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The interaction of mammalian Class C Vps with nSec-1/Munc18-a and syntaxin 1A regulates pre-synaptic release ☆

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Abstract

Membrane docking and fusion in neurons is a highly regulated process requiring the participation of a large number of SNAREs (soluble *N*-ethylmaleimide sensitive factor attachment protein receptors) and SNARE-interacting proteins. We found that mammalian Class C Vps protein complex associated specifically with nSec-1/Munc18-a, and syntaxin 1A both *in vivo* and *in vitro*. In contrast, VAMP2 and SNAP-25, other neuronal core complex proteins, did not interact. When co-transfected with the human growth hormone (hGH) reporter gene, mammalian Class C Vps proteins enhanced Ca^{2+}-dependent exocytosis, which was abolished by the Ca^{2+}-channel blocker nifedipine. In hippocampal primary cultures, the lentivirus-mediated overexpression of hVps18 increased asynchronous spontaneous synaptic release without changing mEPSCs. These results indicate that mammalian Class C Vps proteins are involved in the regulation of membrane docking and fusion through an interaction with neuronal specific SNARE molecules, nSec-1/Munc18-a and syntaxin 1A.

Keywords: Class C Vps; nSec-1/Munc18-a; Syntaxin 1A; SNARE; Synaptic release

In the nervous system, vesicle transport and fusion events are essential for proper synaptic transmission. The modulation of synaptic strength leading to the learning and memory formation is jointly controlled by pre- and post-synaptic mechanisms. Pre-synaptic vesicles containing neurotransmitters fuse to the plasma membrane in a Ca^{2+}-dependent manner to release the contents to the synaptic cleft. The molecular mechanisms regulating transport of specific vesicles to their appropriate target membranes have been intensively characterized. In the neurons, the neurotransmitter release relies on syntaxin 1A and SNAP-25 (target-SNARE; t-SNARE) and on VAMP2 (vesicle-SNARE; v-SNARE) [1,2]. A large number of molecules, including n-Sec1/munc18-a, complexin, munc-13, tomosyn, and SNAP-29, have been suggested to regulate the availability of SNARE proteins to form functional neurotransmitter release machinery [3–6]. However, in spite of the isolation of large numbers of regulatory proteins involved in synaptic vesicle docking and fusion event, it remains largely unclear.

VPS11, *VPS16*, *VPS18*, and the yeast vacuolar S/M gene, *VPS33*, comprise a subset of genes, called the Class C *VPS* genes [7,8]. The Class C Vps proteins are structurally and functionally conserved from yeast to mammals. Vps18 and Vps33 share significant homology with the

☆ *Abbreviations:* GST, glutathione *S*-transferase; HA, hemagglutinin; GFP, green fluorescent protein; SNARE, soluble *N*-ethymaleimide sensitive factor attachment protein receptor; SNAP-25, synaptosome-associated protein of 25 kDa; mEPSC, miniature excitatory post-synaptic current.

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Involvement of $P2X_4$ and $P2Y_{12}$ Receptors in ATP-Induced Microglial Chemotaxis

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KEY WORDS
microglia; ATP; chemotaxis; P2Y12; P2X4

ABSTRACT
We previously reported that extracellular ATP induces membrane ruffling and chemotaxis of microglia and suggested that their induction is mediated by the Gi/o-protein coupled $P2Y_{12}$ receptor ($P2Y_{12}R$). Here we report discovering that the $P2X_4$ receptor ($P2X_4R$) is also involved in ATP-induced microglial chemotaxis. To understand the intracellular signaling pathway downstream of $P2Y_{12}R$ that underlies microglial chemotaxis, we examined the effect of two phosphatidylinositol 3′-kinase (PI3K) inhibitors, wortmannin, and LY294002, on chemotaxis in a Dunn chemotaxis chamber. The PI3K inhibitors significantly suppressed chemotaxis without affecting ATP-induced membrane ruffling. ATP stimulation increased Akt phosphorylation in the microglia, and the increase was reduced by the PI3K inhibitors and a $P2Y_{12}R$ antagonist. These results indicate that $P2Y_{12}R$-mediated activation of the PI3K pathway is required for microglial chemotaxis in response to ATP. We also found that the Akt phosphorylation was reduced when extracellular calcium was chelated, suggesting that ionotropic P2X receptors are involved in microglial chemotaxis by affecting the PI3K pathway. We therefore tested the effect of various $P2X_4R$ antagonists on the chemotaxis, and the results showed that pharmacological blockade of $P2X_4R$ significantly inhibited it. Knockdown of the $P2X_4$ receptor in microglia by RNA interference through the lentivirus vector system also suppressed the microglial chemotaxis. These results indicate that $P2X_4R$ as well as $P2Y_{12}R$ is involved in ATP-induced microglial chemotaxis.

INTRODUCTION

Microglia are the immune effector cells that participate in tissue repair, amplification of inflammatory responses, and neuronal degeneration in the central nervous system (CNS) (Kreutzberg, 1996; Streit, 2002). They are present in the form of ramified cells under normal conditions, but in response to pathological stimuli microglia rapidly transform into a motile ameboid form and migrate toward lesion sites, where they secrete a variety of substances and clear cell debris (Moran and Graeber, 2004; Nakajima and Kohsaka, 2005; Stence et al., 2001). Thus, microglial migration plays a crucial role in the amelioration of a damaged CNS; however, the intracellular signals underlying microglial cell migration are poorly understood.

Extracellular ATP is known to play a role as a neurotransmitter or neuromodulator in the CNS (Illes and Alexandre Ribeiro, 2004), and it regulates various physiological functions of microglia (Inoue, 2002). ATP receptors are classified into two families: the ionotropic P2X receptor (P2XR) family and the GTP-binding (G-) protein coupled P2Y receptor (P2YR) family (Ralevic and Burnstock, 1998), and microglia have been reported to possess functional ATP receptors, including $P2X_4R$, $P2X_7R$, and $P2Y_{12}R$ (Cavaliere et al., 2003; James and Butt, 2002; Sasaki et al., 2003; Tsuda et al., 2003). Davalos et al. (2005) and Nimmerjahn et al. (2005) recently reported that processes of ramified microglia extended toward a confocal laser injury, where ATP is likely to be released by damaged tissue and surrounding astrocytes. These observations suggest that ATP is a primary molecule in the induction of the change in microglial morphology.

We have also previously reported that ATP-induced microglial membrane ruffling and chemotaxis are mediated by Gi/o-protein coupled $P2Y_{12}R$ (Honda et al., 2001; Sasaki et al., 2003); however, the intracellular signaling pathway downstream of $P2Y_{12}R$ following ATP stimulation is not fully understood. Several recent articles have revealed that $P2Y_{12}R$ stimulation results in activation of the phosphatidylinositol 3′-kinase (PI3K) pathway in some cells (Czajkowski et al., 2004; Soulet et al., 2004; Van Kolen and Slegers, 2004). Although PI3K is known to be a crucial enzyme in the regulation of chemotaxis by monocytes and macrophages (Procko and McColl, 2005; Ridley, 2001; Van Haastert and Devreotes, 2004), whether the PI3K pathway participates in ATP-induced microglial chemotaxis remained unclear.

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Vaccination and infection as causative factors in Japanese patients with Rasmussen syndrome: Molecular mimicry and HLA class I

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Abstract

Rasmussen syndrome is an intractable epilepsy with a putative causal relation with cellular and humoral autoimmunity. Almost half of the patients have some preceding causative factors, with infections found in 38.2%, vaccinations in 5.9% and head trauma in 8.9% of Japanese patients. In a patient with seizure onset after influenza A infections, cross-reaction of the patient's lymphocytes with GluRε2 and influenza vaccine components was demonstrated by lymphocyte stimulation test. Database analyses revealed that influenza A virus hemagglutinin and GluRε2 molecules contain peptides with the patient's HLA class I binding motif (HLA − A*0201). The relative risks of HLA class I genotypes for Rasmussen syndrome are 6.1 (A*2402), 6.4 (A*0201), 6.3 (A*2601) and 11.4 (B*4601). The relative risks of HLA class I-A and B haplotypes are infinity (A*2601 + B*5401), 21.1 (A*2402 + B*1501), 13.3 (A*2402 + B*4801) and 5.1 (A*2402 + B*5201). Some alleles and haplotypes of HLA class I may be the risk factors in Japanese patients. Cross-reactivity of cytotoxic T lymphocytes may contribute to the processes leading from infection to the involvement of CNS.

Keywords: *Rasmussen syndrome, HLA, cytotoxic T cells, influenza, vaccination, epilepsy*

Introduction

Rasmussen's encephalitis is a slowly progressive, autoimmune-mediated chronic inflammatory disease of the CNS. The mean age of onset is 7.4 years. The disease may be preceded by some causative factors including infection, and the initial seizure episode manifests various forms such as partial onset generalized tonic-clinic (pGTC) seizures (30%), focal motor seizures (26%) and complex partial seizures (CPS) (26%) (Andermann 1991). One third of the patients have preceding infections within 1 month before onset. Patients with typical Rasmussen's encephalitis manifest frequent intractable partial motor seizures in the acute phase, characteristically epilepsia partialis continua (EPC) (56%). Patients begin to manifest EPC 1.8 years after the onset of epilepsy, but the seizure frequency decreases markedly in the residual stage (Andermann 1991, Bien et al. 2002b) (Figure 1). Patients in the residual stage are affected by hemiplegia (96%), mental deterioration (85%), visual field defect (49%), and cortical sensory defect (29%) (Andermann 1991). Histological examination reveals infiltration of T lymphocytes and microglia cells, astrocytosis, and neuronal loss in the lesion (Aguilar and Rasmussen 1960, Andermann 1991, Farrell et al. 1995). Functional hemispherectomy is the only reliable therapy when the non-dominant side is involved, but hemiparesis and hemianopsia are

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Solo/Trio8, a Membrane-Associated Short Isoform of Trio, Modulates Endosome Dynamics and Neurite Elongation

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With DNA microarrays, we identified a gene, termed *Solo*, that is downregulated in the cerebellum of Purkinje cell degeneration mutant mice. Solo is a mouse homologue of rat Trio8—one of multiple Trio isoforms recently identified in rat brain. Solo/Trio8 contains N-terminal sec14-like and spectrin-like repeat domains followed by a single guanine nucleotide exchange factor 1 (GEF1) domain, but it lacks the C-terminal GEF2, immunoglobulin-like, and kinase domains that are typical of Trio. Solo/Trio8 is predominantly expressed in Purkinje neurons of the mouse brain, and expression begins following birth and increases during Purkinje neuron maturation. We identified a novel C-terminal membrane-anchoring domain in Solo/Trio8 that is required for enhanced green fluorescent protein-Solo/Trio8 localization to early endosomes (positive for both early-endosome antigen 1 [EEA1] and Rab5) in COS-7 cells and primary cultured neurons. Solo/Trio8 overexpression in COS-7 cells augmented the EEA1-positive early-endosome pool, and this effect was abolished via mutation and inactivation of the GEF domain or deletion of the C-terminal membrane-anchoring domain. Moreover, primary cultured neurons transfected with Solo/Trio8 showed increased neurite elongation that was dependent on these domains. These results suggest that Solo/Trio8 acts as an early-endosome-specific upstream activator of Rho family GTPases for neurite elongation of developing Purkinje neurons.

Endosomal membrane trafficking in neurons plays a key role in various neural processes, including neurite elongation (19, 33), synaptic transmission (17), neuronal degeneration (36), and neuronal cell death or survival (7). The early endosome regulates the selective transfer of membrane proteins to other organelles, and thus it is a key organelle for sorting vesicles containing cell surface membrane proteins, including receptors, transporters, channels, and cell adhesion molecules (2, 29, 39, 47).

Several lines of evidence suggest that small GTPases play pivotal roles in regulating early-endosome dynamics (2, 39, 47). For example, Rab5 regulates the motility and fusion of early endosomes (32), whereas Rab4 and Rab5 control vesicle influx and efflux, respectively, in the early-endosome pool (28). Rho family GTPases also regulate early-endosome dynamics. Once such GTPase, Cdc42, controls endocytic transport in polarized cells (20), whereas RhoD specifically localizes to early endosomes and regulates their motility via diaphanous-related formin proteins (13). Upstream regulators of small GTPases that associate with early endosomes have been studied extensively. For example, early-endosome antigen 1 (EEA1) acts as an effector for Rab family small GTPases (5, 45). Although Rho family GTPases are also activated by multivalent upstream effectors (42), the specialized upstream activators that function in early endosomes remain unknown.

Trio, a member of the Dbl homology domain family of guanine nucleotide exchange factors (GEFs), was originally identified by its interaction with the leukocyte common antigen-related protein receptor (6). Trio has an N-terminal sec14-like domain, spectrin-like repeats, two GEF domains (GEF1 and GEF2), an immunoglobulin (Ig)-like domain, and a C-terminal Ser/Thr kinase domain (3). The GEF1 domain activates RhoG and Rac1, whereas GEF2 acts on RhoA, suggesting that Trio is involved in multiple GTPase cascades mediating various cellular processes (3). Genetic analysis of the Trio gene in *Drosophila* embryos implicates this protein in neuronal and retinal axon guidance (3). Mice lacking Trio die during embryogenesis and exhibit a loss of myofiber formation and cellular disorganization in the hippocampus and olfactory bulb (35). Although Trio is highly expressed in the adult brain, heart, liver, skeletal muscle, kidney, placenta, and pancreas (6), its effector function in these adult tissues remains unknown. Several Trio isoforms were recently identified (25), and the expression of each isoform was shown to be regulated in a tissue-specific manner. The functions of these isoforms, however, have not been delineated.

Purkinje cell degeneration (pcd) is an autosomal recessive

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Dopaminergic neuronal loss in transgenic mice expressing the Parkinson's disease-associated UCH-L1 I93M mutant

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Abstract

The I93M mutation in ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1) was reported in one German family with autosomal dominant Parkinson's disease (PD). The causative role of the mutation has, however, been questioned. We generated transgenic (Tg) mice carrying human *UCHL1* under control of the *PDGF-B* promoter; two independent lines were generated with the I93M mutation (a high- and low-expressing line) and one line with wild-type human UCH-L1. We found a significant reduction in the dopaminergic neurons in the substantia nigra and the dopamine content in the striatum in the high-expressing I93M Tg mice as compared with non-Tg mice at 20 weeks of age. Although these changes were absent in the low-expressing I93M Tg mice, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treatment profoundly reduced dopaminergic neurons in this line as compared with wild-type Tg or non-Tg mice. Abnormal neuropathologies were also observed, such as silver staining-positive argyrophilic grains in the perikarya of degenerating dopaminergic neurons, in I93M Tg mice. The midbrains of I93M Tg mice contained increased amounts of insoluble UCH-L1 as compared with those of non-Tg mice, perhaps resulting in a toxic gain of function. Collectively, our data represent *in vivo* evidence that expression of $UCHL1^{I93M}$ leads to the degeneration of dopaminergic neurons.

Keywords: Ubiquitin carboxy-terminal hydrolase L1; Animal model; Parkinson's disease; Dopaminergic neuron

1. Introduction

Parkinson's disease (PD) is the second most common human neurodegenerative disorder after Alzheimer's disease (AD) (Dauer and Przedborski, 2003; Vila and Przedborski, 2004). PD patients exhibit motor dysfunction, including slowed movement (bradykinesia), resting tremor, rigidity, and postural instability (Dauer and Przedborski, 2003). The pathological basis of PD is the progressive loss of dopaminergic neurons in the substantia nigra pars compacta, giving rise to a decrease in dopamine content in the striatum (Dauer and Przedborski, 2003). Although most cases of PD are sporadic, studies of familial PD have provided accumulating evidence for the molecular mechanisms of PD. Thus far, at least six proteins have been identified to cause familial PD: α-synuclein (Chartier-Harlin et al., 2004; Farrer et al., 2004; Ibanez et al., 2004; Kruger et al., 1998; Polymeropoulos et al., 1997; Singleton et al., 2003), UCH-L1 (Leroy et al., 1998), parkin (Kitada et al., 1998), DJ-1 (Bonifati et al., 2003), phosphatase

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