The neurotransmitter DA plays a critical role in a number of physiological processes mediated by the central nervous system (CNS), e.g., locomotion, cognition, and reward. Clearance of DA from the synaptic cleft is primarily accomplished via reuptake of DA by presynaptic DATs localized on the plasma membrane of dopaminergic neurons. The DAT is a member of a superfamily of neurotransmitter transporters, all of which have 12 putative transmembrane domains and translocate neurotransmitters, or other substrates, into cells against their concentration gradients by coupling transport of the neurotransmitter to the cellular electrochemical Na⁺/Cl⁻ gradient (Amara and Kuhar, 1993). Other members of this superfamily include transporters for γ-aminobutyric acid (GAT1), serotonin (SERT), norepinephrine (NET), glycine, glutamate, GABA, and dopamine (Amara and Kuhar, 1993; Giros and Caron, 1993). Since the DAT plays a crucial role in controlling the concentration of extracellular DA, elucidating mechanisms by which DAT activity is regulated is vital to understanding how dopaminergic neurotransmission in the CNS is controlled.

Many of the transporters in this Na⁺/Cl⁻-dependent neurotransmitter transporter superfamily, including DAT, are subject to acute regulation by signaling cascades that often involve activation of cellular protein kinases (Reith et al., 1997; Beckman and Quick, 1998; Blakely and Bauman, 2000). For example, activation of protein kinase C (PKC) by bath-applied phorbol esters down-regulates the number of cell surface, and thus activity of NETs, DATs, and SERTs (Qian et al., 1997; Apparsundaram et al., 1998; Beckman et al., 1998). Likewise, PKC-mediated down-regulation of DAT function has been consistently observed in rat striatal synaptosomes (Copeland et al., 1996) and heterologous expression systems (Kitayama et al., 1994; Huff et al., 1997; Zhang et al., 1997; Pristupa et al., 1998), including Xenopus oocytes expressing the hDAT (Zhu et al., 1997). Recent studies have revealed that PKC-mediated inhibition of DAT activity also...
occurs via increased transporter endocytosis (Daniels and Amara, 1999; Melikian and Buckley, 1999).

Although PKC-induced transporter regulation is well-established, it is not yet clear whether the DAT is acutely regulated by other protein kinases, such as PTKs. PTKs are a vital component in intracellular signaling, and well over a thousand human genes encode for PTKs (for review, see Fruman et al., 1998). Receptor PTKs (RPTKs) are transmembrane receptors with intrinsic tyrosine kinase activity (for review, see van der Geer and Hunter, 1994). RPTKs are well known for controlling cellular growth and differentiation, but they also regulate many other cellular programs. In addition, there are at least nine families of nonreceptor PTKs. Such families include Jak and Src family PTKs (Neet and Hunter, 1996).

Tyrosine kinases appear to regulate Na+/Cl−-dependent neurotransmitter transporters via several different molecular mechanisms. Prasad et al. (1997) have shown that tyrosine phosphorylation indirectly up-regulates gene expression of hSERT in JAR placental choriocarcinoma cells. Furthermore, PTKs can alter transporter trafficking. Acute inhibition of PTKs attenuates GAT1 activity concomitant with a redistribution of the transporter away from the cell surface of hippocampal neurons (Law et al., 2000). Conversely, insulin, which activates RPTKs, increases the maximal velocity of NET in SK-N-SH cells (Apparsundaram and Blakely, 1997). In contrast, brain-derived growth factor (BDNF) dose dependently reduces hSERT activity in certain immortalized B-lymphocyte cell lines, whereas nerve growth factor is without effect (Mössner et al., 2000). Striatal dopaminergic neurons possess a number of RPTKs that represent potential targets for growth factor-mediated regulation of the DAT. These include RET receptors for glial cell line-derived factor, trkA receptors for nerve growth factor, trkB receptors for BDNF and neurotrophin-4/5, and trkC receptors for neurotrophin-3 (Hymen et al., 1994; Eggert et al., 1999; Numan and Seroogy, 1999; Taraviras et al., 1999). Pharmacological manipulation with PTK inhibitors suggests the involvement of PTKs in the acute regulation of DA uptake in mouse striatum (Simon et al., 1997). However, the mechanism by which this regulation occurs and the specific PTK(s) involved is not known.

Here, we used PTK inhibitors to test the hypothesis that PTKs acutely regulate DAT activity by altering the number of DATs present on the cell surface. Our results show that inhibition of PTKs, other than Src-family kinases, rapidly decreased hDAT function in Xenopus oocytes. Our data are also consistent with the idea that inhibition of PTKs produces a redistribution of hDATs away from the cell surface. Thus, PTKs can rapidly alter the number of functional DATs and thereby may play a role in acutely regulating dopaminergic neurotransmission.

Experiment Procedures

Frogs and Oocyte Preparation. Female X. laevis frogs were purchased from either Nasco (Ft. Atkinson, WI) or Xenopus I (Ann Arbor, MI). All animal use procedures were in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee, University of Colorado Health Sciences Center. Stage V and VI X. laevis oocytes were defolliculated by gentle shaking in OR2 buffer (82 mM NaCl, 2.5 mM KCl, 1.0 mM MgCl2, 5 mM HEPES, pH 7.5) containing 1.4 to 2.0 mg/ml collagenase B (Boehringer-Mannheim, Indianapolis, IN) for 1 to 2 h at room temperature.

hDAT cRNA Preparation and Oocyte Expression. Capped cRNA was transcribed from a linear oocyte expression vector pOTV containing the 1.9-kb hDAT cDNA insert (Sonders et al., 1997) using mMessage mMachine with TT polymerase (Ambion, Austin, TX). Oocytes were injected with water-diluted cRNA (~10 ng) and maintained at room temperature in frog Ringer’s buffer (FRB; 96 mM NaCl, 2 mM KCl, 1.8 mM CaCl2, 1 mM MgCl2, and 5 mM HEPES, pH 7.5) supplemented with 2.5 mM sodium pyruvate, 0.5 mM theophylline, 100 U/ml penicillin, 100 μg/ml streptomycin, and 50 μg/ml gentamycin for 3 to 5 days.

DA Uptake. Individual hDAT-expressing oocytes were placed in a 0.5-mL bath and superfused at a rate of approximately 2 mL/min with FRB at room temperature. For uptake measurements, oocytes were either maintained at resting potential or voltage clamped at −80 mV; similar results were obtained using either protocol. Each oocyte was superfused with a relatively high concentration of DA (10 μM) for 3 min. In experiments testing PTK inhibitors, the inhibitors were superfused for approximately 5 min before DA treatment and were present throughout the DA exposure. After the 3-min DA exposure, the oocytes were quickly washed three times in FRB, transferred to 0.8 ml of 2 mM perchloric acid, and stored for not more than 2 weeks at −4°C. Oocytes were sonicated in the perchloric acid, and the suspension was filtered through 0.22-μm nitro. Intracellular DA accumulation was determined in the filtrate by high performance liquid chromatography using electrochemical detection. Retention times of standards were used to identify peaks, and absolute DA content in samples was determined by comparison to the area of standard peaks. Minimum detection for DA was 0.5 pg/injection. Nonspecific DA uptake was determined in water-injected oocytes and was less than 1% of hDAT-injected oocytes.

Two-Electrode Voltage-Clamp Recording. Currents were measured in oocytes using two-electrode voltage clamp; microelectrodes were filled with 3 M KCl (Sonders et al., 1997). A Warner OC-725B amplifier (Warner Instruments, Hamden, CT) was used with a DigiData 1200 interface. pClamp6 software (Axon Instruments, Foster City, CA) was used to control stimulation parameters, for data acquisition, and for analysis. MacLab data acquisition software (AD Instruments, Castle Hill, Australia) and a MacLab/2e interface were simultaneously used to monitor experiments. Currents were low-pass filtered at 100 Hz and digitized at 2048 Hz.

Oocytes were superfused in a 0.5 mL bath at 2 to 3 mL/min with FRB at room temperature. In experiments designed to measure only hDAT-associated leak currents, oocytes were superfused with FRB containing 96 mM LiCl, instead of 96 mM NaCl. Oocytes were voltage clamped at −60 mV and then subjected to a series of 400-ms steps in membrane potential ranging from −120 mV to +40 mV in 10-mV increments. Currents were recorded before and again 1 min after superfusion with 10 μM tyramine or 100 μM R-(+)-3-(3-hydroxyphenyl)-N-propylpipеридине (3-PPP). Steady-state currents were measured and averaged during the last 100 ms of each voltage step. Inward currents induced by the DA substrate tyramine were measured by performing off-line subtraction of currents measured in the absence of drug from those measured in the presence of drug (I_{brug} − I_{buffer}). In experiments using the DAT inhibitor 3-PPP to measure inhibition of inward hDAT-associated leak currents, outward 100 μM 3-PPP-induced currents were measured by performing off-line subtraction of the currents measured in the presence of drug from those measured in the absence of drug (I_{buffer} − I_{brug}). The effects of PTK inhibitors were determined on hDAT-mediated currents by superfusing the inhibitor for 3 to 5 min before addition of either tyramine or 3-PPP and continuing the drug superfusion during the voltage steps. Currents from each oocyte were normalized to the hDAT current measured at −120 mV.

Radioligand Binding to hDAT. hDAT binding sites were quantitated in intact oocytes by superfusion in a 0.5-mL bath with FRB
containing an approximate $K_i$ concentration of 2β-carbomethoxy-3β-(4-fluorophenyl)[3H]tropane ([3H]WIN 35,428; specific activity 84.5 Ci/mmol; 4 nM) at room temperature for 20 min. Oocytes were then quickly washed three times in FRB, dissolved in 0.25 ml of 2% SDS. Radioactivity was measured by liquid scintillation spectroscopy. Nonspecific binding was determined in the presence of 1 μM GBR 12909 and was 50 ± 13% of total binding.

Data Analysis. Apparent values for $V_{max}$, $K_m$, and $K_i$ were determined using nonlinear regression analysis (GraphPad Prism, San Diego, CA). For statistical analysis, transport-associated currents were compared at ~80 mV, whereas leak currents were compared at +20 mV. Statistical significance was determined by unpaired Student’s t test with a significance criterion of $P < 0.05$.

Materials. Lavendustin A and GBR 12909 were purchased from Sigma/Research Biochemicals International (St. Louis, MO). PP2 was a gift from Dr. Alexander Sorkin (University of Colorado Health Sciences Center, Denver, CO) or purchased from Calbiochem (La Jolla, CA). [3H]DA and [3H]WIN 35,428 were purchased from NEN Life Science Products (Boston, MA). All other drugs were purchased from Sigma (St. Louis, MO).

DA Uptake. We used several PTK inhibitors, namely, genistein, lavendustin A, tyrphostin 25, and PP2, to test our hypothesis that PTKs acutely regulate DAT function by altering cell surface expression of the transporter. Numerous PTK inhibitors are available that can be used as tools to characterize the effects of PTKs (for review, see Lawrence and Niu, 1998). The flavonoid genistein, which exhibits little specificity, is often used as a general inhibitor of protein kinases. The potency for inhibition of the epidermal growth factor receptor (EGFR) is one basis for comparison among PTK inhibitors. Both genistein and the erbsstatin analog PTK inhibitor tyrphostin 25 inhibit the EGFR kinase activity with an $IC_{50}$ = ~3 μM. The addition of a glucoside to genistein results in its inactive analog, genistin. Lavendustin A is a more selective PTK inhibitor, with little effect on either protein kinase A or PKC ($IC_{50}$ > 200 μM), and has a substantially higher affinity for the EGFR ($IC_{50}$ = 11 nM). PP2 is a potent inhibitor of the Src family of PTKs (Hanke et al., 1996).

To investigate possible effects of PTKs on DAT function, we first measured DA uptake into individual oocytes expressing the hDAT in the absence or presence of these PTK inhibitors. Oocytes were pre-exposed to the PTK inhibitors for approximately 5 min, and exposure to the inhibitors was continued during the 3-min incubation with 10 μM DA. This is a relatively high concentration of DA and, therefore, changes in uptake are more likely to reflect changes in uptake capacity, rather than affinity. Effects of PTK inhibitors were much more robust and consistent when oocytes were superfused, rather than incubated in a static bath. Although we do not understand the mechanism(s) underlying this observation, all experiments reported here were conducted using superfusion. The velocity of DA uptake in control oocytes was 3.06 ± 0.45 fmol/sec/oocyte ($N$ = 26 oocytes from four batches of oocytes; Fig. 1). Exposure to genistein inhibited DA uptake into oocytes in a concentration-dependent manner with an apparent $K_i$ value of 68 nM (pK$_i$ = 7.17 ± 0.22 M; $N$ = 15–26 oocytes from four batches of oocytes). At the highest concentration of genistein tested (10 μM), the velocity of DA uptake was 58 ± 13% of control. Similarly, two of the other PTK inhibitors tested significantly inhibited the velocity of 10 μM DA uptake into hDAT-expressing oocytes (Fig. 2). Lavendustin A (10 μM) reduced uptake to 41 ± 6% of control ($N$ = 17–18 oocytes from three batches of oocytes), and tyrphostin 25 (10 μM) reduced uptake to 30 ± 7% of control ($N$ = 17–19 oocytes from three batches of oocytes). In contrast, the potent Src PTK inhibitor PP2 (1 μM; Hanke et al., 1996) did not significantly inhibit the velocity of 10 μM DA uptake (Fig. 2).

To confirm that the decreases in uptake velocities induced by the PTK inhibitors reflected reductions in DAT $V_{max}$, direct kinetic analysis of DA uptake was conducted in the absence or presence of 10 μM genistein (Fig. 3). This analysis showed that the $K_m$ was not different between control (7.8 μM; pK$_m$ = 5.1 ± 0.18 M) and genistein-treated oocytes (4.7 μM; pK$_m$ = 5.3 ± 0.30 M; $N$ = 11–17 oocytes from three batches of oocytes). However, the $V_{max}$ was decreased by 50% in the presence of 10 μM genistein (2.8 ± 0.5 fmol/sec/oocyte) compared with control (5.6 ± 0.7 fmol/sec/oocyte). Thus, the magnitude of the inhibition of the DAT $V_{max}$ by 10 μM genistein measured with the full kinetic analysis was similar to that seen in Fig. 1 (58 ± 13% of control) measured with the single 10 μM concentration of DA.

hDAT-Associated Currents. DATs generate currents, and these currents provide additional measures by which the functional state of the transporter can be assessed. To investigate possible effects of PTK inhibitors on hDAT-associated currents, we used two-electrode voltage-clamp recording. Although identical results are seen using either DA or tyramine as a substrate in hDAT-expressing oocytes, tyramine was
used here because it is more stable in solution over time. The current-voltage (I-V) relationship produced by DAT substrates in hDAT-expressing oocytes is comprised of at least two separate currents (Sonders et al., 1997). An inward transport current, induced by translocation of the positively charged substrate and Na\(^+\), predominates at more hyperpolarized membrane potentials (\(-120\) to \(-20\) mV). In addition, a smaller outward current is produced by both DAT substrates and inhibitors. This current results from blockade of a constitutively active inward cation leak over all voltages tested (\(-120\) to \(+40\) mV). However, since DAT substrates such as tyramine produce the two opposing currents, blockade of the leak is more readily evident at more depolarized potentials (\(+10\) to \(+40\) mV).

Individual hDAT-expressing oocytes were clamped at \(-60\) mV, and I-V relationships were generated using the voltage jump protocol described under Experimental Procedures. Tyramine-induced currents in oocytes superfused with \(10\) \(\mu\)M genistein were significantly inhibited at hyperpolarized potentials (Fig. 4A). For example, currents measured at \(-80\) mV in the presence of \(10\) \(\mu\)M genistein were only 23 \(\pm\) 19\% of control currents (\(N = 5\) oocytes from three batches of oocytes). However, at more depolarized potentials where blockade of the leak current is more readily observed, genistein did not significantly alter tyramine-induced currents. The inactive analog of genistein, genistin (\(10\) \(\mu\)M), had no effect on transport-associated currents at hyperpolarized membrane potentials but appeared to induce a small change at more depolarized potentials (Fig. 4B). Lavendustin A also significantly inhibited tyramine-induced currents (Fig. 5A). At \(-80\) mV, tyramine-induced currents in the presence of \(10\) \(\mu\)M lavendustin A were 35 \(\pm\) 22\% of control currents (\(N = 9–10\) oocytes from three batches of oocytes). In contrast to the effects of genistein, lavendustin A also significantly inhibited tyramine-induced currents at more depolarized potentials. For example, currents measured at \(+20\) mV were 35 \(\pm\) 7\% of control currents. These results suggest that lavendustin A inhibited not only the transport-associated current, but also the leak current. Tyramine-induced currents were also significantly inhibited by \(10\) \(\mu\)M tyrphostin 25 at hyperpolarized membrane potentials but appeared to induce a small change at more depolarized potentials (Fig. 5B). Similar to genistein, the predominant effect of tyrphostin 25 was to inhibit the transport-associated current. At \(-80\) mV currents in the presence of tyrphostin 25 were 50 \(\pm\) 9\% of control currents (\(N = 9–10\) oocytes from three batches of oocytes), whereas tyrphostin 25 only slightly decreased currents at depolarized potentials (currents at \(+20\) mV were 77 \(\pm\) 16\% of control currents). The Src inhibitor PP2 (\(10\) \(\mu\)M) had no measurable effect on hDAT-associated currents at any voltage tested (Fig. 5C).

To more fully investigate the effect of PTK inhibitors on hDAT-mediated leak currents, currents induced by the DAT inhibitor 3-PPP were measured in LiCl-substituted FRB.
DAT inhibitors such as 3-PPP block the leak current but do not induce the transport-associated current. Li ions are conducted more readily than Na ions via the hDAT cation leak (Sonders et al., 1997). Therefore, the outward current resulting from the block of the leak conductance by 3-PPP is amplified in the LiCl-substituted FRB, compared with currents measured in the presence of NaCl. Currents induced by 100 μM 3-PPP were markedly inhibited in the presence of 10 μM genistein (Fig. 6). 3-PPP-induced currents were 50 to 80% of control at all membrane potentials tested, with the exception of 0 mV where control and genistein curves intersect. The specificity of this effect may be questioned because, as noted above, the inactive analog genistin (10 μM) also appeared to inhibit the leak current (Fig. 4B). Nonetheless, the 3-PPP data in the LiCl-substituted FRB strongly suggest that, in addition to inhibiting transport-associated currents, PTK inhibitors such as genistein inhibit the hDAT-mediated leak current.

[^3H]WIN 35,428 Binding to Cell Surface DATs. A decrease in the Vmax of the DAT with no change in Km suggests that PTK inhibitors regulate DAT function by reducing the number of functional transporters on the cell surface. To address this more directly, we measured[^3H]WIN 35,428 binding to intact hDAT-expressing oocytes in the absence (control) and presence of 10 μM genestein A (A), 10 μM tyrphostin 25 (B), or 1 μM PP2 (C). Data represent mean ± S.E.M. for 9 to 10 oocytes from three batches of oocytes.
that acute PTK inhibition attenuates function of hDAT expressed in oocytes, suggesting that PTKs can rapidly alter DAT activity. Furthermore, several lines of evidence suggest that this regulation occurs via a change in the number of functional DATs on the cell surface. First, three general PTK inhibitors decreased the velocity of DA uptake. Second, DAT transport-associated currents were reduced by a similar magnitude as DA uptake by these PTK inhibitors, but not by an inactive analog. Third, hDAT-associated leak currents were also inhibited by PTK inhibitors, albeit in a more variable manner. Last, genistein inhibited radioligand binding to cell surface hDATs, suggesting that genistein induces a rapid decrease in the number of hDATs present on the cell surface.

Genistein attenuated DA uptake into individual Xenopus oocytes with a decrease in $V_{\text{max}}$ and no change in $K_m$, consistent with a reduction in the number of functional hDATs on the cell surface. This inhibition of DA uptake into oocytes occurred in a concentration-dependent manner with high affinity ($IC_{50} = 68 \, \text{nM}$). Genistein also attenuated hDAT-mediated currents at hyperpolarized membrane potentials where transport-associated currents predominate, whereas the inactive analog genistin had no effect on transport-associated currents. Like genistein, both lavendustin A and tyrphostin 25 significantly inhibited DA uptake and hDAT-mediated currents at hyperpolarized membrane potentials. Among these three general PTK inhibitors (all tested at 10 $\mu$M), genistein had the highest efficacy, producing the greatest inhibition of both uptake and transport-associated currents. Lavendustin A had intermediate efficacy. Tyrphostin 25 produced the smallest effects. Thus, these inhibitors reduced both functional measures of hDAT activity, uptake and transport-associated currents, to similar extents. Interestingly, the potent inhibitor of Src-family kinases PP2 did not inhibit hDAT uptake or currents. Although genistein is a more general protein kinase inhibitor, it appears to elicit changes similar to the more selective PTK inhibitors lavendustin A and tyrphostin 25. Furthermore, if the changes produced by genistein were a result of inhibition of PKC, an increase in DAT activity would be expected. Together, these data suggest that PTKs, other than Src-family PTKs, regulate hDAT function. In support of this hypothesis preliminary data suggest that hDAT function is increased by the tyrosine phosphatase inhibitor orthovanadate (data not shown).

In previous studies using mouse striatal synaptosomes, genistein inhibited DA uptake, but lavendustin A (up to 50 $\mu$M) and tyrphostin 25 (up to 100 $\mu$M) did not (Simon et al., 1997). The difference between these and the present findings may be due to the presence of different PTKs and/or different phosphorylation states of the proteins involved in brain synaptosomes versus oocytes. Alternatively, there may be differences between mouse DAT and hDAT in terms of susceptibility to phosphorylation by PTKs.

Although PTK inhibitors altered both DA uptake and transport-associated currents with a similar order of potency, their effects were more variable at depolarized membrane potentials where the constitutive leak current is predominant. In normal FRB hDAT-mediated leak currents were significantly inhibited by lavendustin A but not by genistein. The results with genistein suggest that despite decreases in uptake velocity and transport-associated currents, the transporter may still be present on the cell surface and/or that...
heterogeneous pools of hDAT proteins may mediate transport and leak currents. However, these possibilities are unlikely because of our results using LiCl-substituted buffer and the DAT inhibitor 3-PPP, the most definitive way to amplify leak currents and to study them in isolation from transport currents. In these experiments, genistein clearly reduced the magnitude of the leak current, consistent with the idea that there are fewer functional transporters on the cell surface.

It is not completely unexpected that identical PTK inhibitor-induced changes in the transport-associated and leak currents were not observed here. Substrates clearly have differential effects on transport-associated and leak currents; substrates increase transport-associated current, whereas they decrease leak currents (Sonders et al., 1997; Sitte et al., 1998). Studies in our laboratory using hDAT-expressing oocytes have shown an apparent discrepancy between changes in hDAT function and leak currents following pharmacological manipulation (Mayfield and Zahniser, 2000). Furthermore, a difference in ion selectivity between transport-associated and leak currents suggests multiple permeation pathways within a single transporter (Sonders and Amara, 1996). Thus, it is plausible that the different PTK inhibitors have unique effects on leak current permeation in the hDATs remaining on the cell surface and therefore, the magnitude of the leak current may not reflect the actual number of cell surface hDATs after PTK inhibitor treatment.

According to Michaelis-Menten kinetics, a decrease in $V_{\text{max}}$ with no change in $K_m$ may be the result of a reduction in the number of active transporters. The simplest explanation for this would be that PTK inhibition decreases the number of active hDATs on the cell surface. The genistein-mediated decrease in $[^3H]WIN\,35,428$ binding to cell surface hDATs supports this conclusion. Changes in membrane potentials or ion gradients could also cause a decrease in $V_{\text{max}}$. We ruled out altered membrane potentials by performing all of our electrophysiological and some of our uptake measurements under voltage clamp. Also, we observed no change in resting membrane potential in unclamped oocytes after genistein treatment (data not shown), suggesting that genistein does not alter ion gradients. Although it is possible that PTK inhibitors could induce a conformational change that altered the binding site such that binding was decreased but no change in the number of cell surface DATs occurred, the PTK inhibitor-induced reduction in $[^3H]WIN\,35,428$ binding and functional DAT measures collectively suggest a redistribution of active hDATs away from the cell surface.

PKC activation similarly decreases DAT $V_{\text{max}}$ with no change in $K_m$, the hDAT-associated transport current and hDAT cell surface binding (Kitayama et al., 1994; Huff et al., 1997; Zhang et al., 1997; Zhu et al., 1997; Pristupa et al., 1998). PTK inhibition of hDAT activity could occur via increased endocytosis, similar to the mechanism by which PKC activation regulates hDAT activity (Daniels and Amara, 1999; Melikian and Buckley, 1999). Law et al. (2000) recently found that acute exposure to PTK inhibitors (genistein and K252a) attenuates the function of GAT1 in hippocampal neurons via a redistribution of the transporter away from the cell surface. Since both GAT1 and DAT belong to the same superfAMILY of Na$^+$/Cl$^-$-dependent neurotransmitter transporters, it is possible that PTK inhibitors regulate GAT1 and DAT by a common mechanism. Interestingly, however, the effects of PTK inhibitors and PKC activators on GAT1 function were additive, suggesting multiple independent pathways accomplish down-regulation of the transporter by these different protein kinases.

Although we have shown that PTK inhibitors can rapidly regulate hDAT, the identity of the PTK(s) involved is not yet known. We have ruled out the involvement of Src family kinases as the potent Src inhibitor PP2 did not alter hDAT function. PTK-mediated regulation of GAT1 is produced in hippocampal neurons by BDNF (Law et al., 2000). Thus, although we cannot rule out numerous receptor and cytosolic PTKs, it is conceivable that DAT regulation can occur in dopaminergic neurons via BDNF activation of trkB receptors. However, further studies using neurons must be conducted to confirm this hypothesis.

Whether PTK inhibition regulates hDAT function by altering the tyrosine phosphorylation state of the hDAT itself or another protein involved in hDAT trafficking is also not known. A correlation between PKC-mediated direct phosphorylation of the hDAT and decreased transport $V_{\text{max}}$ has been observed (Huff et al., 1997). Furthermore, the PTK-mediated redistribution of GAT1 away from the cell surface is associated with increased phosphorylation of the transporter (Law et al., 2000). However, a previous study examining overall phosphotyrosine levels in mouse striata did not detect an effect of genistein on the amount of phosphoproteins from 60 to 80 kDa, where DAT would have been expected (Simon et al., 1997). Thus, although more sensitive methods may ultimately reveal direct tyrosine phosphorylation of hDAT, it is not yet clear whether PTK inhibitors regulate hDAT function by decreasing hDAT phosphorylation. Alternatively, PTK inhibitors may regulate hDAT function by inhibiting the phosphorylation state of another protein that interacts with the hDAT. Indeed, the same study revealed differences in the level of unidentified 110- and 180-kD phosphoproteins (Simon et al., 1997). Furthermore, GAT1 trafficking is influenced by the phosphorylation state of Munc18, a syntaxin 1A binding partner, which regulates the association of syntaxin 1A with the GAT1 (Beckman et al., 1998).

Post-translational modulation of DAT is of physiological and therapeutic importance. Growth factors well known for controlling cell cycle differentiation and survival of dopaminergic neurons may also participate in acute control of dopaminergic neuronal function. If receptor PTK inhibition produces down-regulation of DAT function, stimulation of these kinases by growth factors may acutely increase DAT activity and DA clearance, thereby transiently decreasing synaptic activity. Understanding regulation of the DAT will provide new insights regarding normal dopaminergic function. This will ultimately enhance our understanding of pathological conditions in which DA is implicated, such as Parkinson’s disease, schizophrenia, and drug addiction.

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