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Research Report

Matrix-degrading enzymes tissue plasminogen activator and matrix metalloprotease-3 in the hypothalamo-neurohypophysial system

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Abstract

The hypothalamo-neurohypophysial system (HNS), synthesizing arginine vasopressin (AVP) and oxytocin (OXT), is well known to show structural plasticity during chronic physiological stimulation such as salt loading and lactation. In the present study, we undertook in the HNS to study localization and activity-dependent changes in the expression of matrix-degrading enzymes such as tissue plasminogen activator (tPA) and matrix metalloprotease-3 (MMP-3). Double labeling confocal microscopy demonstrated that the immunoreactivity of tPA was localized at AVP-positive dendrites in the supraoptic nucleus (SON) and AVP-positive terminals in the neurohypophysis (NH). The immunoreactivity of tPA was also seen at astrocytic processes in the HNS. Likewise, the immunoreactivity of MMP-3 was observed at AVP-positive dendrites and terminals. High magnification observation further revealed punctate distribution of tPA and MMP-3 immunoreactivity at dendrites and terminals, suggesting that they are localized at neurosecretory granules. Salt loading, known as the chronic stimulation to cause the structural plasticity, increased protein and mRNA levels of tPA in the SON but reduced protein levels of it in the NH. The chronic stimulation also increased protein levels of urokinase plasminogen activator in the SON, but the stimulation did not change protein levels of MMP-3 in the SON and NH. Depolarizing agent KCl released tPA from isolated neurosecretosomes, and this depolarization-dependent release was abolished by verapamil, a Ca^{2+} channel blocker. These results demonstrate that tPA and MMP-3 are localized mainly at dendrites and terminals of AVP-expressing magnocellular neurons and tPA is released in an activity-dependent manner, suggesting that matrix-degrading proteases are candidate molecules to be concerned with the structural plasticity in the HNS.

Theme: Endocrine and autonomic regulation
Topic: Osmotic and thermal regulation

Keywords: Protease; tPA; Supraoptic nucleus; Neurohypophysis; Oxytocin; Vasopressin

1. Introduction

The hypothalamo-neurohypophysial system (HNS) is a well-known peptidergic part of central nervous system (CNS). Magnocellular neurons synthesize arginine vasopressin (AVP) and oxytocin (OXT) in the supraoptic nucleus (SON) and paraventricular nucleus and release them into blood capillary in the neurohypophysis (NH) (for a review, see [4]). Magnocellular neurons show marked structural plasticity in response to chronic physiological stimulation such as salt loading and lactation (for reviews, see [14,15,24,53]). In the SON, the structural plasticity has been caused by the retraction of glial cells between magnocellular neurons, which results in the formation of direct apposition of neuronal membrane and multiple synapses to promote neuronal excitation and synchronization [16,25,32,42]. In the NH, similarly, astrocytes are likely to surround or engulf axonal terminals under normal conditions, whereas astrocytes release axonal terminals in response to chronic stimulation by changing their morphology [22,23,27]. These morphological changes permit the neurosecretory terminals to contact directly with the basal lamina of the capillary vessels to facilitate peptide release [27].

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The structural plasticity of neuronal, glial, and synaptic architectures are reversible, and therefore such changes must accompany with dynamic cell interactions which are probably mediated via cell adhesion molecules (CAMs) and extracellular matrix (ECM). At present time, many CAMs and ECM molecules have been demonstrated to be expressed highly or restrictively in the HNS as compared with other adult brain regions, and therefore they are considered as candidate molecules involving in structural plasticity (for reviews, see [24,57]). Although neural cell adhesion molecule (NCAM) is highly sialylated in embryonic tissues and most adult tissues contain NCAM with little polysialic acid (PSA), the HNS still expresses high levels of PSA-NCAM [54]. The specific enzymatic removal of PSA from NCAM inhibits the neuronal–glial changes occurring in the magnocellular neurons [56]. The expression of F3 varies significantly in response to chronic stimulation [41]. In salt-loaded and lactating rats, F3 mRNA and protein levels were singularly increased in magnocellular neurons, while they decreased in the NH. Other adhesion molecules that may participate in the structural plasticity in the HNS are OBCAM [26,29], Kilon [26], L1 [12], and Thy-1 [26,27]. In addition to CAMs, ECM molecules are of great importance to the structural plasticity in the HNS. An extracellular matrix glycoprotein, tenascin-C, is highly expressed in the HNS [52,55]. Tenascin-C is observed in the astrocytes, and the expression level of tenascin-C is decreased with chronic salt loading. Chondroitin sulfate proteoglycans, phosphacan, and receptor type protein–tyrosine phosphatase β are also abundant in the magnocellular nuclei and are localized to constitute perineuronal nets around magnocellular somata and dendrites [28,31].

Extracellular proteolysis provides an attractive mechanism by which neuronal processes could remodel their synaptic connections and release of a protease could promote neuronal plasticity by degrading ECM proteins (for reviews, see [43,51]). One likely candidate for such a molecule is tissue plasminogen activator that is known to convert the ubiquitous zymogen plasminogen to plasmin [45], which in turn functions to degrade ECM components [59]. Accumulating evidences suggest that tPA plays crucial functions in plasticity and development of the central nervous system (CNS) [20]. The expression of tPA can be found in many brain regions, including the cerebellum [11], amygdala [40], and hippocampus [47]. The expression of tPA has been implicated in migration of cerebellar granular cells during development [11,48], long-term potentiation [3,10,19,36], cerebellar motor learning [49], NMDA receptor-mediated signaling [7,37], and synaptic plasticity [35,40]. Transcription of tPA mRNA is induced after synaptic [8,46,49] and glutamate stimulation [50]. It is shown that membrane depolarization induces a secretion of tPA in PC12 cells [13,39] and cultured cortical and hippocampal neurons [7,37,50].

MMP-3 belongs to a family of zinc-binding endopeptidases and degrades various ECM molecules such as fibronectin, collagen, and laminin [1]. MMP-3 is shown to digest phosphacan, neurocan, versican, brevican, and NG2 [34]. It is shown that MMP-3 enhances plasminogen activation by binding to tPA and plasminogen [2] and inactivating plasminogen activator inhibitor-1 [21]. MMP-3 is demonstrated to be produced in vivo by some developing or adult neurons [58]. MMP-3 by some growth cones promotes axon growth in vitro [6,34,38,61].

To date, however, there have been no studies to demonstrate the presence of tPA and MMP-3 and their possible correlation with the structural plasticity in the HNS, in spite of their important participation of CNS plasticity. Therefore, we examined the light microscopic localization of tPA and MMP-3 and activity-dependent changes in expression of tPA and MMP-3 in the HNS and depolarization-induced release of tPA from isolated neurosecretosomes. Our results showed that tPA and MMP-3 are specifically expressed mainly at magnocellular dendrites and terminals, and tPA is released from terminals in an activity-dependent manner. We therefore suggest that tPA and MMP-3 are important molecules to degrade the ECM and thereby induce the structural plasticity in the HNS.

2. Materials and methods

2.1. Animals

Wistar male rats (8–12 weeks old) were used for the present experiments. Rats were housed under standard conditions with a 12:12 h dark and light cycle. They were divided into two groups according to our previous method [27,31]: (1) unstimulated control rats that were free access to food and water ad libitum; (2) chronically stimulated (salt-loaded) males whose drinking water had been replaced with 2% NaCl for 1 week. All experimental protocols were performed in accordance with the guidelines for animal research of the Neuroscience Society of Japan to minimize the number of animals used and their suffering.

2.2. Antibodies

Biotinylated anti-mouse IgG was obtained from Vector Laboratory (Burlingame, CA). Fluorescein isothiocyanate (FITC)-conjugated anti-rabbit IgG and horseradish-peroxidase-labeled anti-mouse and rabbit IgG were purchased from Kirkegaard and Perry Laboratories (Gaithersburg, MD). The primary antibodies used were follows: anti-AVP-neurophysin (PS41; mouse IgG, from Dr. Gainer, NIH), anti-glial acidic fibrillary protein (GFAP; Chemicon, Temecula, CA), anti-MMP-3 (sheep IgG, Oncogene, Cambridge, MA, or goat IgG, Santa Cruz, Santa Cruz, CA), anti-OXT-neurophysin (PS38; mouse IgG, from Dr. Gainer, NIH), and anti-tPA (rabbit IgG, Molecular Innovations, Southfield, MI) antibodies.