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Ceramide-induced alterations in dopamine transporter function

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Abstract

The purpose of this study was to determine the effects of ceramide on dopamine and serotonin (5-HT, 5-hydroxytryptamine) transporters. Exposure of rat striatal synaptosomes to C2-ceramide caused a reversible, concentration-dependent decrease in plasmalemmal dopamine uptake. In contrast, ceramide exposure increased striatal 5-HT synaptosomal uptake. This increase did not appear to be due to an increased uptake by the 5-HT transporter. Rather, the increase appeared to result from an increase in 5-HT transport through the dopamine transporter, an assertion evidenced by findings that this increase: (1) does not occur in hippocampal synaptosomes (i.e., a preparation largely devoid of dopamine transporters), (2) occurs in striatal synaptosomes prepared from *para*-chloroamphetamine-treated rats (i.e., a preparation lacking 5-HT transporters), (3) is attenuated by pretreatment with methylphenidate (i.e., a relatively selective dopamine reuptake inhibitor) and (4) is inhibited by exposure to exogenous dopamine (i.e., which presumably competes for uptake with 5-HT). Taken together, these results reveal that ceramide is a novel modulator of monoamine transporter function, and may alter the affinity of dopamine transporters for its primary substrate.

Keywords: Dopamine; 5-HT (5-hydroxytryptamine, serotonin) transporter; Ceramide; Dopamine transporter

1. Introduction

The discovery of inhibition of protein kinase C by sphingosine (Hannun et al., 1986) led to the suggestion that sphingolipid-derived products act as lipid second messengers. Subsequent studies demonstrated that ceramide, a cleavage product of sphingomyelin, alters the phosphorylation state of a variety of proteins. These effects on phosphorylation are undoubtedly complex, as ceramide modifies the activity of a variety of enzymes including cytosolic serine/threonine protein phosphatases (Dobrowsky and Hannun, 1992), protein kinase C (Tanabe et al., 1998) and stress-activated protein kinases (Westwick et al., 1995).

Of interest are recent findings that ceramide can alter the function of several distinct transporter molecules, including *p*-glycoproteins (for review, see Sietsma et al., 2001). Although never reported, ceramides would predictably alter monoamine transport as well, since: (1) kinases such as protein kinase C (Copeland et al., 1996; Vaughan et al., 1997) and PI-3 kinase (Carvelli et al., 2002) regulate dopamine uptake, and (2) phosphorylation of the dopamine (Vaughan et al., 1997; Huff et al., 1997; Zhu et al., 1997) and serotonin (5-HT; 5-hydroxytryptamine) transporters (Qian et al., 1997; Ramamoorthy and Blakely, 1999) decreases these carriers' activities. Accordingly, the purpose of the present study was to determine if ceramide alters monoamine transport. Specifically, the effects of applying C2-ceramide (a cell-permeable analog of ceramide) were assessed. Results reveal that C2-ceramide profoundly *decreases* dopamine uptake into rat striatal synaptosomes. In contrast, C2-ceramide *increases* synaptosomal 5-HT uptake. Interestingly, this increase in 5-HT uptake appeared to occur via the dopamine transporter. These data provide the first demonstration that ceramide alters monoamine transport and that a lipid second messenger may change the substrate-specificity of a monoamine transporter.

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2. Materials and methods

2.1. Animals and treatments

Male Sprague–Dawley rats (300–350 g; Simonsen Laboratories, Gilroy, CA) were maintained under conditions of controlled temperature and lighting, with food and water provided ad libitum. Animals were sacrificed by decapitation. All experiments were conducted in accordance with the National Institutes of Health guidelines.

2.2. Drugs and chemicals

(−)-Cocaine hydrochloride and (±)-methylphenidate were provided generously by the National Institute on Drug Abuse. *Para*-chloroamphetamine was purchased from Sigma (St. Louis, MO). Citalopram hydrochloride was supplied kindly by H. Lundbeck, and pargyline hydrochloride was obtained from Abbott Laboratories (North Chicago, IL). [7,8-^{3}H]Dopamine (46 Ci/mmol) was purchased from Amersham Life Sciences (Arlington Heights, IL). 5-[1,2,-^{3}H(*N*)]hydroxytryptamine (25.5 Ci/mmol), [*N*-methyl-^{3}H]WIN35428 [(−)-2-β-carbomethoxy-3-β-(4-fluorophenyl)tropane 1,5-naphthalenedisulfonate; 84.5 Ci/mmol] and γ[^{32}P] ATP (3000 Ci/mmol) were purchased from New England Nuclear (Boston, MA). C2-ceramide and C2-dihydroceramide were purchased from Calbiochem (San Diego, CA). Brain ceramide was purchased from Avanti Polar Lipids (Alabaster, AL). The sn-1,2-diacylglycerol assay reagents system was purchased from Amersham (Piscataway, NJ).

2.3. Synaptosomal [^{3}H]neurotransmitter uptake and [^{3}H] WIN35428 binding

Synaptosomal uptake of [^{3}H]neurotransmitter was determined as described by Fleckenstein et al. (1997). C2-ceramide was added to reaction tubes and incubated at 37 °C for 10 min prior to the 3-min incubation with [^{3}H]neurotransmitter. [^{3}H]WIN35428 binding experiments were performed as described by Kokoshka et al. (1998).

For ceramide washout experiments, P2 pellets were resuspended in ice-cold modified Kreb's buffer and incubated with 100 μM ceramide for 10 min. After incubation, tubes were placed on ice and then centrifuged (22,000 × *g* for 15 min; 4 °C). The supernatant was discarded and pellets were resuspended in modified Kreb's buffer containing 1 μM pargyline prior to preincubation and addition of [^{3}H]dopamine.

2.4. Ceramide determinations

Rat striatal tissue was homogenized in a phosphate-free buffer containing 50 mM HEPES and 1 mM NaCl. Lipids were extracted by the method of Bligh and Dyer (1959), and then used to quantitate ceramide and total lipid phosphate. Ceramide levels were measured by a modification of the diacylglycerol kinase assay (Okazaki et al., 1990; Preiss et al., 1986) using the diacylglycerol assay system (Amersham) according to the manufacturers instructions with two modifications. First, brain ceramide (0–2500 pmol) was used for the standard curve instead of diacylglycerol, and second, lipids were separated by thin layer chromatography using a $CHCl_3$/methanol/acetic acid (325:75:25) solvent system. Ceramide levels were normalized to total lipid phosphate levels determined from the lipid extracts as previously described (Whatley et al., 1993).

2.5. Data analysis

Statistical analyses between two groups was conducted by a two-tailed Student's *t*-test. Analyses among three or more groups were conducted with analysis of variance followed by Fisher's test. Differences among groups were considered significant if the probability of error was less than 5%.

3. Results

Results presented in Fig. 1 demonstrate that incubation of rat striatal synaptosomes at 37 °C for 10 min with C2-ceramide in vitro produced a concentration-dependent decrease in dopamine uptake. Concentrations of 1 and 10 μM had no effect, while 50 and 100 μM produced an 18% and 60% decrease in dopamine uptake, respectively. In contrast, incubation with the inactive C2-ceramide precursor, C2-dihydroceramide, had no effect on [^{3}H]dopamine uptake at concentrations up to 100 μM (data not shown). The ceramide-induced decrease occurred as early as 5 min and persisted for 20 min (control 1.07 ± 0.09, 5 min 0.42 ± 0.08*, 10 min 0.19 ± 0.01* and 20 min 0.05 ± 0.01* fmol/μg protein; $n = 3$; *$P \leq 0.05$). Effects of ceramide application for greater time periods were not assessed owing to a loss of

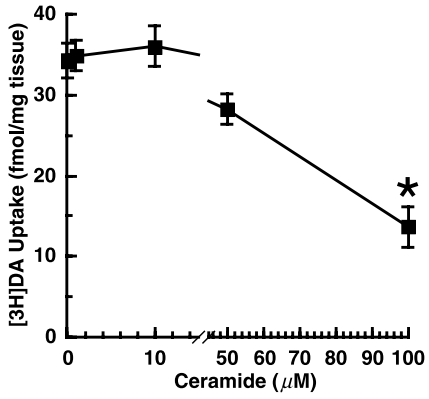

Fig. 1. Synaptosomes were incubated with C2-ceramide or vehicle for 10 min prior to the addition of [^{3}H]dopamine. Values represent means and vertical lines 1 S.E.M. of determinations from three independent experiments, with samples in each experiment run in triplicate. *Value for C2-ceramide-treated synaptosomes that is significantly different from control ($P \leq 0.05$).

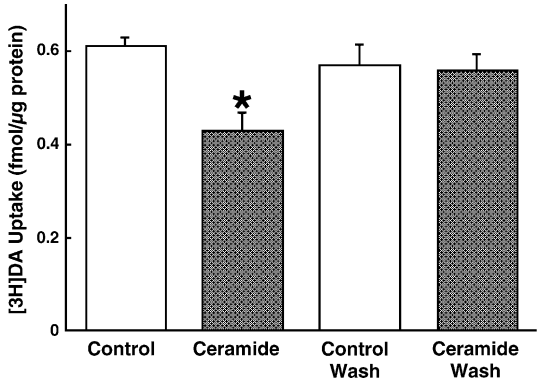

Fig. 2. Synaptosomes were incubated with 100 μM C2-ceramide or vehicle for 10 min prior to the addition of [^{3}H]dopamine. "Washed" tissue was incubated with 100 μM C2-ceramide for 10 min prior to wash, as described in Section 2. Columns represent means and vertical lines 1 S.E.M. of determinations from three independent experiments, with samples in each experiment run in triplicate. *Value for C2-ceramide-treated synaptosomes that is significantly different from control ($P \leq 0.05$).

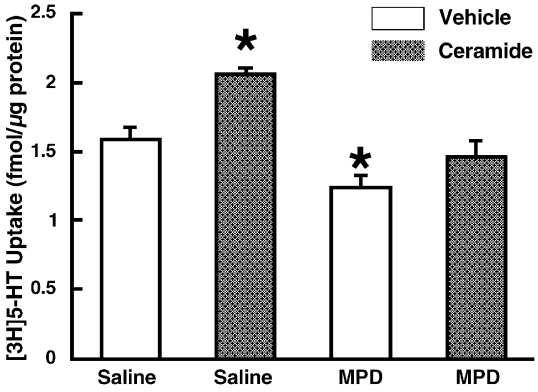

Fig. 4. Synaptosomes were incubated with C2-ceramide, methylphenidate (MPD) and/or vehicle for 10 min prior to the addition of [^{3}H]5-HT. Columns represent means and vertical lines 1 S.E.M. of determinations from three independent experiments, with samples in each experiment run in triplicate. *Values for treated synaptosomes that are significantly different from control ($P \leq 0.05$).

viability of the synaptosomes (data not shown). To investigate the physiological relevance of this phenomenon, ceramide levels were assessed and determined to be present in rat striatum (mean concentrations of 20.46 ± 1.56 pmol ceramide/nmol phosphate).

At a concentration of 100 μM, C2-ceramide incubation for 10 min produced a 60% decrease in [^{3}H]dopamine uptake, effects similar in magnitude to application the protein kinase C-activating phorbol esters (Vaughan et al., 1997). For this reason, 100 μM C2-ceramide was used in the remaining experiments. The ceramide-induced decrease in [^{3}H]dopamine uptake caused by incubation with 100 μM ceramide was attributable to a decrease in V_{max} (1495.9 vs. 619.1 fmol/mg/min for control and C2-ceramide-treated synaptosomes, respectively) and no effect on K_m (73.3 vs. 63.9 nM for control and C2-ceramide-treated synaptosomes, respectively). Moreover, both C2-ceramide and C2-dihydroceramide treatment did not affect the B_{max} (250 ± 57.8, 227.3 ± 36 and 247.3 ± 41 pmol/g for control, C2-ceramide and C2-dihydroceramide, respectively) or K_d (9.3 ± 0.7, 16.7 ± 3.3 and 11.1 ± 1.5 nM for control, C2-ceramide and C2-dihydroceramide, respectively) of [^{3}H]WIN35428 binding. Results presented in Fig. 2 indicate that the ceramide-induced decrease in [^{3}H]dopamine uptake was reversible since washing of C2-ceramide-treated synaptosomes eliminated the decrease in [^{3}H]dopamine uptake.

In contrast to effects on [^{3}H]dopamine uptake, C2-ceramide application to striatal synaptosomes increased [^{3}H]5-HT uptake. The inactive ceramide analog, C2-dihydroceramide, had no significant effect on 5-HT uptake at a concentration of 100 μM (values for striatal synaptosomal 5-HT uptake were: control 147.2 ± 15.3, C2-ceramide 229.1 ± 6.4* and C2-dihydroceramide 135.2 ± 10.7 fmol/mg tissue; $n = 3$; *$P \leq 0.05$).

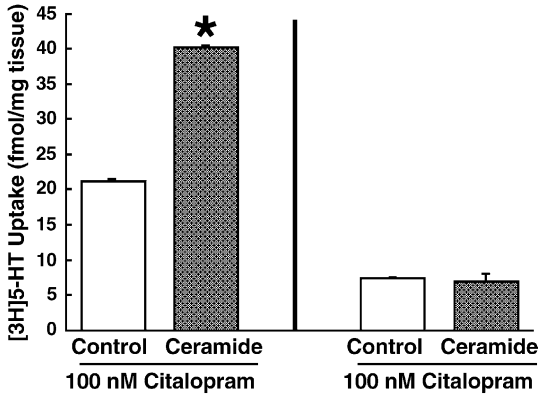

Fig. 3. Synaptosomes were incubated with C2-ceramide, citalopram and/or vehicle for 10 min prior to the addition of [^{3}H]5-HT. Columns represent means and vertical lines 1 S.E.M. of determinations from two independent experiments, with samples in each experiment run in triplicate. *Value for C2-ceramide-treated synaptosomes that is significantly different from control ($P \leq 0.05$).

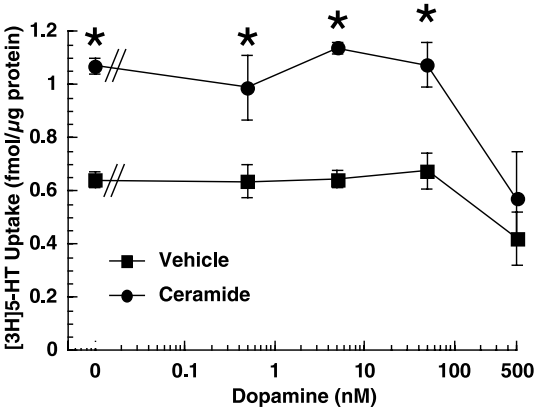

Fig. 5. Synaptosomes were incubated with C2-ceramide, DA and/or vehicle for 10 min prior to prior to the addition of [^{3}H]5-HT. Values represent means and vertical lines 1 S.E.M. of determinations from three independent experiments, with samples in each experiment run in triplicate. *Values for C2-ceramide-treated synaptosomes that are significantly different from concentration-matched controls ($P \leq 0.05$).

Because the striatum contains both dopamine and 5-HT transporters, the next experiments were designed to elucidate which transporter (dopamine or 5-HT) was responsible for the increase in synaptosomal 5-HT uptake. In a first experiment, 100 nM and 100 μM concentrations of citalopram were employed to selectively prevent 5-HT transport through the 5-HT transporter (IC_{50} 1.8 nM) and/or dopamine transporter (IC_{50} 41 μM; Hyttel, 1982). Results presented in Fig. 3 demonstrate that the ceramide-induced increase in 5-HT uptake is blocked by 100 μM citalopram (i.e., a concentration sufficient to block both dopamine and 5-HT transporter function). The ceramide-induced increase in 5-HT uptake was not blocked by 100 nM citalopram (i.e., a concentration sufficient to selectively inhibit 5-HT transporter function). In contrast, application of a relatively selective concentration (100 nM) of the dopamine reuptake inhibitor, methylphenidate (IC_{50} for dopamine and 5-HT uptake of 165 and 26000 nM, respectively (Fleckenstein et al., 1999)), prevented the ceramide-induced increase in 5-HT uptake (Fig. 4). It is noteworthy that this concentration of methylphenidate was not entirely selective, as it decreased 5-HT uptake per se by 22% (Fig. 4).

To further address the issue of substrate specificity, the ability of dopamine to compete for ceramide-affected 5-HT uptake was examined. Results presented in Fig. 5 demonstrate that dopamine dose-dependently competes for this transport. Specifically, 500 nM dopamine attenuated the ceramide-induced increase in 5-HT uptake. Noteworthy, however, are findings that this concentration decreased 5-HT uptake per se by approximately 33% (Fig. 5).

Results presented in Fig. 6 demonstrate that the ceramide-induced increase still occurs, even when applied to striatal synaptosomes prepared from rats treated previously with *para*-chloroamphetamine (7.5 mg/kg, i.p.), a dosing regimen

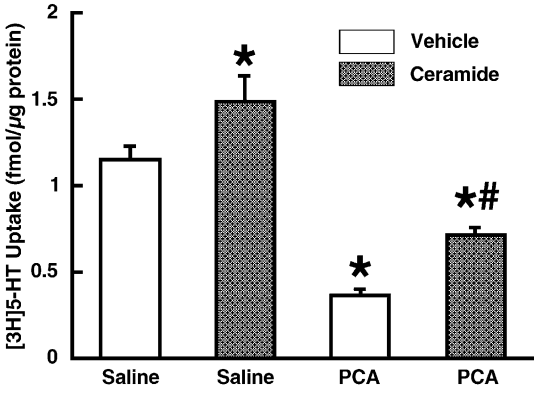

Fig. 6. Rats received *para*-chloroamphetamine (PCA; 7.5 mg/kg, s.c.) or saline vehicle (1 ml/kg, s.c.) 1 week prior to decapitation. Synaptosomes were prepared from the PCA- and saline-treated rats, and then incubated with 100 μM C2-ceramide or vehicle for 10 min prior to the addition of [^{3}H]5-HT. Columns represent means and vertical lines 1 S.E.M. of determinations in five to six rats. *Values for PCA-treated rats and/or ceramide-treated synaptosomes that are significantly different from control. #Value for PCA- and ceramide-treated groups that are significantly from PCA-treated rats ($P \leq 0.05$).

that destroyed 5-HT neurons (and presumably 5-HT transporters) as evidenced by a 57% decrease in [^{3}H]5-HT uptake 7 days after drug treatment.

In contrast to effects in striatal synaptosomes, C2-ceramide did not increase plasmalemmal [^{3}H]5-HT uptake in hippocampal synaptosomes. Instead, ceramide treatment decreased [^{3}H]5-HT uptake in synaptosomes prepared from hippocampal tissue (values for hippocampal synaptosomal 5-HT uptake were: control 2.04 ± 0.10, C2-ceramide 1.23 ± 0.17 fmol/μg protein; $n = 3$).

4. Discussion

Considerable attention has been directed towards elucidating the mechanism underlying the regulation of dopamine and 5-HT transporters. In particular, it has been demonstrated that phosphorylation decreases the activity, and leads to internalization, of dopamine and 5-HT transporters (Vaughan et al., 1997; Qian et al., 1997; Pristupa et al., 1998; Ramamoorthy and Blakely, 1999) The data presented in this manuscript demonstrate that the lipid second messenger, ceramide, alters the function of dopamine transporters. The physiological importance of the ceramide-induced changes in dopamine uptake is evidenced by findings that it is reversible. Specifically, application of C2-ceramide decreased striatal dopamine uptake and this phenomenon was reversed by washing ceramide from the preparation.

In contrast to its effects on dopamine uptake, ceramide application increased 5-HT uptake into striatal synaptosomes (which contain both dopamine and 5-HT transporters). The dopamine transporter transports dopamine readily, but has little affinity for 5-HT (i.e., the K_i for 5-HT inhibition of dopamine uptake is >10 μM; Giros et al., 1991). Blockade of both dopamine and 5-HT transporters by 100 μM citalopram completely prevented the ceramide-induced increase in 5-HT uptake. However, blockade of the 5-HT transporter, but not the dopamine transporter, by 100 nM citalopram did not prevent the increased 5-HT uptake. Hence, these data suggest that the increased uptake of 5-HT by striatal synaptosomes may be occurring through the dopamine transporter.

Consistent with the hypothesis that the ceramide-induced increase in 5-HT uptake is occurring through the dopamine transporter are findings that the increase is prevented upon application of a relatively selective concentration (100 nM) of the dopamine reuptake inhibitor, methylphenidate (the IC_{50} of methylphenidate for dopamine and 5-HT uptake are 165 and 26000 nM, respectively; Fleckenstein et al., 1999). These data are confounded, however, by the fact that these concentrations of methylphenidate were not entirely selective (e.g., application of 100 nM methylphenidate decreased 5-HT uptake by 22%). Accordingly, these data suggest either some uptake of 5-HT by the dopamine transporter, or that 100 nM methylphenidate is preventing a small amount of 5-HT uptake by the 5-HT transporter. Lower concentrations of methylphenidate (1 nM) did not prevent the ceramide-

induced increase in 5-HT uptake (data not shown). Unfortunately, this concentration was without effect on dopamine uptake per se and, therefore, render the 5-HT data inconclusive.

Since neither citalopram nor methylphenidate are perfectly selective as inhibitors of their respective transporters, and because reuptake inhibitors with absolute selectivity are not available, additional studies were conducted to test the hypothesis that ceramide is effecting 5-HT transport via the dopamine transporter. Accordingly, results presented in Fig. 6 demonstrate that ceramide still increases 5-HT uptake, even after a substantial number of 5-HT transporters have been destroyed as a result of *para*-chloroamphetamine administration. These data are confounded by the fact the *para*-chloroamphetamine lesion did not destroy all 5-HT projections. Still, the finding that ceramide increased 5-HT uptake by a similar magnitude in both saline-and *para*-chloroamphetamine-treated rats is consistent with the hypothesis of altered substrate recognition by the dopamine transporter.

Additional data presented in Fig. 5 support the hypothesis that ceramide-induced increases in 5-HT uptake are mediated via the dopamine transporter in that dopamine per se competed for this uptake. Even more compelling is the finding that ceramide does not increase 5-HT uptake in hippocampal synaptosomes: these data are predictable since the hippocampus is largely devoid of dopamine transporters and therefore lacking targets upon which ceramide might act. Rather, ceramide treatment decreased 5-HT uptake. These data suggest that ceramide may decrease 5-HT transport via 5-HT transporters, per se, or via other transporters found in the hippocampus (i.e., the norepinephrine transporter).

Since ceramides activate both protein kinases and phosphatases, the ceramide-induced changes in dopamine uptake may be due to altered phosphorylation of the dopamine transporter. The dopamine transporter contains many consensus phosphorylation sites and, as noted above, activation of protein kinase C by phorbol esters decreases dopamine transporter activity and leads to internalization of the transporter. Accordingly, the ceramide-induced decrease in dopamine uptake could be explained by phosphorylation and internalization of the dopamine transporter. This hypothesis is not, however, sufficient to explain all of the data since the ceramide-induced *increase* in 5-HT uptake would not occur if transporters were internalized. Moreover, the lack of effect of ceramide on WIN35428 binding is not consistent with internalization, assuming that WIN35428 is not membrane-permeable. It is possible that differing phosphorylation sites mediate differing effects (i.e., internalization vs. substrate recognition). In addition, the ceramide-induced effects on the dopamine transporter may represent a novel phosphorylation-independent mechanism of transporter regulation. Further studies are necessary to elucidate mechanisms whereby this lipid second messenger affects monoamine transporter function.

In summary, we conclude that ceramide decreases dopamine uptake through the dopamine transporter. In addition, ceramide appears to increase 5-HT uptake through the dopamine transporter. Noteworthy are recent studies demonstrating 5-HT transport into dopamine neurons (Suarez-Roca and Cubeddu, 2002; Zhou et al., 2002). The present data extend these findings by suggesting that such uptake may be enhanced by ceramide treatment. These data are the first to demonstrate that transporter function can be rapidly and reversibly regulated by a lipid second messenger.

Acknowledgements

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References

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911–917.

Carvelli, L., Moron, J.A., Kahlig, K.M., Ferrer, J.V., Sen, N., Lechleiter, J.D., Leeb-Lundberg, L.M., Merrill, G., Lafer, E.M., Ballou, L.M., Shippenberg, T.S., Javitch, J.A., Lin, R.Z., Galli, A., 2002. PI 3-kinase regulation of dopamine uptake. J. Neurochem. 81, 859–869.

Copeland, B.J., Vogelsberg, V., Neff, N.H., Hadjiconstantinou, M., 1996. Protein kinase C activators decrease dopamine uptake into striatal synaptosomes. J. Pharmacol. Exp. Ther. 277, 1527–1532.

Dobrowsky, R.T., Hannun, Y.A., 1992. Ceramide stimulates a cystolic protein phosphatase. J. Biol. Chem. 267, 5048–5051.

Fleckenstein, A.E., Metzger, R.R., Wilkins, D.G., Gibb, J.W., Hanson, G.R., 1997. Rapid and reversible effects of methamphetamine on dopamine transporters. J. Pharmacol. Exp. Ther. 282, 834–838.

Fleckenstein, A.E., Haughey, H.H., Metzger, R.R., Kokoshka, J.M., Riddle, E.L., Hanson, J.E., Gibb, J.W., Hanson, G.R., 1999. Differential effects of psychostimulants and related agents on dopaminergic and serotonergic transporter function. Eur. J. Pharmacol. 382, 45–49.

Giros, B., Mestikawy, S.E., Bertrand, L., Caron, M.G., 1991. Cloning and functional characterization of a cocaine-sensitive dopamine transporter. FEBS Lett. 295, 149–154.

Hannun, Y.A., Loomis, C.R., Merrill, A., Bell, R.M., 1986. Sphingosine inhibition of protein kinase C activity and of phorbol dibutyrate binding in vitro and in human platelets. J. Biol. Chem. 261, 12604–12609.

Huff, R.A., Vaughan, R.A., Kuhar, M.J., Uhl, G.R., 1997. Phorbol esters increase dopamine transporter phosphorylation and decrease transport V_{max}. J. Nuerochem. 68, 225–232.

Hyttel, J., 1982. Citalopram—pharmacological profile of a specific serotonin uptake inhibitor with antidepressant activity. Prog. Neuropsychopharmacol. Biol. Psychiatry 6, 277–295.

Kokoshka, J.M., Vaughan, R.A., Hanson, G.R., Fleckenstein, A.E., 1998. Nature of methamphetamine-induced rapid and reversible changes in dopamine transporters. Eur. J. Pharmacol. 361, 269–275.

Okazaki, T., Bielawska, A., Bell, R.M., Hannun, Y.A., 1990. Role of ceramide as a lipid mediator of 1α,25-dihydroxyvitamin D_3-induced HL-60 cell differentiation. J. Biol. Chem. 265, 15823–15831.

Preiss, J., Loomis, C.R., Bishop, W.R., Stein, R., Niedel, J.E., Bell, R.M., 1986. Quantative measurement of sn-1,2-diacylglycerols present in platelets, hepatocytes, and ras- and cis-transformed normal rat kidney cells. J. Biol. Chem. 261, 8597–8600.

Pristupa, Z.B., McConkey, F., Liu, F., Man, H.Y., Lee, F.J., Wang, Y.T., Niznik, H.B., 1998. Protein kinase-mediated bidirectional trafficking and functional regulation of the human dopamine transporter. Synapse 30, 79–87.

Qian, Y., Galli, A., Ramamoorthy, S., Risso, S., DeFelice, L.J., Blakely,

N.R., 1997. Protein kinase C activation regulates human serotonin transporters in HEK-293 cells via altered cell surface expression. J. Neurosci. 17, 45–57.

Ramamoorthy, S., Blakely, R.D., 1999. Phosphorylation and sequestration of serotonin transporters differentially modulated by psychostimulants. Science 285, 763–766.

Sietsma, H., Veldman, R.J., Kok, J.W., 2001. The involvement of sphingolipids in multidrug resistance. J. Membr. Biol. 181, 153–162.

Suarez-Roca, H., Cubeddu, L.X., 2002. The selective serotonin reuptake inhibitor citalopram induces the storage of serotonin in catecholaminergic terminals. J. Pharmacol. Exp. Ther. 302, 174–179.

Tanabe, F., Cui, S., Ito, M., 1998. Ceramide promotes calpain-mediated proteolysis of protein kinase C β in murine polymorphonuclear leukocytes. Biochem. Biophys. Res. Commun. 242, 129–133.

Vaughan, R.A., Huff, R.A., Uhl, G.R., Kuhar, M.J., 1997. Protein kinase C-mediated phosphorylation and functional regulation of dopamine transporters in striatal synaptosomes. J. Biol. Chem. 272, 15541–15546.

Westwick, J.K., Bielawska, A.E., Dbaibo, G., Hannun, Y.A., Brenner, D.A., 1995. Ceramide activates the stress-activated protein kinases. J. Biol. Chem. 270, 22689–22692.

Whatley, R.E., Stroud, E.D., Bunting, M., Zimmerman, G.A., McIntyre, T.M., Prescott, S.M., 1993. Growth-dependent changes in arachidonic acid release from endothelial cells are mediated by protein kinase C and changes in diacylglycerol. J. Biol. Chem. 268, 16130–16138.

Zhou, F.C., Lesch, K.P., Murphy, D.L., 2002. Serotonin uptake into dopamine neurons via dopamine transporters: a compensatory alternative. Brain Res. 942, 109–119.

Zhu, S., Kavanaugh, M.P., Sonders, M.S., Amara, S.G., Zahniser, N.R., 1997. Activation of protein kinase C inhibits uptake, currents and binding associated with the human dopamine transporter expressed in xenopus oocytes. J. Pharmacol. Exp. Ther. 282, 1358–1365.