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

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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.

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

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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

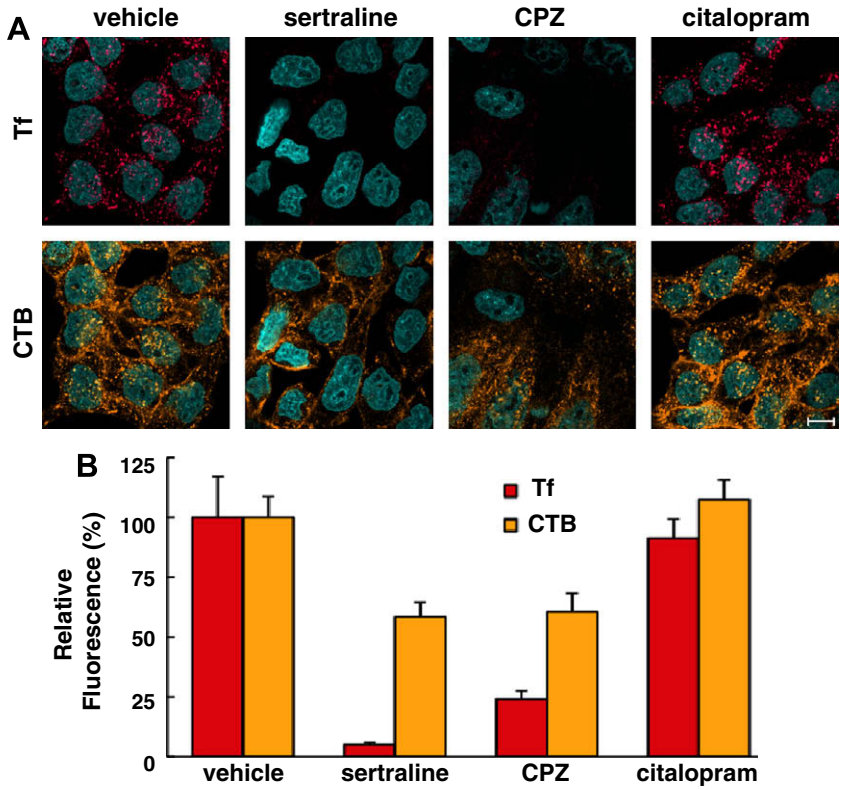

Fig. 1. Sertraline selectively blocks internalization of Tf in HeLa cells. Inhibition of endocytosis markers by sertraline in HeLa cells. (A) HeLa cells were treated for 30 min at 37 °C with vehicle, 20 μM sertraline, 20 μM CPZ or 20 μM citalopram. Upper panels, internalization of Tf (red); lower panels, internalization of CTB (orange). Nuclei were stained with DAPI (blue). Scale bar = 10 μm. (B) Relative fluorescence intensity. Internalized markers in the presence of vehicle, sertraline, CPZ and citalopram were measured by application of NIH-ImageJ (vehicle, $n = 13$; sertraline, $n = 19$; CPZ, $n = 13$; citalopram, $n = 25$). Relative fluorescence intensity was calculated in arbitrary units, based on the value for vehicle-treated cells as 100%. Red and orange columns show relative fluorescence intensity of Tf and CTB uptake, respectively. Error bars indicate SEM.

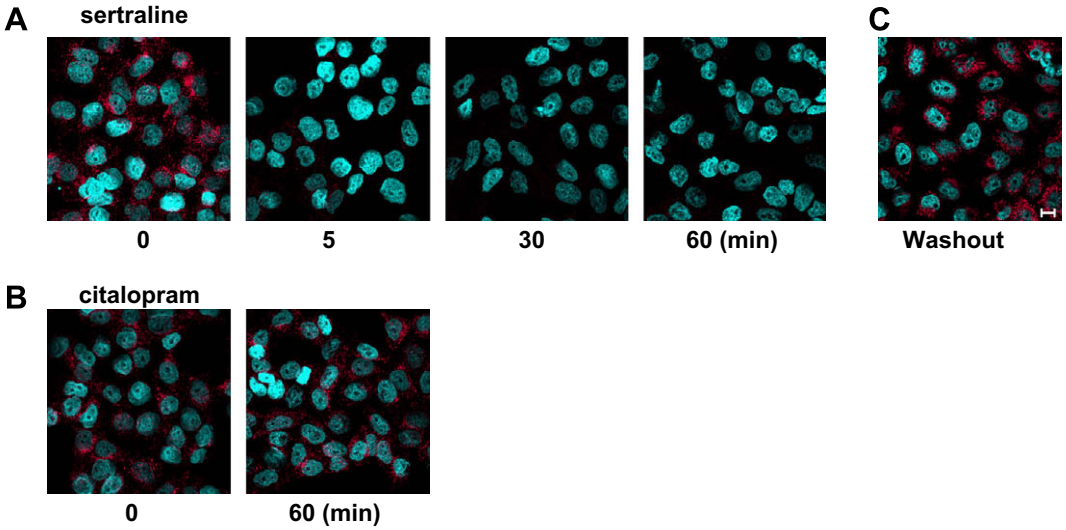

Fig. 2. The inhibitory effect of sertraline on Tf uptake in HeLa cells is reversible. Time course of inhibition of Tf (red) internalization by sertraline or citalopram. (A) Treatment with 20 μM sertraline for 0, 5, 30 or 60 min. (B) Treatment with 20 μM citalopram for 0 and 60 min. (C) Washout procedure: cells were treated with sertraline (30 min, 37 °C) and then with DMEM (20 min, 37 °C) to wash out sertraline. The washed cells were incubated with Alexa-Tf (5 min, 37 °C). Nuclei were stained with DAPI (blue). Scale bar = 10 μm.

the target of sertraline. We found that Dyn 1 was expressed in SH-Sy5Y cell extract, but was undetectable in HeLa cell extract (Fig. 3B). Dyn 2 and clathrin heavy chain (CHC) were expressed in both HeLa and SH-Sy5Y cells, as was β-actin, used as a loading control (Fig. 3B). Since sertraline blocked endocytosis equally effectively in HeLa and SH-Sy5Y cells (Figs. 1A and 3A), these results suggest that the effect of sertraline on endocytosis of Tf is mediated by inhibition of Dyn 2, not Dyn 1, at least in non-neuronal cells.

To investigate whether sertraline inhibits Dyn 2 GTPase activity, the Malachite Green GTPase assay was conducted. Sertraline inhibited Dyn2-His_6 with an IC_{50} value of 3.7 ± 1.3 μM as shown in Fig. 3C. In our previous study, the IC_{50} value of sertraline for Dyn

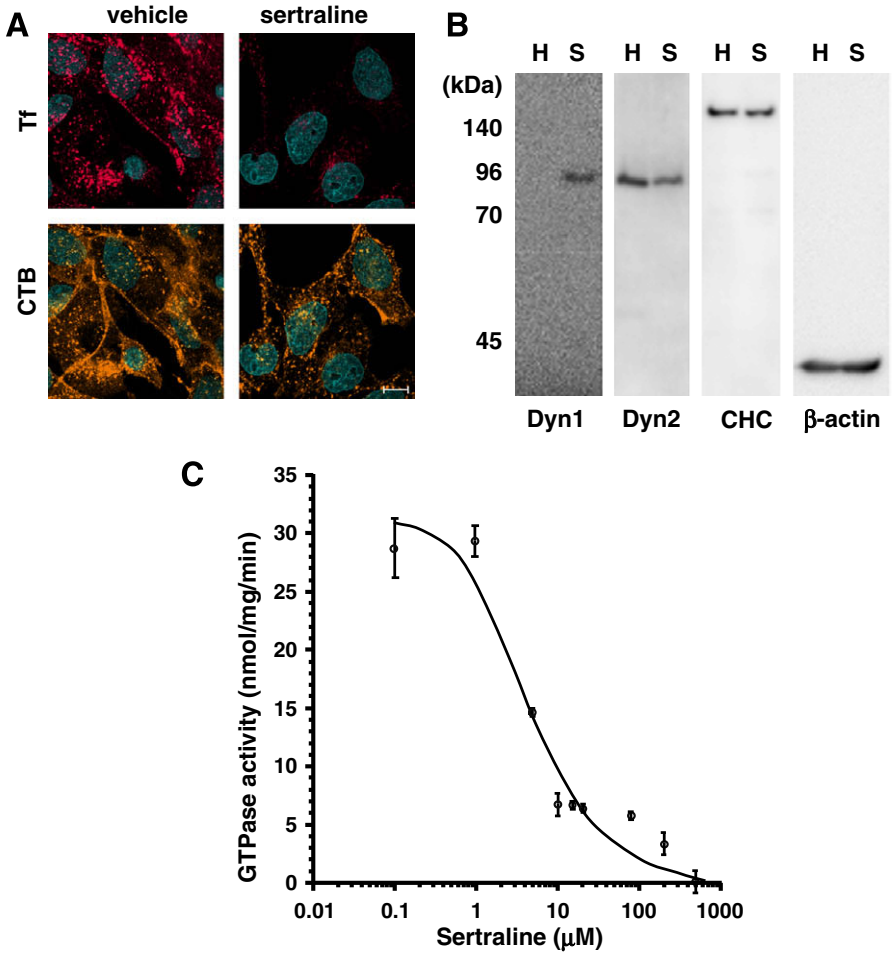

Fig. 3. Sertraline inhibits endocytosis in neuronal cultured cells as well as HeLa cells. (A) Inhibition of endocytosis markers by sertraline in SH-Sy5Y cells. Left panels, vehicle; right panels, 20 μM sertraline; upper panels, internalization of Tf (red); lower panels, internalization of CTB (orange). Nuclei were stained with DAPI (blue). Scale bar = 10 μm. (B) Western blotting for endogenous Dyn 1, Dyn 2 and CHC in HeLa (H) and SH-Sy5Y (S) cells. β-Actin was used as an internal control. (C) Sertraline inhibits the GTPase activity of Dyn 2. PS-stimulated GTPase activity of purified Dyn2-His_6 (100 nM) was determined in the presence of various concentration of sertraline.

1 was 7.3 ± 1.0 μM [20]. These results suggest that sertraline inhibits Dyn 2 GTPase as well as Dyn 1.

Effect of sertraline on endocytosis in HeLa and SH-Sy5Y cells

Dyn 1 K44A, a GTP binding-defective variant [2,16,17], was employed to further examine the mechanism of inhibition of endocytosis by sertraline. We confirmed that purified DynK44A-His_6 did not show GTPase activity *in vitro*, as expected (Supplementary Fig. 1). Then, HeLa and SH-Sy5Y cells were transfected with GFP-Dyn1 *wt* or K44A to investigate whether expression of the GTP binding-defective variant would influence endocytosis. Transient transfection of K44A suppressed the internalization of endocytosis markers, while transient transfection of *wt* had no effect (Fig. 4A and B). These findings correspond well with a previous report that the proline-rich domain peptide of Dyn 1 disrupts the interaction between Dyn and amphiphysin and that the PRD peptide of Dyn 1 inhibits Dyn-dependent endocytosis [15]. These results suggest that K44A variant-expressing cells can be used as a negative control of dynamin-dependent endocytosis.

The transfection of Dyn 1 K44A inhibited 83.0 ± 3.2% of Tf uptake (Fig. 4C, column 5 versus 1) and 63.1 ± 6.6% of CTB uptake (Fig. 4C, column 6 versus 2) by HeLa cells. In SH-Sy5Y cells, the corresponding values were 49.2 ± 6.9% of Tf uptake (Fig. 4C, column 9 versus 1) and 22.1 ± 11.2% of CTB uptake (Fig. 4C, column 10 versus 2). In both cell types, Tf uptake was reduced more markedly than CTB uptake (Fig. 4C, column 5 versus 6 and column 9 versus 10). On the other hand, sertraline inhibited 94.8 ± 0.8% of Tf uptake (Fig. 4C, column 7 versus 1) and 50.8 ± 7.7% of CTB uptake (Fig. 4C, column 8 versus 2) in HeLa cells. In SH-Sy5Y cells, the corresponding values were 80.0 ± 3.7% (Fig. 4C, column 11 versus 1) and 35.9 ± 7.5% (Fig. 4C, column 12 versus 2), respectively. The Tf uptake was much more markedly reduced than the CTB uptake in both cell lines (Fig. 4C, column 7 versus 8 and column 11 versus 12). In contrast, the transfection of Dyn 1*wt* did not inhibit Tf or CTB uptake (Fig. 4C, column 3 versus 1, column 4 versus 2).

Sertraline has little effect on the interaction between PHD of Dyn 1 and PS-liposomes

SPR analyses were performed to investigate whether sertraline inhibits the interaction between Dyn 1 and PS-liposomes. Dyn-His_6 and DynK44A-His_6 showed specific interactions with PS-liposomes in the absence of sertraline, unlike DynΔPHD-His_6. Moreover, addition of 50 μM sertraline had little effect on the binding of PS-liposomes to either Dyn-His_6 or DynK44A-His_6 (Supplementary Fig. 2). These results show that the PHD of Dyn 1 is necessary for binding to PS-liposomes and that sertraline does not directly affect the active site of Dyn 1 GTPase.

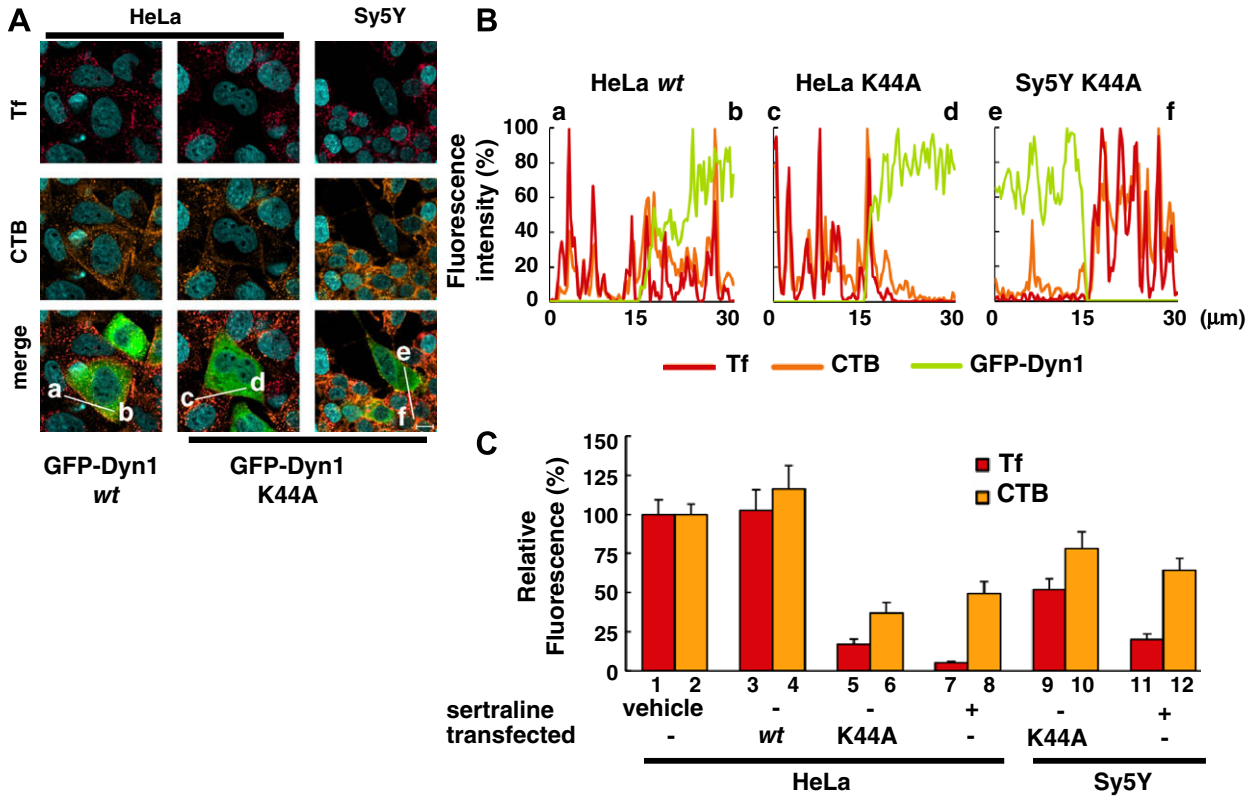

Fig. 4. Overexpression of Dyn 1 K44A inhibits internalization of Tf. (A) Endocytosis was inhibited by overexpression of Dyn 1 K44A in HeLa and SH-Sy5Y cells. Left panels, Dyn 1 *wt*-expressing HeLa cells; center panels, Dyn 1 K44A-expressing HeLa cells; right panels, Dyn 1 K44A-expressing SH-Sy5Y cells; Upper panels, internalization of Tf (red); center panels, internalization of CTB (orange); lower panels, GFP-Dyn 1 *wt* or K44A (light green) merged with CTB and Tf. Nuclei were stained with DAPI (blue). Scale bar = 10 μm. (B) Fluorescence intensity of endocytosis markers and GFP-Dyn 1 (*wt* or K44A). Letters (a–f) in both graphs correspond to lines a–b, c–d and e–f in (A). Red line, Tf; orange line, CTB; green line, GFP-Dyn1. (C) Relative fluorescence intensity. Internalized markers was measured with NIH-ImageJ from the images in Figs. 1A, 3A, and 4A (vehicle, $n = 40$; Dyn 1*wt*-expressing HeLa cells, $n = 15$; K44A-expressing HeLa cells, $n = 19$; sertraline-treated HeLa cells, $n = 13$; K44A-expressing SH-Sy5Y cells, $n = 12$; sertraline-treated SH-Sy5Y cells, $n = 10$). Relative fluorescence intensity was calculated in arbitrary units, based on the value for column 1 or 2 (vehicle) as 100%. Red and orange columns show Tf and CTB uptake, respectively, in terms of relative fluorescence intensity. Error bars indicate SEM.

Discussion

We previously reported that Dyn 1 GTPase activity is inhibited by sertraline *in vitro* [20]. PS-liposomes were used instead of plasma membrane, and membrane protein was not employed in our previous GTPase assay. Here, we examined the effect of sertraline on Tf and CTB uptake in cultured cells. It has been reported that Tf is internalized by receptor-mediated, clathrin- and Dyn-dependent endocytosis [17,27,28]. It is known that the endocytic pathway of CTB involves lipid-raft-dependent internalization *via* glycosphingolipid binding [29]. In this study, internalization of Tf was blocked by 20 μM sertraline in HeLa cells (Fig. 1A and B) and SH-Sy5Y cells (Fig. 3A). Accordingly, we concluded that sertralin may act as an inhibitor of Dyn-dependent endocytosis in both neuronal and non-neuronal cultured cells.

In our previous study, only Dyn 1 was employed for *in vitro* GTPase assay. Thus, it remained unknown whether sertraline inhibits other Dyn isoforms. Here, we found that sertraline inhibited endocytosis in HeLa cells (Fig. 1), which contain Dyn 2, but lack endogenous Dyn 1 (Fig. 3B). Furthermore, sertraline inhibited Dyn 2 GTPase activity *in vitro* (Fig. 3C). These results indicate that sertraline inhibited Dyn 2 GTPase in HeLa cells. Thus, our results indicate that sertraline suppresses Dyn-dependent endocytosis *via* inhibition of both Dyn 1 and Dyn 2 GTPase activities. Moreover, inhibition of Tf uptake by sertraline in HeLa cells was different from that in SH-Sy5Y cells (Fig. 4C, columns 7 versus 11). This difference may be a consequence of the difference in expression of Dyn isoforms between the two cell lines.

Sertraline inhibited Tf uptake within 5 min (Fig. 2A). The inhibition lasted for more than 60 min (Fig. 2A) and could be reversed by washing the cells (Fig. 2C). These results indicate that sertraline is a rapid, continuous and reversible inhibitor of Dyn-dependent endocytosis of Tf in HeLa cells. In this study, SPR analysis showed that sertraline had little effect on the interaction between the PHD of Dyn 1 and PS-liposomes (Supplementary Fig. 2). The results of SPR analysis are consistent with our previous finding that sertraline is not a competitive inhibitor with respect to PS-liposomes [20]. Sertraline is not a long-chain compound, and therefore is not expected to interfere with liposome formation or to disrupt the membrane structure of cultured cells.

It is known that Dyn 1 K44A is a variant deficient in GTP binding [2,16,17]. There are two possible pathways through which Dyn 1 K44A expression may cause inhibition of endocytosis. First, Dyn 1 K44A has the *PXXP* motif, like Dyn 1 *wt*, at the C-terminus. It is well known that the *PXXP* motif of Dyn 1 interacts with SH3 domain-containing proteins such as amphiphysin [13]. Hence, Dyn 1 K44A may compete with endogenous Dyn for binding to SH3 domain-containing proteins *in vivo* [15]. Second, Dyn 1 K44A showed no GTPase activity *in vitro* (Supplementary Fig. 1), in agreement with several previous reports [2,16,17]. Moreover, it was reported that tetramerization of Dyn is necessary for GTPase activity [12]. Therefore, Dyn 1 K44A may act as an inhibitor of endocytosis by forming inactive tetramers with Dyn 1 *wt* [10]. The transfection of Dyn 1 K44A indeed reduced Tf uptake in HeLa and SH-Sy5Y cells, respectively, but sertraline resulted in greater reductions of Tf uptake (Fig. 4C). These differences between the effects of Dyn 1 K44A

transfection and sertraline on internalization of Tf and CTB indicate that different mechanisms of inhibition of endocytosis are involved.

Although citalopram is a SSRI, like sertraline [21,26], it did not inhibit the uptake of Tf or CTB (Fig. 1A and B). This result may be consistent with the previous finding that citalopram did not influence the regulation of norepinephrine [30]. The uptake of Tf was more markedly reduced by sertraline than by CPZ, whereas sertraline inhibited the uptake of CTB as effectively as did CPZ in HeLa cells (Fig. 1B). It is well known that CPZ is a clathrin-dependent endocytosis inhibitor [25]. The difference between sertraline and CPZ in the inhibition of Tf uptake is consistent with our previous report that the IC_{50} values of sertraline and CPZ for Dyn 1 GTPase were 7.3 ± 1.0 and 47.2 ± 23.1 μM, respectively [20]. Thus, it might appear that the difference between inhibition of Tf uptake by sertraline and CPZ can be attributed to the difference of inhibitory potency towards Dyn 1 GTPase. In conclusion, this report presents evidence that sertraline suppresses endocytosis in cultured cells.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2009.11.067.

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