region of the hippocampus. Sections were stained by anti-TCTP antibody (a, b), anti-synapsin II antibody (c, d), anti-MAP2 antibody (e, f), or anti-NMDAR2B (g, h). Immunohistochemical signals were visualized using DAB and cell nuclei were stained with hematoxylin. Arrows in b indicate “mossy fiber terminals”. b, d, f, and h: high magnification of the boxed areas in a, c, e and g. Or, stratum oriens; Pyr, stratum pyramidale; Rad, stratum radiatum; SLu, stratum lucidum; LMol, stratum lacunosum-moleculare; Mol, stratum moleculare. Scale bars: 200 μ m (A, C, E, G), and 20 μ m (B, D, F, H).

magnification, TCTP was found to be primarily located in axon terminals of the mossy fibers, which were originated from dentate granule cells (Fig. 3b). CA3 pyramidal cells and dendritic processes were not stained by TCTP (Fig. 3a and 3b). Anti-synapsin II staining labeled axon terminals of the mossy fibers (Fig. 3c and 3d). Anti-synapsin II staining in stratum oriens and stratum

radiatum in the CA3 area was relatively higher compared to that in the stratum lucidum (Fig. 3c). MAP2 was located in the dendritic processes of CA3 pyramidal neurons (Fig. 3e and 3f). Anti-NMDAR2B appeared to label the cell membrane of the dendritic processes of CA3 pyramidal neurons and processes of astrocytes in the CA3 area (Fig. 3g and 3h). Thus, subcellular distributions of

MAP2 and NMDAR2B were not related to that of TCTP.

Localization of TCTP in the CA1 region of the hippocampus

In the CA1 region, high-level TCTP immunoreactivity was

found in the stratum oriens, stratum radiatum and stratum moleculare. In contrast, TCTP immunoreactivity was not detected in the stratum pyramidale which contained cell bodies of the pyramidal neurons (Fig. 4a and 4b). Dendritic processes of CA1

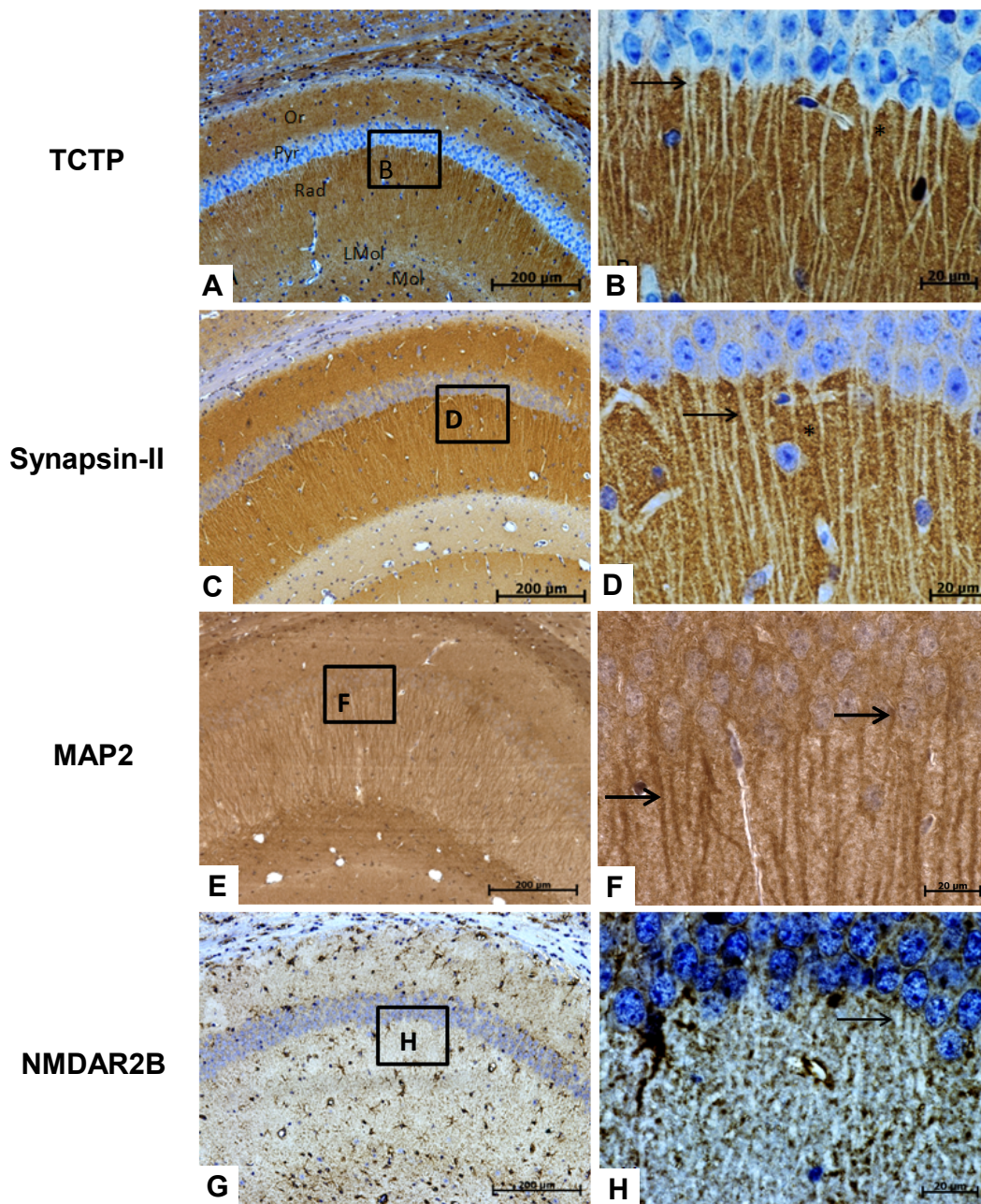


Fig. 4. Photomicrographs showing the localization of TCTP in the CA1 region of the hippocampus. Sections were stained by anti-TCTP antibody (a, b), anti-synapsin II antibody (c, d), anti-MAP2 antibody (e, f), or anti-NMDAR2B (g, h). Immunohistochemical signals were visualized using DAB and cell nuclei were stained with hematoxylin. Dendritic shafts of pyramidal cells in the CA1 were marked by arrows: In staining with anti-TCTP antibody (b) or anti-synapsin II (d), dendritic shafts from pyramidal cells were not stained at all, while in staining with anti-MAP2, dendritic shafts from pyramidal cells appeared with the internal side stainings (f). Anti-NMDAR2B immunoreactivity was detected in cell membrane of dendritic processes of CA1 pyramidal cells (h). b, d, f, and h: high magnification of the boxed areas in a, c, e and f. Or, stratum oriens; Pyr, stratum pyramidale; Rad, stratum radiatum; LMol, stratum lacunosum moleculare; Mol, stratum moleculare. Scale bars: 200 μm (A, C, E, G), and 20 μm (B, D, F, H).

pyramidal cells were not stained by TCTP (Fig. 4a and 4b). To be convinced with the exact location of TCTP in CA1 subregions, we performed immunohistochemistry with additional neuronal markers. Synapsin II (axonal marker) labeled axonal terminals of neurons in most brain regions including the hippocampus, and synapsin II distribution in the CA1 was similar to that of TCTP (Fig. 4c and 4d). Anti-MAP2 (dendritic marker) stained dendrites of pyramidal cells (Fig. 4e and 4f), while anti-NMDAR2B labeled the membrane of dendritic processes (Fig. 4g and 4h). Thus, anti-MAP2 and anti-NMDAR2B staining were far different from that of TCTP or synapsin II. Taken together, these results indicate that TCTP was principally located in axon terminals rather than in dendrites and cell bodies of pyramidal cells. Because the MAP2 staining seems to be broad in CA1 regions, it is possible that TCTP is dendritically localized in other CA1 neurons.

DISCUSSION

This study is the first to characterize the detailed localization of TCTP in the hippocampus, which is well-characterized for cell types, the location of cell body and axons, and internal neural circuits. Previous studies revealed that TCTP was expressed in the human nervous tissue [13]. However, the functional significance of TCTP in brain function has been speculated on the basis of information mostly from non-neuronal cells. In the present study, we demonstrated specific localization of TCTP in axons of neurons in most brain regions including the cerebral cortex, hippocampal subregions and corpus callosum (Fig. 1).

In the CA1 region of the hippocampus, TCTP was primarily localized in axon terminals of the Schaffer collateral fibers (Fig. 4a and 4b). In the CA3 region, TCTP was preferentially localized in the stratum lucidum (Fig. 3a and 3b), the region that receives synaptic inputs of the mossy fibers and the nonmyelinated axons of the DG granule cells [14]. In the DG, TCTP was detected in the inner molecular layer (Fig. 2a and 2b), the region that receives synaptic inputs from the entorhinal cortex. In a previous study, we showed that TCTP was also highly expressed in the myenteric nerve ganglia, whereas TCTP was almost absent in perikaryon [12]. Overall, our results suggest that TCTP was predominantly localized in the axon terminals of neurons in all hippocampal subregions, than in the dendrites and cell bodies. However, the present study is primarily based on immunostaining with anti-TCTP and following light-microscopic analysis, we believe that further studies on the role of TCTP in synaptic terminals or in other subcellular regions, if any, are necessary. Also, co-localization studies using either axonal or dendritic markers are needed to define the distribution of TCTP expression in distinctive neuronal

structures in the hippocampus.

The mossy fiber synapses between DG granule cells and pyramidal neurons in the CA3 region are important part of the hippocampal trisynaptic circuit [15]. The mossy fibers have large sized boutons which contain a maxi-pool of releasable vesicles and form the strong synapses with CA3 pyramidal neurons [16]. Considering that TCTP was remarkably localized in axon terminals of the mossy fibers (Fig. 3b), there is a possibility that TCTP might be involved in neurotransmitter release in mossy fibers. Given the role of microtubule stabilization and calcium-binding activities [17], we do not exclude the possibility that TCTP is involved in maintaining synaptic terminals by stabilizing cytoskeletal elements and synaptic machineries within synaptic terminals.

TCTP was shown to inhibit Na^+ , K^+ -ATPase by interacting with the 3rd cytoplasmic domain of Na^+ , K^+ -ATPase $\alpha 1$ subunit [18]. TCTP is also known to be involved in several diseases such as hypertension and cataract, through inhibition of Na^+ , K^+ -ATPase activity and elevating $[\text{Ca}^{2+}]_i$ levels [19]. Of interest, the studies on the role of Na^+ , K^+ -ATPase in the synaptic transmission has been found, especially, the changes in neuronal membrane properties and the synaptic function induced by the partial inhibition of the Na^+ , K^+ -ATPase [20]. Inhibition of Na^+ , K^+ -ATPase by dihydroouabain was shown to depolarize the presynaptic terminals, which may change $[\text{Ca}^{2+}]_i$ [21]. Therefore, it is possible that TCTP plays a role in neurotransmitter release of axons in the hippocampus, possibly through the regulation of Na^+ , K^+ -ATPase. Also, as suggested by Roque et al. in the embryonic visual system [22], TCTP possibly has a role in axonal development through the regulation of the survival signaling and axonal mitochondrial function. Further studies, including behavioral analysis using the TCTP-transgenic mouse model, might be necessary to clarify the specified roles and mechanisms of TCTP in hippocampal functions.

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