Evidence suggests that protein kinase C (PKC) and intracellular calcium are important for amphetamine-stimulated outward transport of dopamine in rat striatum. In this study, we examined the effect of select PKC isoforms on amphetamine-stimulated dopamine efflux, focusing on Ca\textsuperscript{2+}-dependent forms of PKC. Efflux of endogenous dopamine was measured in superfused rat striatal slices; dopamine was measured by high-performance liquid chromatography. The non-selective classical PKC inhibitor G66976 inhibited amphetamine-stimulated dopamine efflux, whereas rottlerin, a specific inhibitor of PKC\(\delta\), had no effect. A highly specific PKC\(\beta\) inhibitor, LY379196, blocked dopamine efflux that was stimulated by either amphetamine or the PKC activator, 12-O-tetradecanoylphorbol-13-acetate. None of the PKC inhibitors significantly altered \(^{3}H\)dopamine uptake. PKC\(\beta\) and PKC\(\beta_{II}\), but not PKC\(\alpha\) or PKC\(\gamma\), were co-immunoprecipitated from rat striatal membranes with the dopamine transporter (DAT). Conversely, antisera to PKC\(\alpha\) and PKC\(\beta_{II}\), but not PKC\(\alpha\) or PKC\(\gamma\), were able to co-immunoprecipitate DAT. Amphetamine-stimulated dopamine efflux was significantly enhanced in hDAT-HEK 293 cells transfected with PKC\(\beta_{II}\) as compared with hDAT-HEK 293 cells alone, or hDAT-HEK 293 cells transfected with PKC\(\alpha\) or PKC\(\beta_{II}\). These results suggest that classical PKC\(\beta\) is physically associated with DAT and is important in maintaining the amphetamine-stimulated outward transport of dopamine in rat striatum.

The plasmalemmal dopamine transporter (DAT)\(^1\) is a presynaptic protein that belongs to the SLC6 family of Na\textsuperscript{+}/Cl\textsuperscript{−}-dependent neurotransmitter transporters. It is the primary regulator of the duration and strength of the dopamine signal in the synapse (1). DAT binds extracellular dopamine, Na\textsuperscript{+}, and Cl\textsuperscript{−} and transports them intracellularly, clearing dopamine from the synaptic cleft. The transporter is also the site of action of psychostimulant drugs such as amphetamine, which is a DAT substrate. Following its transport into the synaptic terminal, amphetamine stimulates a reversal of the transporter eliciting dopamine efflux. DAT is characterized as having 12 transmembrane segments and a large second extracellular loop with the N- and C-terminals located intracellularly. There are also putative phosphorylation sites for protein kinase C (PKC), protein kinase A, and Ca\textsuperscript{2+}/calmodulin-stimulated protein kinase II (2). PKC has been implicated in various aspects of DAT function and regulation, such as trafficking (3), transport activity (4, 5), and direct phosphorylation (Refs. 6 and 7, and see references in Ref. 8).

Evidence strongly suggests that PKC activity is important for amphetamine-stimulated outward transport. General PKC inhibitors blocked dopamine efflux induced by amphetamine in rat striatal slices (9). Accordingly, a PKC inhibitor blocked amphetamine-mediated locomotor behavior when injected directly into rat nucleus accumbens (10). The phorbol ester PKC activator, 12-O-tetradecanoylphorbol-13-acetate (TPA), mimicked the effect of amphetamine on the reverse transport by eliciting an efflux of dopamine that was cocaine-sensitive and independent of extracellular Ca\textsuperscript{2+} (11). Finally, a deletion of the N-terminal 22 amino acids of DAT, containing the distal N-terminal serines that are strongly considered to be substrate sites of PKC (6), abrogated amphetamine-induced dopamine efflux from hDAT-HEK 293 cells (human embryonic kidney 293 cells) without altering inward transport (12). Mutation of the distal N-terminal serines of DAT to alanines similarly abolished amphetamine-stimulated dopamine efflux, whereas mutation of the N-terminal serines to aspartates restored sensitivity of the reverse transport to amphetamine.

The PKC isoform altering DAT-mediated dopamine efflux is not known, but it could be a Ca\textsuperscript{2+}-sensitive isoform. Although amphetamine-induced dopamine efflux does not require extracellular Ca\textsuperscript{2+}, it does require intracellular Ca\textsuperscript{2+} (13, 14). Moreover, amphetamine, acting at DAT, increases intracellular Ca\textsuperscript{2+} from thapsigargin-sensitive stores (14, 15). There are three major classes of PKC isoforms: classical, non-classical, and atypical. The classical PKC isoforms (\(\alpha, \beta_{1}, \beta_{2}, \gamma\)) are Ca\textsuperscript{2+}- and diacylglycerol-dependent. The non-classical PKC isoforms (\(\delta, \epsilon, \eta, \theta\)) are insensitive to Ca\textsuperscript{2+} and diacylglycerol. There is a limited number of inhibitors that can distinguish among the different isoforms. G66976 is a potent inhibitor of the classical PKC isoforms (16).LY379196, structurally similar to LY333531, is a highly selective inhibitor of the PKC\(\beta\) isoform (17), and rottlerin is a selective inhibitor of the PKC\(\delta\) isoform (18).

Using these selective inhibitors in combination with co-immunoprecipitation studies and the transfection of PKC isoforms into hDAT human embryonic kidney 293 (hDAT-HEK 293) cells, we investigated the role of two classical PKC isoforms focusing on Ca\textsuperscript{2+}-dependent forms of PKC.
isozymes in regulating outward transport of dopamine by amphetamine and their association with DAT.

**EXPERIMENTAL PROCEDURES**

**Materials**—G60976 and rotterlin were purchased from Calbiochem. d-Amphetamine, dopamine, and TPA were purchased from Sigma. LY379196 was a generous gift from Lilly. hDAT cDNA was a gift from Dr. Z. B. Pristupa, Centre for Addiction and Mental Health, Department of Psychiatry, University of Toronto, Canada; cDNAs for PKCα, PKCβ1, and PKCβII were generous gifts from Dr. Stephen Ferguson, John P. Robarts Research Institute, London, Ontario, Canada.

*Amphetamine-* or TPA-mediated Dopamine Efflux—Rat striata were homogenized in 10 volumes of homogenization buffer containing 0.32 M sucrose, 1 mM EDTA, and a mixture of protease inhibitors (Complete Mini, Roche Applied Sciences), pH 7.4. Homogenates were centrifuged at 1000 g for 30 min to remove cell debris. The supernatant was saved, and the pellet was resuspended in homogenization buffer and centrifuged again. The combined supernatant fractions were centrifuged at 30 min at room temperature before the addition of 2 µM amphetamine (500 µl, 5-min fractions) were collected into vials containing 25 µl of internal standard solution (0.05 M HClO4, 4.55 mM dihydroxybenzylamine (internal standard), 1 mM ascorbic acid, and 0.1 M EDTA). Dopamine was measured by high performance liquid chromatography with electrochemical detection.

To measure dopamine efflux from hDAT-HEK 293 cells, cells cultured on coverslips were incubated with 15 µl unlabeled dopamine for 30 min at 37°C. Following the incubation, cells were washed two times with a Krebs-Ringer-Hepes (KRH) buffer (25 mM Hepes, pH 7.4, 125 mM NaCl, 4.8 mM KCl, 1.2 mM KH2PO4, 1.3 mM CaCl2, 1.2 mM MgSO4, 5.6 mM glucose, and 1 mM trotopline) and resuspended in 300 µl of KRH. The cells (200 µl) were placed in the Brandel superperfusion apparatus and washed for 30 min at room temperature before the addition of 2 µM amphetamine for 2.5 min. Dopamine was measured in the eluates as described above.

**Synaptosome Preparation**—Rat striata were homogenized in 10 volumes of homogenization buffer containing 0.32 M sucrose, 1 mM EDTA, and a mixture of protease inhibitors (Complete Mini, Roche Applied Science), pH 7.4. Homogenates were centrifuged at 1000 g for 10 min to remove cell debris. The supernatant was saved, and the pellet was resuspended in homogenization buffer and centrifuged again. The combined supernatant fractions were centrifuged for 30 min at 15,000 g at 4°C. The supernatant was removed, and the pellet (P2) was resuspended in KRB for [3H]dopamine uptake assay.

**[3H]Dopamine Uptake**—Synaptosomes were resuspended in 1.2 ml of KRB and incubated in the presence or absence of drugs (130 mM G60976, 10 µM rotterlin, or 100 nM LY379196) for 60 min at 37°C. [3H]Dopamine was measured in the absence of the drug to 0.13 ± 0.05 pmol/mg of protein in the presence of the drug (p < 0.05, n = 3) (Fig. 1A). Inhibition of amphetamine-stimulated dopamine efflux, in the absence of the drug to 0.13 ± 0.05 pmol/mg of protein in the presence of the drug (p < 0.05, n = 3). These results were used to examine the effectiveness of a single classical PKC isozyme, PKCβ, LY379196 is a highly selective inhibitor of PKCβ (IC50 of 30 nM) (17); we used this compound to examine the role of a single classical PKC isozyme, PKCβ, LY379196 demonstrates a 10-fold selectivity over other classical PKCs and 10–100-fold selectivity over non-classical PKCs. In the presence of 100 nM LY379196, amphetamine-stimulated dopamine efflux was re-
duced from 0.28 ± 0.08 pmol/mg of protein in the absence of
drug to 0.06 ± 0.03 pmol/mg of protein (p < 0.03, n = 8) (Fig. 1B). The value for dopamine efflux in the presence of LY379196
was not different from base line (0.10 ± 0.02, n = 3; B, fraction 7, *, p ≤ 0.02, n = 8; C, fraction 11, n.s., n = 3.

**Effect of the PKC Inhibitors on [^3]H]Dopamine Uptake**—To
determine whether the PKC inhibitors prevented inward
transport of substrate, [^3]H]dopamine uptake was assessed.
Striatal synaptosomes were incubated with Go 6976, LY379196, and rottlerin at 37 °C for 40 min prior to the addition
of [^3]H]dopamine to mimic the conditions in the efflux
assay. Preincubation of the synaptosomes with 130 nM Go 6976, 100 nM LY379196, or 10 μM rottlerin had no effect on [^3]H]dopamine uptake as compared with vehicle controls (Fig. 2).

**Effect of LY379196 on TPA-mediated Dopamine Efflux**—We
have shown previously that activation of PKC with TPA mimicked
the ability of amphetamine to induce dopamine efflux from rat striatal slices (9, 11). If TPA and amphetamine are
using the same signaling pathway, TPA-mediated dopamine
efflux should be inhibited by the selective PKCβ inhibitor,
LY379196, in rat striatal slices. As shown in Fig. 3, LY379196
reduced TPA-mediated dopamine efflux from 3.3 ± 0.83-fold of
base-line dopamine values to 1.3 ± 0.43-fold base-line dopamine
values (p < 0.05, n = 4).
immunoprecipitated using a peptide affinity-purified polyclonal antibody developed in our laboratory. The DAT immunoprecipitates were immunoblotted for PKCa, PKCβI, PKCβII, and PKCy. To ensure that the co-immunoprecipitation was specific for DAT, we preincubated the antibody with the DAT N-terminal antigen. The data in Fig. 4 demonstrate that both PKCβI and PKCβII co-immunoprecipitated with DAT in the absence but not the presence of the peptide antigen. On the contrary, neither PKCa nor PKCy co-immunoprecipitated with DAT. As shown in Fig. 4, all four forms of PKC were present in the lysate. To further test the apparent association, immunoprecipitation was conducted using antisera to the PKC isoforms, and the presence of DAT in the immunoprecipitates was tested with immunoblotting. As shown in Fig. 5, DAT co-immunoprecipitated with PKCβI and PKCβII but not with PKCa or PKCy. We have validated these results with two different PKCβ2 antibodies. We also detected co-immunoprecipitation of hDAT and PKCβII in hDAT-PC12 cells following immunoprecipitation with either anti-DAT or anti-PKCβII (data not shown). However, we would use caution in interpreting the relative amounts of PKCβ isoforms that immunoprecipitated with DAT. We performed all experiments with two to three different antibodies to PKCβI and PKCβII, and although the results were qualitatively similar, we found that the relative amounts of the detected isoform could vary depending on the efficacy of the antibody for immunoprecipitation or immunoblotting.

PKCβII Potentiates Amphetamine-stimulated Dopamine Efflux—Because both PKCβI and PKCβII co-immunoprecipitated with DAT, one or both of the isoforms could be responsible for enhancing amphetamine-stimulated dopamine efflux. LY379196 does not discriminate between PKCβI and PKCβII. To distinguish between the two PKCβ isoforms we created stable hDAT-HEK 293 cell lines overexpressing PKCα, PKCβI, or PKCβII. As shown in Fig. 6A, hDAT-HEK 293 cells endogenously contain PKCa and PKCβII but have very little PKCβI. However, the stable cell lines contained high concentrations of their respective PKC isoforms. The cells were loaded with 15 μM amphetamine for 30 min to measure dopamine efflux by superfusion. The dopamine efflux in response to 3 μM amphetamine was similar in the hDAT-HEK 293 cells, the PKCs-hDAT-HEK 293 cells, and the PKCβI-hDAT-HEK 293 cells (Fig. 6B). However, the dopamine efflux in response to amphetamine was much greater in the PKCβII-hDAT-HEK 293 cells than in the other cell lines. The average "fold" base line of amphetamine response in the PKCβII-hDAT-HEK 293 cells was 2.8 ± 0.5 (n = 7), whereas that in the other lines was maximal at 1.57 ± 0.3 (for hDAT-HEK 293 cells, n = 6), 1.47 ± 0.3 (for PKCβI-hDAT-HEK 293 cells, n = 7), and 1.3 ± 0.2 (for PKCβI-hDAT-HEK 293 cells, n = 7). Base-line values (in pmol of dopamine/5 min/mg of protein) in the untransfected hDAT-HEK 293 cells and the stable HEK 293 lines containing

![Figure 3](http://example.com/image3.png)

**FIG. 3.** Effect of the selective PKCβ inhibitor, LY379196, on TPA-mediated dopamine efflux. Rat striatal slices were incubated with 100 nM LY379196 for 40 min prior to the addition of 300 nM TPA. For fraction 6, ***p < 0.05*** as determined by two-tail Student’s *t* test.

![Figure 4](http://example.com/image4.png)

**FIG. 4.** Co-immunoprecipitation of DAT with PKCβI and PKCβII but not with PKCα or PKCy. A rat striatal synaptosomal preparation was lysed in RIPAE buffer and precleared with protein A-Sepharose as described under “Experimental Procedures.” 50 μg of protein were then incubated with anti-DAT antibody preincubated with (IP+P) or without (IP) the immunogenic peptide (40 μg, corresponding to three times the concentration of antibody) as discussed under “Experimental Procedures.” The immunoprecipitates were eluted into sample buffer. Eluted proteins and original lysates (Ly) were analyzed by immunoblotting (IB) for the classical PKC isozymes.

![Figure 5](http://example.com/image5.png)

**FIG. 5.** Immunoprecipitation of striatal synaptosomes with either anti-PKCβI or anti-PKCβII but not anti-PKCγ or anti-PKCα co-immunoprecipitates DAT. Rat striatal synaptosomes were lysed in RIPAE buffer and precleared as described under “Experimental Procedures.” 50 μg of protein were incubated with either anti-PKCα, anti-PKCβI, anti-PKCβII, or anti-PKCγ antibody. The protein from the immunoprecipitates (IP) was eluted in sample buffer. Eluted proteins and original lysates (Ly) (20 μg of protein for lysate in left panel and 12.5 μg of protein in lysate in right panel) were analyzed by immunoblotting (IB) for DAT.

![Figure 6](http://example.com/image6.png)

**FIG. 6.** Effect of overexpression of PKC isoforms on amphetamine-stimulated dopamine efflux. A, PKC isoform content of untransfected (U) hDAT-HEK 293 cells and in hDAT-HEK 293 cells overexpressing (T, transfected) either PKCα, PKCβI, or PKCβII. B, amphetamine (AMPH, 3 μM)-stimulated dopamine efflux in hDAT-HEK cells and cells overexpressing PKCa, PKCβI, and PKCβII. All cells were preloaded with 15 μM dopamine for 30 min at room temperature. Cells were loaded into the superfusion apparatus, washed, and perfused with 3 μM amphetamine for 2.5 min. Results are expressed as -fold base line ± S.E. Base-line values are given under PKCβI, Potentiates Amphetamine-stimulated Dopamine Efflux. Analysis of variance for all groups at fraction 7, *p < 0.01*. In the post hoc Tukey-Kramer multiple comparisons test, results for PKCβII-hDAT-HEK 293 cells differed from those for PKCα hDAT-HEK 293 at *p < 0.01* and for PKCβI hDAT-HEK 293 and hDAT-HEK 293 cells at *p < 0.05*. n = 6–7. C, surface expression of hDAT in the untransfected hDAT-HEK 293 cells, PKCα hDAT-HEK 293 (α), PKCβI-hDAT-HEK 293 (βI) and PKCβII-hDAT-HEK 293 (βII) cells. Cells were biotinylated as described under “Experimental Procedures” and immunoblotted (IB) for hDAT. C demonstrates the biotinylated fraction (surface DAT).
Dopamine Efflux and Protein Kinase C

PKCa, PKCβ1, and PKCβ2 were 9.4 ± 1.6, 12.8 ± 1.5, 9.4 ± 1.2, and 10.0 ± 1.3, respectively. The average concentration of dopamine present in the cells at the start of the perfusion was, in nmol/mg of protein: 1.6 ± 0.5 in hDAT-HEK 293 cells; 1.2 ± 0.3 in PKCa-hDAT-HEK 293 cells; 1.5 ± 0.3 in PKCβ1-hDAT-HEK 293 cells; and 1.9 ± 0.6 in PKCβ1-hDAT-HEK 293 cells (n = 6, p = 0.76, one-way analysis of variance). Similar numbers of cells were used in all assays. Therefore the increase in amphetamine-stimulated dopamine efflux in the PKCβ1-hDAT-HEK 293 cells was not due to a significantly greater initial concentration of dopamine in the cells. To ensure that transport of amphetamine into the different cell lines was not altered by the transfection of the PKC isoforms, we measured uptake of [3H]dopamine in the four cell lines. Uptake of 25 nM [3H]dopamine was, in pmol/3 min/mg of protein: 0.16 ± 0.02 in hDAT-HEK 293 cells; 0.22 ± 0.09 in PKCα-hDAT-HEK 293 cells; 0.19 ± 0.07 in PKCβ1-hDAT-HEK 293 cells; and 0.23 ± 0.1 in PKCβ1-hDAT-HEK 293 cells (n = 5, p = 0.7, one-way analysis of variance). To determine whether the amount of hDAT on the surface of the four cell lines was altered by transfection of the PKC isoforms, surface biotinylation of hDAT was measured. As shown in Fig. 6C, the enhancement in amphetamine-stimulated dopamine efflux in PKCβ1-hDAT-HEK 293 cells is also not due to the presence of significantly greater levels of surface hDAT as compared with the other cell types.

DISCUSSION

We report that classical PKC isoforms regulate the mechanism of action of amphetamine in eliciting outward transport of dopamine. We made the original observations that PKCβ and, more specifically, PKCβ1, promotes amphetamine-induced outward transport through the dopamine transporter and that PKCβ isoforms are selectively associated with DAT. The regulation of DAT by PKC is obviously complex. We have demonstrated that brief (<3 min) incubations with TPA elicit efflux of dopamine that is blocked by cocaine but independent of extracellular Ca2+ (11). On the contrary, longer incubations with TPA (>5 min) increase inward trafficking of DAT (8). The TPA-induced internalization of DAT also involves classical PKCs; G66976 attenuated TPA-induced reduction of DAT-associated transport currents (4), an effect attributed to the enhanced endocytosis of DAT. Because TPA can activate many PKC isoforms, and there are four isoforms of classical PKC isoforms (PKCα, β1, β11, and γ), different classical PKC isoforms could be mediating these two effects. Opposing regulation of cell surface transporters by different patterns of PKC activation and different PKC isoforms has been reported. Brief activation with TPA increases activity of the Na+/HCO3- transporter in proximal convoluted tubules, whereas longer incubations decreases inward transport (22). Angiotensin II elicits biphasic effects on bicarbonate transport in the proximal tubule, and both effects appear to be mediated by PKC (22–24). Na+/K+-ATPase is another transporter that is regulated by PKC. A PKCδ-dependent phosphorylation of the Na+/K+-ATPase α subunit recruits the transporter to the cell surface, whereas a PKCε-dependent phosphorylation of the same subunit induces endocytosis of the transporter (25–27).

Inhibition of TPA-mediated dopamine efflux by the highly specific PKCβ inhibitor LY379196 strongly suggests that PKCβ is the specific isoform that is responsible for DAT-mediated efflux of dopamine. The effects of PKCa and PKCγ on amphetamine-mediated dopamine efflux were not investigated because of the lack of selective inhibitors for these isoforms. However, the co-immunoprecipitation studies and the studies with overexpression of PKCγ strongly suggest that these isoforms neither bind to DAT nor affect DAT-mediated dopamine efflux. All four classical PKC isoforms are reported to exist in the dopamine cell body areas of Sprague-Dawley rats, the strain used in these studies (28), although the predominant form was PKCβ1.

This study demonstrated that both the PKCβ1 and PKCβ11 isoforms readily co-immunoprecipitated with DAT suggesting that they associate in the terminal. We do not know whether they bind directly to DAT or whether there is an intermediary protein. The receptor for activated C kinases (RACK1) has recently been demonstrated to bind to the N terminus of DAT (29). PKCβ11 interacts specifically with RACK1, which consequently determines the localization and functional activity of PKCβ11 (30). The PKCα-binding protein, PICK 1 (31), has been demonstrated to form a stable complex with the PDZ domain in the C terminus of DAT (32). It is unclear at this time whether PKCa plays any role in the ability of PICK 1 to increase the number of plasma membrane DATs (32). If so, a complex of PKCa, PICK 1, and DAT may not have been retained through our immunoprecipitation protocol, or PKCa may only bind when DAT is activated. PKCα complexes with the neuronal glutamate transporter EAAC1 upon PKC activation in C6 glioma cells and rat brain synaptosomes, and this binding correlates with the PKC-dependent redistribution of EAAC1 (33). Our study suggests that PKCβ is associated with DAT even under "resting" conditions. Co-immunoprecipitation studies revealed complexes between protein phosphatase 2Ac and DAT as well as the norepinephrine and serotonin transporters under basal conditions (34). Therefore it appears that DAT and other transporters can exist in complexes in the membrane that include scaffolding and signal transduction molecules.

Our experiments using hDAT-HEK 293 cells transfected with PKC isoforms suggest that the PKCβ1 isoform is important for amphetamine-stimulated efflux of dopamine through DAT. Overexpression of hDAT-HEK 293 cells (PKCa or PKCβ1) did not significantly alter the amphetamine-stimulated dopamine efflux from that of untransfected HEK 293 cells. Although one could argue that overexpression of PKCa may not have a sufficient effect because the HEK 293 cells already contained some level of PKCa, that argument would not be true for PKCβ1. The HEK 293 cells had low levels of PKCβ1 and almost no PKCβ11. Only co-transfection with PKCβ1 significantly increased the dopamine efflux in response to amphetamine.

The mechanism by which PKCβ alters amphetamine-stimulated dopamine efflux is currently under investigation. PKCβ could be directly phosphorylating DAT and altering efflux. A PKC-dependent phosphorylation of DAT has been demonstrated at sites that appear to be the most distal N-terminal serine (6). Recently we demonstrated that phosphorylation of the N-terminal serine in DAT is required for maximal amphetamine-stimulated efflux (12), but [3H]dopamine uptake is not affected. An alternative explanation is that PKCβ could participate in the transporter vesicle recycling or maintenance of surface expression of DAT. PKCβ increases the surface expression of both glucose transporter 1 and rodent proximal tubule Na+/K+-ATPase (25, 26). PKCβ11, as opposed to PKCβ1, has been demonstrated to associate with the actin cytoskeleton (35) and therefore could be involved in DAT trafficking. We are presently examining this possibility.

We and others have reported that amphetamine can activate PKC in rat striatum (36–39) and that Ca2+ seems to be involved in this activation. The amphetamine-induced activation of PKC involves activation of phospholipase C and Na+/Ca2+ exchange (38). Further, amphetamine can increase intracellular Ca2+ (14, 15). However, there is no direct evidence that indicates which isoforms of PKC are activated. We demonstrated that amphetamine increases PKC activity and stimulates in vivo and in vitro phosphorylation of GAP-43 on serine-
41, which is the PKC substrate site (36, 40). There is evidence that GAP-43 is phosphorylated more actively by PKCβ, especially PKCβII, as compared with PKCα or PKCγ (41), suggesting that the amphetamine-stimulated increase in GAP-43 phosphorylation could be mediated by activation of PKCβII.

By elucidating which PKC isoforms are involved in regulating amphetamine-mediated dopamine efflux we can better understand the mechanism by which amphetamine acts to reverse the transport of dopamine. These studies have demonstrated that PKCβII is complexed with DAT and is important in the reverse transport of dopamine through DAT.

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