Dopamine transporters (DATs) are members of a family of Na\textsuperscript{+}- and Cl\textsuperscript{−}-dependent neurotransmitter transporters responsible for the rapid clearance of dopamine from synaptic clefts. The predicted primary sequence of DAT contains numerous consensus phosphorylation sites. In this report we demonstrate that DATs undergo endogenous phosphorylation in striatal synaptosomes that is regulated by activators of protein kinase C. Rat striatal synaptosomes were metabolically labeled with \[^{32}\text{P} \]orthophosphate, and solubilized homogenates were subjected to immunoprecipitation with an antisera specific for DAT. Basal phosphorylation occurred in the absence of exogenous treatments, and the phosphorylation level was rapidly increased when synaptosomes were treated with the phosphatase inhibitors okadaic acid or calyculin. Treatment of synaptosomes with the protein kinase C activator phorbol 12-myristate 13-acetate (PMA) also increased the level of phosphate incorporation. This occurred within 10 min and was dose-dependent between 0.1 and 1 \(\mu\)M PMA. DAT phosphorylation was also significantly increased by two other protein kinase C activators, (−)-indolactam V and 1-oleoyl-2-acetyl-sn-glycerol. The inactive phorbol ester 4α-phorbol 12,13-didecanoate at 10 \(\mu\)M was without effect, and PMA-induced phosphorylation was blocked by treatment of synaptosomes with the protein kinase C inhibitors staurosporine and bisindolylmaleimide. These results indicate that DATs undergo rapid in vivo phosphorylation in response to protein kinase C activation and that a robust mechanism exists in synaptosomes for DAT dephosphorylation. Dopamine transport activity in synaptosomes was reduced by all treatments that promoted DAT phosphorylation, with comparable dose, time, and inhibitor characteristics. The change in transport activity was produced by a reduction in \(V_{\text{max}}\) with no significant effect on the \(K_{\text{m}}\) for dopamine. These results suggest that synaptosomal dopamine transport activity is regulated by phosphorylation of DAT and present a potential mechanism for local neuronal control of synaptic neurotransmitter levels and consequent downstream neural activity.

Dopamine transporters (DATs)\(^1\) are integral membrane neuronal proteins that function to terminate dopaminergic neurotransmission by the rapid reuptake of synaptic dopamine into presynaptic neurons. As the primary mechanism for the clearance of synaptic dopamine, DAT is the main determinant that regulates the intensity and duration of dopaminergic neurotransmission (1). DAT is implicated in the etiology of psycho-stimulant drug abuse, as binding of cocaine and amphetamine to the protein inhibit dopamine transport (2), and the resulting elevation of synaptic dopamine levels is believed to underlie the reinforcing properties of these drugs (3, 4). DAT is also a dopaminergic-specific mode of entry for the neurotoxins 6-hydroxydopamine and 1-methyl-4-phenylpyridinium (5, 6), implicating it in mechanisms of neurotoxicity that serve as the best current models for Parkinsonian neurodegeneration.

DAT is a member of a large class of neurotransmitter and amino acid transporters, including carriers for norepinephrine, serotonin, \(\gamma\)-amino butyric acid, glycine, proline, taurine, and betaine, which drive reuptake of transmitter by cotransport of Na\textsuperscript{+} and Cl\textsuperscript{−} down electrochemical gradients (7–9). Molecular cloning of DAT demonstrates the presence of 12 potential transmembrane domains, with a presumed topology orienting the N and C termini intracellularly. DAT and the other proteins in this group are extensively glycosylated, and their sequences contain numerous consensus phosphorylation sites for PKA, PKC, and Ca\textsuperscript{2+}-calmodulin kinase (10–14).

The presence of potential phosphorylation sites on these proteins suggests that they may be subject to phosphorylation-induced functional regulation, and several studies have shown that transport of neurotransmitters is affected by protein kinase activators. Treatment of striatal synaptosomes and heterologous expression systems with phorbol esters or other protein kinase activators reduces dopamine transport activity of mouse, rat, and human DATs (15–18). Activation of arachidonic acid pathways can also decrease hDAT activity (19), whereas elevated striatal dopamine uptake occurs after treatment with Ca\textsuperscript{2+} pathway activators (20). PMA also regulates activity of several other neurotransmitter transporters, including glutamate transporters expressed in in cultured glia and transfected HeLa cells (21), \(\gamma\)-amino butyric acid transporters expressed in \(Xenopus\) oocytes (22), and glycine transporters expressed in cultured embryonic kidney cells (23). Serotonin transport can also be acutely modulated by calmodulin in placent al choriocarcinoma cells (24), PMA in HEK 293 cells (25), and cGMP and nitric oxide in rat basophilic leukemia cells (26).

\(^{1}\) The abbreviations used are: DAT, dopamine transporter; DA, dopamine; PMA, phorbol 12-myristate 13-acetate; 4αPDD, 4α-phorbol 12,13-didecanoate; OA, okadaic acid; OAG, 1-oleoyl-2-acetyl-sn-glycerol; PKC, protein kinase C; PKA, protein kinase A; \[^{125}\text{I}\]DEEP, \[^{125}\text{I}\]-1-[2-(diphenyl methoxy)ethyl]-4-[2-(4-azido-3-iodophenyl)ethyl]piperazine.

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Evidence for the potential involvement of transporter phosphorylation in producing these effects has been obtained only for dopamine and glutamate transporters, which undergo in vivo phosphorylation regulated by PMA in rDAT-LLC-PK1 cells and C6 glioma cells, respectively (16, 21). Purified glutamate transporters and serotonin transporter N- and C-terminal tail fusion proteins have also been shown to be in vitro substrates for PKA and/or PKC (21, 27). Thus, although several studies have indicated that protein kinase activators regulate neurotransmitter transport activity, only a small number of studies utilizing in vitro or heterologous expression systems have demonstrated direct transporter phosphorylation, and to date little work has been done examining these properties in the brain. In this report we describe the endogenous phosphorylation and dephosphorylation of DAT in rat striatal synaptosomes and show that the phosphorylation state of the protein is regulated by protein kinase C. Dopamine transport activity is reduced by all treatments that increase DAT phosphorylation, with similar dose, kinetic, and inhibitor characteristics, suggesting that in brain DAT is subject to phosphorylation-induced functional regulation.

EXPERIMENTAL PROCEDURES

Tissue Preparation—Male Sprague Dawley rats, 150–300 g, were decapitated, and the striata were rapidly removed and weighed. The tissue was homogenized in a Teflon-glass homogenizer in 10 ml of cold 0.32 M sucrose and centrifuged at 800 × g for 10 min. The supernatant was recentrifuged at 12,500 × g for 10 min, and the resulting synapto- 

somal pellet was resuspended in 0.32 M sucrose at a concentration of 120 mg/ml original wet weight for phosphorylation experiments or 30 mg/ml original wet weight for uptake assays (0.5–2 mg/ml protein).

Proteins were assayed using the Pierce protein assay kit with bovine 
milk as a standard.

Phosphorylation—Kreb's bicarbonate buffer (25 mM NaHCO3, 124 
mM NaCl, 5 mM KCl, 1.5 mM CaCl2, 5 mM MgSO4, 10 mM glucose, pH 7–3) was saturated with 95% O2/5% CO2, and [32P]orthophosphate was added to a final concentration of 1.5 μCi/ml. The synaptosomal suspension was diluted 4-fold in this buffer to a tissue concentration of 30 mg/ml original wet weight in a final volume of 300 μl. Treatment compounds were added in 5-μl volumes, and incubations were carried out at 30 °C for 45 min with shaking at 100 rpm. At the end of the incubation, reaction tubes were transferred to an ice water bath to reduce enzyme activity, and samples were centrifuged at 4,000 × g for 2 min at 4 °C. Supernatants were removed, and the tissue was solubi- 

lized with 100 μl of 0.5% SDS and four passages through a 26-gauge needle. The solubilized samples were centrifuged at 20,000 × g for 10 min at 4 °C, and supernatants were removed for analysis.

Immunoprecipitation—Solubilized tissue samples were immunopre- 

cipitated with antibody 16, directed against amino acids 42–59 of the 
deduced DAT primary sequence (28). This antibody has been shown by immunoprecipitation, immunoblot, and immunohistochemistry to be highly specific for DAT (28, 29). Solubilized, [32P]labeled synapto- 

somal tissue was diluted with 50 mM Tris-HCl, pH 7.5, containing 0.1% 

Triton X-100 and serum 16 diluted 1:100. Samples were incubated at 4 °C for 1 h followed by the addition of 20 μl of protein Sepharose CL4B (Pharmacia Biotech Inc.) for an additional hour. Immune complexes were washed twice with the Tris-Triton buffer, and samples were eluted with SDS-polyacrylamide gel electrophoresis loading buffer (60 mM Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, and 5 mM dithiothreitol). Samples were electrophoresed on 7% polyacrylamide gels followed by autoradiography using Kodak BioMax film for 1–5 days. Amersham Rainbow molecular mass markers were standards on all gels. Positive controls for immunoprecipitations were provided by parallel precipita- 

tion of [32P]DEEP photosifinity labeled dopamine transporters (30). For peptide blocking experiments, diluted antiserum was preincubated with other 50 μg/ml peptide 16 or peptide 18 (15 amino acids 580–608) prior to addition of the sample. Levels of DAT phosphorylation were quantitated by Cerenkov counting. All results were obtained in three or more independent experiments.

Dopamine Uptake—For preincubations with test compounds or veh- 
hicle, synaptosomal samples were distributed into 500-μl volumes and treated at 30 °C with shaking at 100 rpm for 20 min or other indicated times. After preincubation, 50-μl aliquots were distributed into assay tubes prepared as follows, and [3H]DA was added to initiate transport.

Dopamine uptake was performed in a final volume of 1000 μl, in a 

buffer consisting of 16 mM Na2PO4, 126 mM NaCl, 4.8 mM KCl, 1.3 mM 

CaCl2, 1.4 mM MgSO4, 1 mM ascorbic acid, and 1 μM pargyline. Final 

solution concentration was 1.5 mg/ml original wet weight, and [3H]DA 

was 1 nM except for saturation analyses where [3H]DA was increased to 

10 nM. Nonlinear compounds were added in 3-μl volumes and 

were prepared as follows, and [3H]DA was added to initiate transport. Non-specific 

uptake was defined with 100 μM (-)-cocaine. Uptake assays were 

carried out for 3 min at 30 °C, followed by filtration under vacuum 

through Whatman GF/B filters soaked in 0.05% polyethyleneimine. 

Filters were washed rapidly with 5 ml of ice-cold 0.3 M sucrose using a 

Brandle M48R filtering manifold. Radioactivity bound to filters was 

counted using a Beckman LS 6000 liquid scintillation counter at 45– 

50% efficiency. Specific dopamine uptake in control preparations aver- 

aged 1.18 ± 0.38 pmol/min/mg protein, and data were analyzed by 

EBDA and LIGAND computer software. For purposes of comparison 

across experiments, uptake results were converted to percentages of 

control values and analyzed statistically with paired t-tests using Stat- 

view version 4D by Abacus Concepts, Inc. (Berkley, CA). Because of the 

modest effects produced by the test compounds, the experimental de- 

sign incorporated substantial assay redundancy. In each experiment, 

preincubations with test compounds or vehicle were performed on 

duplicate tissue samples. Each tissue sample was analyzed in six assay 

and the resulting values were averaged. Each data point thus 

consists of the mean of 12 independent determinations.

Alanine Uptake—[3H]Alanine uptake was examined using the same 

conditions as for dopamine transport, except that [3H]alanine was 

10 nm.

Materials—PMA, 4α-PDD, okadaic acid, calyculin, microcystin, 

cyclosporin A, staurosporine, bisindolylmaleimide I HCl, (-)-indolactam 

V, OAG, forskolin, 8-Br-cAMP, and isobutylmethylxanthine were ob- 

tained from Calbiochem (San Diego, CA). Compounds were dissolved at 

high concentrations in Me2SO or water, and stock solutions were di- 

luted for use in Krebs bicarbonate buffer, producing final Me2SO con- 

centrations of up to 0.5%. Me2SO alone at this level did not affect DAT 

phosphorylation or dopamine transport, but in all experiments control 

samples were treated with matching concentrations of vehicle. 

[32P]Orthophosphate, carrier-free, was from DuPont NEN. [3H]Dopa- 

mine (specific activity 48 Ci/mmol) and [3H]alanine (specific activity 54.0 

Ci/mmol) were from Amersham Corp. [32P]DEEP was radioiodinated by 

Dr. John Lever (Johns Hopkins Medical School, Baltimore, MD).

RESULTS

DAT Phosphorylation—The identification of the dopamine transporter from rat brain as a phosphoprotein is shown in Fig. 1. Serum specific for DAT immunoprecipitated a [32P]labeled 80-kDa protein from rat striatal synaptosomes (lane 6). Preim- 

mune serum did not recognize this protein (lane 5), and preab- 

sorption of the immune serum with the immunizing peptide (lane 7), but not an irrelevant peptide (lane 8), blocked extrac- 

tion of the protein. The [32P]labeled protein exactly co-mi- 

grated on gels with authentic [32P]DEEP-labeled DAT, which exhibited the identical serum recognition and peptide preab- 

sorption profile (lanes 1–4), and the phosphorylated protein was not extracted from the cerebellum, a brain region devoid of 

DATs (lane 9). These results identify this phosphoprotein as 

DAT, with the possible caveat that the observed phosphoprotein 

is a contaminant that co-precipitates only in the presence of 

DAT.

Although DATs were phosphorylated at a basal level in untreated 

synaptosomes, inclusion of the phosphatase inhibitor okadaic acid (OA) during [32P]labeled resulted in a dramatic increase 

in the level of DAT phosphorylation (Figs. 2 and 6). This effect 

was dose-dependent, increasing up to 10 μM, the highest concentation tested (Fig. 2, top). Time course studies showed increased levels of phosphorylated DAT by 5 min of 


treatment, with a maximum reached by 15–20 min (Fig. 2, 

bottom). Treatment with 10 μM okadaic acid is depicted in 

all subsequent experiments unless otherwise indicated.

Treatment of synaptosomes with PMA in the presence of OA 

during [32P]labeled increased DAT phosphorylation levels a 

further 3–5-fold (Fig. 3). This effect was dose-dependent, 

increasing steeply between 0.1 and 1 μM (top). The time course 

of PMA stimulation showed increases in phosphorylation by 5–10
min, with maximum levels reached by 15–20 min (bottom). The same relative response to PMA was produced in the absence of phosphatase inhibitors (not shown), although the lower baseline phosphorylation made this difficult to observe. The inactive phorbol ester 4αPDD at 10 μM had no effect on DAT phosphorylation (see Fig. 5). To further verify the involvement of PKC in this response, the effects of two other protein kinase C activators, (-)-indolactam V and OAG, were also examined (Fig. 4). A slight increase above basal was produced by 0.1 μM (-)-indolactam V, whereas 1 and 10 μM treatments increased DAT phosphorylation to comparable or slightly higher levels than PMA. OAG, a diacylglycerol analog, caused enhancement of DAT phosphorylation at 0.3 and 1 μM.

These results suggest that activation of protein kinase C results in increased phosphorylation of DAT. Additional evidence in support of this conclusion was obtained using the PKC inhibitors staurosporine and bisindoyl maleimide (Fig. 5). 1 μM staurosporine blocked the increased DAT phosphorylation produced by 1 μM PMA, whereas 10 μM staurosporine inhibited even basal phosphorylation. The phosphorylation of a 20-kDa contaminant present in precipitated samples was not affected by staurosporine, indicating that the reduction of DAT phosphorylation below basal levels was not due to toxicity or non-specific effects. PMA-induced phosphorylation of DAT was also blocked by bisindoyl maleimide, a kinase inhibitor much more specific for PKC than staurosporine. Partial inhibition of stimulated phosphorylation was observed at 100 nM, and almost complete inhibition of the stimulated increase was obtained at 1 μM, the highest concentration tested.

Additional characterization of DAT dephosphorylation was done using three other phosphatase inhibitors, calyculin, miccroystin, and cyclosporin A (Fig. 6). Calyculin was as effective as okadaic acid at preventing DAT dephosphorylation in the dose range of 1–10 μM. Conversely, neither 10 μM miccroystin (Fig. 6) nor 10 μM cyclosporin A (not shown) showed significant effects. Fig. 6 also shows that protein kinase A activators had no effect on DAT phosphorylation. In four separate experiments, neither 50 μM forskolin in the presence of the cAMP phosphodiesterase inhibitor isobutylmethylxanthine (Fig. 6) nor 10 μM 8-Br-cAMP (not shown) caused stimulation of phos-
Dopamine Transporter Phosphorylation

**Phosphorylation activators reduce \([^3]H/DA\) uptake**

Synaptosomes were treated with the indicated compounds for 20 min at 30 °C prior to analysis for dopamine transport. Results are shown as the relative amount of DA transported compared with control samples treated with MeSO.

| Treatment          | Control ± S.E. | n |
|--------------------|----------------|---|
| PMA                | 86.9 ± 3.0 a  | 11|
| OA                 | 86.5 ± 3.0 b  | 10|
| PMA + OA           | 75.7 ± 2.5 a  | 14|
| 4aPDD              | 97.8 ± 3.2 a  | 10|
| (-)-indolactam V   | 92.8 ± 1.5 a  | 5 |
| OAG                | 79.0 ± 2.8 a  | 3 |

a Different letters indicate means which are significantly different (p < 0.05). n, number of independent experiments with each compound. The final concentration of all test compounds was 10 μM, except for OAG which was 1 mM.

**Table II**

Protein kinase inhibitors block PMA-induced decrease in DA transport

Synaptosomes were treated with the indicated compounds for 20 min at 30 °C prior to analysis for dopamine transport. Results are shown as the relative amount of DA transported compared with control samples treated with MeSO.

| Treatment                          | Control ± S.E. | n |
|------------------------------------|----------------|---|
| PMA                                | 85.0 ± 4.4 a  | 4 |
| PMA + staurosporine                | 100.0 ± 3.6    | 4 |
| PMA                                | 90.0 ± 1.7 b  | 3 |
| PMA + bisindoylmaleimide           | 105.0 ± 2.6    | 3 |

a Indicates means significantly different from control (p < 0.05). n, number of independent experiments directly matching each treatment. PMA and bisindoylmaleimide were 1 μM; staurosporine was 3 μM.

whereas control and treated \(V_{\text{max}}\) values were 139.7 ± 19.3 and 72.2 ± 5.8 pmol/min/mg protein (p < 0.05).

**Alanine Transport**—To assess whether the reduced transport activity in response to PMA and OA was due to an alteration of Na⁺ electrochemical gradients across membranes, we examined the effects of these compounds on Na⁺-dependent transport of alanine (26). Synaptosomes were treated with 10 μM PMA plus 10 μM OA or with vehicle, and aliquots of each were assayed in parallel for transport of \([^3]H/\)alanine or \([^3]H/\)alanine. In four independent experiments, alanine transport in treated tissue was not significantly different from control transport (normalized uptake = 93.0 ± 4.4%, p > 0.05), whereas dopamine transport activity in the treated tissue was 62.8 ± 3.6% compared with control (p < 0.01). This indicates that the reduction in DA transport observed after treatment with PMA and OA is unlikely to be due to loss of transmembrane ion gradients or perturbations of membrane integrity.

**DISCUSSION**

**Dopamine Transport**—This report demonstrates that dopamine transporters expressed in brain undergo endogenous phosphorylation and characterizes the DAT phosphorylation and dephosphorylation properties. The appearance of phosphorylated DAT on gels is somewhat different from its appearance in photoaffinity labeling or Western blots, in which DAT is visualized as a broad, homogenous band. Although basally phosphorylated DAT had this appearance, PMA-stimulated DAT often exhibited reduced electrophoretic mobility. In some experiments, radiolabel was concentrated at the protein’s trailing edge, with reduced label intensity at the leading edge (Figs. 1 and 3; Fig. 5, left), whereas in other experiments the appearance of the stimulated band remained more homogenous but displayed a discrete upward shift relative to the basal band (Fig. 4; Fig. 5, right). Many phosphoproteins migrate as dou-
bly that DAT phosphorylation is controlled by protein kinase C. DAT phosphorylation was strongly increased by PMA, OAG, and (−)-indolactam V, three different protein kinase C activators. Phosphorylation was not affected by the inactive phorbol ester 4αPDD, and the protein kinase C inhibitors staurosporine and bisindoylmaleimide blocked PMA-induced increases in DAT phosphorylation. These results are strong evidence that activation of PKC leads to increased phosphorylation of DAT, although it is not known if PKC phosphorylates DAT directly or induces phosphorylation via a downstream event. PKC-induced increases occurred in the presence of okadaic acid, demonstrating that the effects of OA and PKC activators on phosphorylation are additive and indicating the potential for in vivo regulation of DAT phosphorylation to occur through alterations in activity levels of kinases, phosphatases, or both. In contrast to the results with PKC activators, treatment of synaptosomes with the PKA activators forskolin and 8-Br-CAMP did not induce DAT phosphorylation, indicating the lack of involvement of PKA with DAT.

**Dopamine Transport**—The possibility that dopamine transport activity is regulated by phosphorylation of DAT is indicated by the finding that dopamine uptake in synaptosomes was reduced by all treatments that promoted DAT phosphorylation. PMA and okadaic acid each reduced dopamine transport to about 87% of control values, and use of both compounds together resulted in further reduction to about 76% of control values, mimicking the additivity of these compounds on DAT phosphorylation. Uptake was also reduced by (−)-indolactam V and OAG, 4αPDD had no effect on uptake, and staurosporine and bisindoylmaleimide were both able to block PMA-induced reductions in transport activity. The reduction in uptake occurred with dose and time courses similar to those for phosphorylation and was produced by a reduction in transport V_{max} with no apparent change in the K_{m} for dopamine. The close correlation of phosphorylation and transport characteristics strongly suggests that dopamine transport activity is regulated by DAT phosphorylation.

Results similar to these were found when mouse striatal synaptosomes were treated with OA, PMA, and diacylglycerol analogs (18). However, PKA activators did not affect DA transport, consistent with our finding that PKA activators do not induce DAT phosphorylation. The possibility that the reduced dopamine transport obtained in these studies was a nonspecific
effect produced by an alteration of transmembrane Na⁺ gradients has been addressed by several different approaches. Our results show that in synaptosomes and rDAT-LLC-PK₁ cells (16), Na⁺-dependent alanine transport was not altered by PMA and OA treatments that substantially reduced DA uptake. In addition, PMA does not affect synaptosomal viability or alter the activity of synaptosomal Na⁺-K⁺ ATPase, which controls transmembrane Na⁺ gradients (18). These results suggest that it is unlikely that the phosphorylation activators examined in these studies are inducing alterations in Na⁺ electrochemical gradients or affecting membrane integrity. The effects of these compounds on DA uptake therefore appear to be specific for DA transport and physiologically meaningful.

Although these results strongly indicate that DAT activity is regulated by phosphorylation, the mechanisms underlying this relationship remain to be elucidated. Possible modes of action include direct effects on substrate translocation or changes in DAT surface expression as has been found for heterologously expressed γ-amino butyric acid and serotonin transporters (22, 25). Additional aspects of DAT function such as ion flux or binding of cocaine or other uptake blockers may also be affected by phosphorylation.

Implications—While the pathways controlling DAT phosphorylation in vivo remain to be elucidated, the finding that the diacylglycerol analog OAG stimulates DAT phosphorylation indicates the feasibility for involvement of phospholipase C-activated dopamine receptors. Although dopamine receptors have classically been characterized as coupling to cAMP production (33), recent studies indicate that dopamine agonists increase striatal dopamine transport (37). If activation of D₂ receptors reduces PKC activity, DAT phosphorylation kinetics of DAT phosphorylation and dephosphorylation are compatible with receptor-mediated actions. One possible control mechanism is by feedback through presynaptic dopamine autoreceptors. Although dopamine receptors have classically been characterized as coupling to cAMP production (33), recent studies indicate that dopamine receptors can couple either positively (34, 35) or negatively (36) with PKC pathways. The latter may be compatible with the observation that D₂ receptor agonists increase striatal dopamine transport (37). If activation of D₂ receptors reduces PKC activity, DAT phosphorylation would be reduced and DA transport activity would be increased. Coupling of dopamine receptors to DAT phosphorylation would also provide a mechanism for cocaine involvement in regulation of transport activity via cocaine’s effects on synaptic dopamine levels. It is also possible that other phospholipase C-coupled receptors may regulate DAT phosphorylation and that this may be a convergence point for multiple signaling pathways.

Although the transport of many neurotransmitters is affected by protein kinase activators, until recently there has been little direct evidence that phosphorylation of transporter proteins mediates these effects. The most well documented example of neurotransmitter transporter phosphorylation is DAT, which has now been characterized as a phosphoprotein both in neuronal tissue and heterologous expression systems (16, 38). The phosphorylation of DATs in LLC-PK₁ cells displays activator, inhibitor, and kinetic characteristics that parallel those shown here for neuronal DATs, indicating that heterologous expression systems provide valid models for examining DAT phosphorylation and that results from studies such as deletion or site-directed mutagenesis using transfected DATs will be relevant to the neuronal form of the protein.

Regulation of DA transport activity by phosphorylation would provide neurons with a previously unappreciated mechanism for fine temporal and spatial control of synaptic dopamine concentrations. Such functional regulation could have profound effects on the intensity and duration of dopaminergic synaptic transmission, actions of psychostimulant drugs, and mechanisms of neurotoxicity and neurodegeneration.

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