Biochemical and Biophysical Research Communications 391 (2010) 382–387

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Suppression of dynamin GTPase activity by sertraline leads to inhibition of dynamin-dependent endocytosis

Kiyofumi Takahashi [a,1], Hiroshi Miyoshi [b,1,*], Masahiro Otomo [a,1], Kenichi Osada [a], Noboru Yamaguchi [a], Hideki Nakashima [b]

[a] Department of Neuropsychiatry, St. Marianna University School of Medicine, 2-16-1 Sugao, Miyamae, Kawasaki 216-8511, Japan
[b] Department of Microbiology, St. Marianna University School of Medicine, 2-16-1 Sugao, Miyamae, Kawasaki 216-8511, Japan

ARTICLE INFO

Article history:
Received 5 November 2009
Available online 12 November 2009

Keywords:
GTPase
Dynamin
Endocytosis
Inhibitor
Sertraline

ABSTRACT

Dynamin (Dyn) 1 plays a role in recycling of synaptic vesicles, and thus in nervous system function. We previously showed that sertraline, a selective serotonin reuptake inhibitor (SSRI), is a mixed-type inhibitor of Dyn 1 with respect to both GTP and L-α-phosphatidyl-L-serine (PS) *in vitro*, and we suggested that it may regulate the neurotransmitter transport by modulating synaptic vesicle endocytosis *via* inhibition of Dyn 1 GTPase. Here, we investigated the effect of sertraline on endocytosis of marker proteins in human neuroblastoma SH-Sy5Y cells and HeLa cells. Sertraline inhibited endocytosis in both cell lines. Western blotting showed that SH-Sy5Y expresses Dyn 1 and Dyn 2, while HeLa expresses only Dyn 2. GTPase assay showed that sertraline inhibited Dyn 2 as well as Dyn 1. Therefore, the effect of sertraline on endocytosis was mediated by Dyn 2, at least in HeLa cells, as well as by Dyn 1 in cell lines that express it. Moreover, the inhibition mechanism of transferrin (Tf) uptake by sertraline differed from that in cells expressing Dyn 1 K44A, a GTP binding-defective variant, and sertraline did not interfere with the interaction between Dyn 1 and PS-liposomes.

© 2009 Elsevier Inc. All rights reserved.

Introduction

Dynamin (Dyn) has GTPase activity and plays a critical role in clathrin- and caveolae-dependent endocytosis [1,2]. A major role of Dyn GTPase activity in endocytosis is to produce a mechanical force for membrane fission during clathrin-coated vesicle budding, either by constriction or expansion of the collar surrounding the neck of the invaginated vesicle [3].

Mammalians have three Dyn isoforms with different tissue distributions [4,5]. Dyn 1 is only expressed in neurons and has been implicated in presynaptic vesicle recycling [4]. It is thought to play a role in the clathrin-dependent endocytotic pathway at neuronal synapses [6,7]. Dyn 1 has four functional domains: an N-terminal GTPase domain, a pleckstrin homology domain (PHD), a proline/arginine-rich domain (PRD), and a GTPase effector domain [3,8–11]. L-α-Phosphatidyl-L-serine (PS) or phosphatidylinositol-4,5-bisphosphate binds the PHD of Dyn [8,9], stimulates the GTPase activity, and induces cooperative helix assembly [10,12]. Moreover, after deletion of the PHD, Dyn shows elevated GTPase activity independently of those lipids [8,9]. PRD of Dyn binds to Src homology 3 (SH3) domain-containing proteins, such as amphiphysin [13], and the complex is necessary for adaptation of clathrin [14]. PRD peptide disrupts the interaction between Dyn and amphiphysin and inhibits endocytosis [15].

The K44A variant of Dyn lacks GTPase activity owing to defects in both GTP binding and hydrolytic activity [2,16]. As a result, overexpression of the K44A variant inhibits clathrin-dependent endocytosis in neuronal cells [2,16,17].

The GTPase activities of Dyn 1 and 2 are inhibited by cationic surfactants such as myristyl trimethyl ammonium bromide [18], and by 3-(2-chloro-10*H*-phenothiazin-10-yl)-*N*,*N*-dimethylpropan-1-amine (chlorpromazine), which is an antipsychotic [19]. We previously reported that (1*S*)-*cis*-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-*N*-methyl-1-naphthalenamine (sertraline) inhibits the GTPase activity of Dyn 1 [20]. Our previous results indicated that the inhibition of Dyn 1 GTPase by sertraline may regulate the endocytic pathway at neuronal synapses.

Sertraline is a selective serotonin reuptake inhibitor (SSRI) [21]. The serotonin transporter is proposed to modulate a variety of brain functions, including mood, anxiety and sleep, by the

Abbreviations: SSRI, selective serotonin reuptake inhibitor; His_6, polyhistidine segment 6 residues in length; Dyn, dynamin; Dyn-His_6, dynamin 1 with a His_6 tag fused to the C-terminus; PHD, pleckstrin homology domain; PRD, proline/arginine-rich domain; SH3, Src homology 3; PS, L-α-phosphatidyl-L-serine; Pi, orthophosphate; SPR, surface plasmon resonance; CTB, cholera toxin subunit B; Tf, transferrin; CPZ, chlorpromazine; DAPI, 4′,6-diamidino-2-phenylindole; CHC, clathrin heavy chain

* Corresponding author. Fax: +81 44 977 7818.
E-mail address: hmiyoshi@marianna-u.ac.jp (H. Miyoshi).

[1] These authors contributed equally to this work.

0006-291X/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2009.11.067

elimination of the neurotransmitter serotonin from the synaptic cleft [22]. Depressive disorder has been postulated to be associated with continuously low levels of serotonin [23]. However, our finding that some SSRIs inhibit Dyn 1 GTPase [20] was unexpected, and furthermore, the relationship between inhibition of Dyn 1 GTPase and regulation of endocytosis by sertraline is still unknown. In this study, we further examined the mechanism of sertraline's inhibitory action on endocytosis.

Materials and methods

Materials. Sources of materials were: restriction enzymes (Takara Bio); pEGFP-C1 (Clontech); pET 21a (Merck); Alexa Fluor®555 conjugate-CTB, Alexa Fluor®633 conjugate-Tf, Prolong Gold and Lipofectamin™2000 (Invitrogen); L-α-phosphatidyl-L-serine (Sigma Aldrich); Dyn 1 antibody (Epitomics); Dyn 2 (C-18) antibody, clathrin HC (C-20) antibody, rabbit anti-goat antibody and goat anti-mouse antibody (Santa Cruz Biotechnology); β-actin rabbit antibody (Cell Signaling); sertraline, citalopram and chlorpromazine (Sigma); ECL Western blotting detection reagents (GE Healthcare).

Cloning and construction of expression plasmids. Dyn 2 gene (gi:87299636) was amplified by PCR using appropriate primers. The amplified gene was ligated into the NdeI and SalI sites in pET 21a to create an expression vector for Dyn 2 *wt* bearing a polyhistidine segment 6 residues in length (His_6 tag) fused to the C-terminus (Dyn2-His_6). Cloning and construction of pETDyn1, an expression vector for Dyn 1 *wt* bearing a His_6 tag fused to the C-terminus (Dyn-His_6), were reported previously [20]. Dyn 1 *wt* gene was subcloned into pBluescript2SK(+) from pETDyn1 to reconstruct expression plasmids for mammalian cells. The subcloned vector was named pBlueDyn1*wt*. Site-directed mutagenesis was performed by PCR to create an expression vector for Dyn K44A bearing a His_6 tag fused to the C-terminus (Dyn K44A-His_6). This was digested with NdeI and XhoI, and ligated into the same sites in both pET 21a and pBlueDyn1*wt*, affording pETDyn1K44A and pBlueDyn1K44A, respectively. A pETDyn1 plasmid was digested with BstBI and BspEI, blunt-ended and ligated to delete 530–550 amino acid residues (Dyn ΔPHD-His_6), affording pETDyn1ΔPHD. pBlueDyn1 plasmid was digested with BglII and EcoRI, and ligated into the same sites in pEGFP-C1 to create an expression vector for Dyn 1 linked at the N-terminus to GFP, designated pEGFP-Dyn1. pEGFP-Dyn1K44A plasmid was constructed similarly to pEGFP-Dyn1.

Expression and purification of Dyn. Expression and purification of Dyn-His_6 were reported previously [20]. DynK44A-His_6, DynΔPHD-His_6, and Dyn2-His_6 were expressed and purified similarly.

GTPase assay and preparation of PS-liposomes. The Malachite Green GTPase assay of Dyn was performed as described previously [20]. A solution of PS in chloroform/methanol (95:5) (10 mg ml^{-1}, 40 μl) was evaporated to about 5 μl, resuspended in 1 ml of 30 mM Tris–HCl pH 7.4, and sonicated for 2 min on ice to afford a working solution of 400 μg ml^{-1}.

Surface plasmon resonance (SPR). SPR analyses were essentially performed as described previously [9]. SPR analyses were performed on a Biacore 3000 with a Sensorchip NTA (Biacore K.K.), using Buffer A (10 mM Tris–HCl, 10 mM NaCl, 2 mM $MgCl_2$, 0.05% Tween 80, pH 7.4) as the eluent (20 μl min^{-1}), at 25 °C. All four sensor sites were treated with 20 μl of 100 μM $NiSO_4$, then Dyn-His_6, DynK44A-His_6, and DynΔPHD-His_6 (50 μl, 10 μg ml^{-1}) were trapped *via* the His_6 tag on sites 1, 2, and 3, respectively. Site 4 was the blank control (Buffer A only). PS-liposomes (100 μl; 100 μg ml^{-1}) were injected simultaneously over all four sites, with or without 50 μM sertraline. Sensorgrams for specific interactions were obtained by subtracting the sensorgram for Buffer A from those for Dyn-His_6, DynK44A-His_6, and DynΔPHD-His_6 with BIA Evaluation Software.

Cell culture and fluorescence imaging. HeLa and SH-Sy5Y cells were cultured with 5% or 10% fetal bovine serum in DMEM, seeded onto collagen IV-coated coverslips, and transfected with pEGFP-Dyn1 or pEGFP-Dyn1K44A using Lipofectamin™2000. Adhering cells were treated with drugs (30 min, 37 °C) and with 1 μg ml^{-1} Alexa-CTB (30 min, on ice), then incubated with 5 μg ml^{-1} Alexa-Tf (5 min, 37 °C). The cells were washed with ice-cold 150 mM glycine (pH 2.0), fixed with 4% paraformaldehyde, rinsed with PBS, air-dried, mounted on Prolong Gold antifade reagent with DAPI, and observed with a Carl Zeiss LSM510 confocal microscope (63×/1.4 oil immersion objective). Excitation wavelengths of DAPI, GFP, Alexa-CTB, and Alexa-Tf were 405, 488, 543 and 633 nm, respectively.

Image analysis. Microscopic images were analyzed using ImageJ software version 1.42q (NIH, USA: http://rsb.info.nih.gov/ij/index.html) [24]. Outlines of cells were traced using the polygon selection tool, and fluorescence intensity was obtained for each cell.

Western blotting. Cells were lysed with SDS–PAGE sample buffer and lysates were subjected to 8% SDS–PAGE. Gels were blotted on PVDF membrane, which was incubated in 5% non-fat dried milk in TBST, then with anti-Dyn 1 (1:250), anti-Dyn 2 (1:250), anti-clathrin HC (1:250), or anti-β-actin (1:1000), followed by HRP-conjugated secondary antibody. Bands were evaluated with the ECL Western Blotting Detection System using a LAS-3000 (Fuji Photo Film).

Results

Sertraline preferentially and reversibly inhibits endocytosis of Tf

Chlorpromazine (CPZ) is well known, not only as an antipsychotic [19], but also as an inhibitor of clathrin-dependent endocytosis [25]. Citalopram is a SSRI, like sertraline [26]. Moreover, we previously reported that sertraline potently inhibited Dyn1 GTPase activity *in vitro* and that sertraline, CPZ and citalopram inhibited Dyn 1 GTPase with IC_{50} values of 7.3 ± 1.0, 47.2 ± 23.1 and >100 μM, respectively [20]. Therefore, we first compared the effects of sertraline, CPZ and citalopram on endocytosis of marker proteins in HeLa cells (Fig. 1). Sertraline and CPZ inhibited uptake of transferrin (Tf) and cholera toxin subunit B (CTB) into HeLa cells, while citalopram was ineffective (Fig. 1A and B). Interestingly, sertraline was strongly Tf-selective, while CPZ was less so (Fig. 1B). These results suggested that Dyn-dependent endocytosis of Tf was blocked *via* inhibition of Dyn GTPase by sertraline.

Time course analyses showed that most of the internalization of Tf was blocked within 5 min after addition of sertraline (Fig. 2A), while the Tf uptake was unaffected by addition of citalopram (Fig. 2B). At 20 min after washout of sertraline, endocytosis was found to have returned to control levels (Fig. 2C), suggesting that sertraline inhibition is rapidly reversible.

Sertraline also inhibits endocytosis of Tf in neuronal cells

We next examined the effect of sertraline on endocytosis in human neuroblastoma SH-Sy5Y cells. Internalization of Tf, but not CTB, was strongly inhibited by 20 μM sertraline in SH-Sy5Y cells (Fig. 3A). These results are consistent with the suppression of endocytosis observed in HeLa cells, as shown in Fig. 1A.

Sertraline inhibits Dyn 2 GTPase

Endogenous expression of Dyn isoforms in both HeLa and SH-Sy5Y cells was investigated by Western blotting in order to identify

Download English Version:

https://daneshyari.com/en/article/10765434

Download Persian Version:

https://daneshyari.com/article/10765434

Daneshyari.com