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Estrogen priming modulates autoreceptor-mediated potentiation of dopamine uptake

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Abstract

The ability of a physiological dose of estrogen (estradiol benzoate, estrogen: 10 μg 48 and 24 h prior) to modulate autoreceptor-mediated changes in dopamine transport properties was investigated in a synaptosomal preparation prepared from the nucleus accumbens of ovariectomized rats. Quinpirole (1–100 μM)-mediated potentiation of [^{3}H]dopamine uptake was attenuated in synaptosomes from estrogen-primed animals. Haloperidol (10 μM) inhibited basal uptake and effectively prevented quinpirole potentiation of uptake in both ovariectomized and estrogen-primed samples. The ability of selective protein phosphatase inhibitors to modulate autoreceptor-mediated potentiation of dopamine uptake was also examined. Pretreatment with protein phosphatase 2B (deltamethrin, cypermethrin) or protein phosphatase 1 (tautomycin) inhibitors attenuated basal and quinpirole-potentiated dopamine uptake in ovariectomized but not estrogen-primed tissue. These data suggest that autoreceptor-mediated activation of dopamine transport can be regulated by physiological doses of estrogen and implicate a role for protein phosphorylation in autoreceptor-mediated potentiation of dopamine uptake.

Keywords: Steroid; Protein phosphatase; Dopamine D_2 receptor agonist; Dopamine D_2 receptor antagonist; Uptake

1. Introduction

We have shown previously that exposure to estrogen can alter parameters of dopamine clearance in the nucleus accumbens in vivo (Thompson and Moss, 1994; Thompson, 1999). Specifically, estrogen (estradiol benzoate, estrogen) priming results in an attenuation of stimulated dopamine release and the reuptake of both endogenous and exogenous dopamine as measured by in vivo voltammetry. In addition, an alteration in both release and reuptake has been observed over the estrous cycle (Thompson and Moss, 1997). Physiologically, the action of the dopamine transporter is primarily responsible for removing released dopamine from the synaptic cleft (Horn, 1990; Garris and Wightman, 1995). The ability of estrogen to modulate dopamine transporter-binding properties has been demonstrated in vitro (Morissette and DiPaolo, 1993a,b; reviewed by DiPaolo, 1994; Attali et al 1997). The dopamine transporter is a member of the high affinity Na^+-dependent neurotransmitter transporter family (Hitri et al., 1994). While inhibition of the dopamine transporter by stimulants, such as cocaine or amphetamine, cause an increase in extracellular dopamine and behavioral activation, little is known about the normal regulation of this protein. The dopamine transporter is a membrane spanning protein (approximately 69 kDa), which has five putative phosphorylation sites on the cytoplasmic domain. There are three consensus sites for protein kinase A and two consensus sites for protein kinase C, one of which overlaps with a calmodulin kinase II site (Giros and Caron, 1993). Agents that result in increased protein kinase C activity have been shown to alter dopamine uptake in cells expressing the transfected transporter (Kitayama et al., 1994; Zhang et al., 1997). Phorbol ester-mediated phosphorylation of the transporter protein has been documented using a stably expressing LLC-PK1 cell line (Huff et al., 1997) and striatal synaptosomes (Vaughan et al., 1997). In all instances, conditions that promote phosphorylation of the protein by protein kinase C are associated with a decrease

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in dopamine uptake characterized by a reduction in V_{max}. In a recent report, Batchelor and Schenk (1998) have reported an upregulation of dopamine transporter activity in synaptosomes in response to pharmacological elevation of cAMP characterized by an increase in V_{max} with no change in K_m. These data suggest that phosphorylation may be an important regulatory mechanism for the activation/deactivation of the dopamine transporter.

The activity of the dopamine transporter also appears to be regulated by the dopamine D_2 autoreceptor. Evidence collected over the last several years suggests that dopamine D_2 autoreceptor activation can potentiate dopamine uptake (Parsons et al., 1993; Meiergerd et al., 1993; Cass and Gerhardt, 1994; Batchelor and Schenk, 1998). The cellular mechanism(s) mediating this response are not known but the modulation of G-protein-dependent processes with corresponding changes in second messengers is likely to be involved.

The regulation of dopamine uptake by estrogen provides a mechanism for controlling the extrasynaptic availability of dopamine. This regulation may be mediated indirectly through a modulation of autoreceptor activation and subsequent autoreceptor-mediated cellular processes or directly through an alteration in the activity of the transporter by a change in dopamine-transporter density or affinity. The present study was designed to examine steroid modulation of autoreceptor-mediated regulation in dopamine uptake.

2. Materials and methods

2.1. Materials

Estradiol benzoate was purchased from Sigma (St. Louis, MO). Quinpirole, sulpiride, haloperidol and *S*(−)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride (SCH23390) were all purchased from RBI (Natick, MA). Protein phosphatase inhibitors deltamethrin, cypermethrin, tautomycin and cantharidin were purchased from CalBiochem (LaJolla, CA). [^{3}H]dopamine was obtained from American Radiolabeled Chemicals (St. Louis, MO).

2.2. Subjects

Adult (200–300 g) female Sprague–Dawley rats were used. All animals underwent surgical ovariectomies under metofane anesthesia. Through a single incision, the bicornuate uterus was located, the ovaries were ligated and resected bilaterally. The animals were allowed to recover for at least 7 days. Some animals received 10 μg estrogen (in corn oil) 24 and 48 h prior to experiment. Animals were housed three to four per cage with free access to food and water. All animal procedures were preapproved by the Institutional Animal Care Advisory Committee at Mercer and in strict accordance with the National Institute of Health guidelines.

2.3. Synaptosomal preparation and [^{3}H]dopamine uptake

Animals were decapitated and nucleus accumbens tissue rapidly dissected. Pooled tissue (from two to three animals) was homogenized in 40 volumes of 0.32 M sucrose, 5 mM HEPES, pH 7.4, and centrifuged at $1000 \times g$ for 5 min. The supernatant was centrifuged at $20{,}000 \times g$ for 20 min to sediment the P_2 fraction. The P_2 pellet was resuspended (approximately 10 mg/ml original wet weight) in a HEPES-buffered medium [145 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 10 mM glucose, 1 mM ascorbate (freshly prepared), 10 mM HEPES, pH 7.4], bubbled briefly with 95% O_2–5% CO_2 and an aliquot of pargyline added to produce a final concentration of 0.1 mM. Tissue suspensions (300 μl) were preincubated at 35°C for 5 min in the presence of quinpirole (10 μM) and/or dopamine receptor antagonists (10 μM sulpiride, haloperidol and SCH23390) prior to addition of [^{3}H]dopamine (0.065 μM final concentration). During each experiment, 4°C blanks were included and these counts were subtracted from the total to represent specific uptake. Final assay volume was 350 μl. In some experiments, synaptosomes were incubated for 5 min in the presence of phosphatase inhibitors (deltamethrin: 1 μM, 0.1 μM; cypermethrin: 1 μM, 0.1 μM; tautomycin: 0.75 μM, 0.1 μM; or cantharidin: 1 μM) prior to incubation with quinpirole. Inhibitors were dissolved in 50% ethanol and diluted to final concentration in assay buffer. Vehicle controls were included in all assays. In these experiments, uptake was allowed to proceed for 1 min and terminated by the addition of 4 ml ice-cold HEPES-buffered medium and rapid filtration using a Millipore filter apparatus. Filters were washed two times with 4 ml buffer, air-dried and radioactivity measured by liquid scintillation counting.

In some experiments, both uptake time and quinpirole dose were varied. In these studies, tissue suspensions (final volume 500 μl) were preincubated as above in the presence of 0, 1, 10 or 100 μM quinpirole prior to the addition of [^{3}H]dopamine. An aliquot of the suspension (100 μl) was removed at 20, 40 and 60 s and placed directly on glass-fiber filters in a Millipore filter apparatus. Filters were washed three times with 4 ml buffer, air-dried and radioactivity measured by liquid scintillation counting.

2.4. Dopamine kinetics

[^{3}H]dopamine uptake kinetics were also evaluated using synaptosomes prepared from control and estrogen-primed animals ($n = 4$ experiments/group with each done in quadriplicate). In order to assess K_m and V_{max} values,

[^{3}H]dopamine uptake was measured over 1 min in the presence of increasing concentrations of cold dopamine [0.016–4.35 μM]. Uptake was terminated by the addition of 4 ml ice-cold HEPES-buffered medium and rapid filtration using a Millipore filter apparatus. Filters were washed two times with 4 ml buffer, air-dried and radioactivity measured by liquid scintillation counting as described above. K_m and V_{max} values were calculated by graphic analysis of Lineweaver–Burk double reciprocal plots.

2.5. Statistical analysis

Data from uptake experiments are expressed as either counts per minute/mg (c.p.m./mg) original wet tissue weight or percent control. In all cases, values represent the mean ± S.E.M. of at least three experiments done in quadruplicate. N is the number of individual experiments. Data were averaged and the variability expressed as the standard error of the mean (± S.E.M.). One- or two-way analysis of variance (ANOVA) followed by pairwise multiple comparisons (Student–Newman–Keuls) or paired t-tests were used to determine statistical significance. A P value < 0.05 was considered significant.

3. Results

3.1. Dopamine kinetics

No statistical difference was observed in either the affinity or the rate of transport following exposure to steroid over a range of dopamine concentrations (Fig. 1). The average V_{max} and K_m for ovariectomized animals was 11.7 ± 0.87 pmol/mg/min and 0.73 ± 0.05 μM, respectively. Similarly, for estrogen-primed animals the V_{max} was 9.72 ± 0.51 pmol/mg/min and the K_m was 0.55 ± 0.07 μM. These values, averaged from four experiments, were not significantly different.

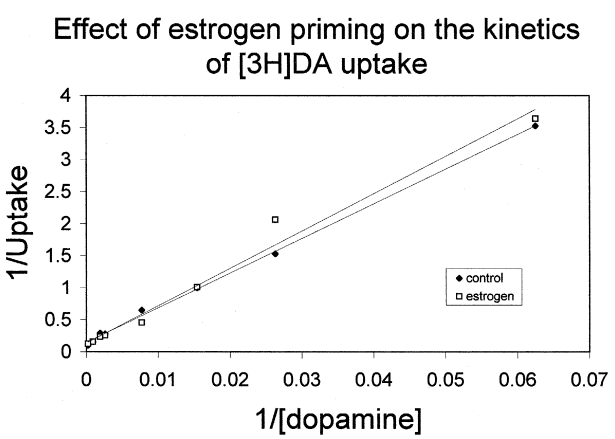

Fig. 1. [^{3}H]dopamine uptake: Lineweaver–Burk plot. Effect of estrogen priming on the kinetics of [^{3}H]dopamine uptake was determined ($n = 4$ for each condition). Synaptosomes were prepared from control and estrogen (10 μg 24 and 48 h prior) primed animals and uptake measured as described. No statistical differences were detected in K_m or V_{max} between the two groups.

3.2. Effect of quinpirole dose and uptake time on [^{3}H]dopamine accumulation

The ability of quinpirole to potentiate [^{3}H]dopamine uptake was determined in a synaptosomal-enriched fraction prepared from the nucleus accumbens of ovariectomized and estrogen-primed animals.

[^{3}H]dopamine uptake was enhanced in tissue from both ovariectomized and estrogen-primed animals. (Table 1). In ovariectomized animals, significant increases were observed in response to 10 and 100 μM quinpirole at all times tested. While 1 μM quinpirole also appeared to potentiate [^{3}H]dopamine uptake, it did not quite reach statistical significance. Tissue prepared from estrogen-primed animals was also responsive to quinpirole. Significant increases in uptake were observed at 20 and 60 s in response to 10 or 100 μM quinpirole, respectively. Basal dopamine uptake was not significantly different in tissue from ovariectomized and estrogen-primed animals; however, a significant difference in quinpirole potentiation of uptake was determined ($P < 0.05$). The magnitude of this difference was variable depending on both the dose of quinpirole examined and the total uptake time. At 20 s, and in the presence of 100 μM quinpirole, [^{3}H]dopamine uptake was almost 60% higher in tissue prepared from ovariectomized animals as compared to tissue from estrogen-primed animals. At other times and doses, [^{3}H]dopamine uptake was 25–45% greater in synaptosomes prepared from ovariectomized animals.

3.3. Effect of dopamine receptor agonist and antagonist treatment on [^{3}H]dopamine uptake in vitro

The ability of dopamine receptor antagonists to block quinpirole (10 μM, 60 s uptake) potentiation of [^{3}H]dopamine uptake in tissue from ovariectomized and estrogen-primed animals was also examined (Fig. 2). Haloperidol (10 μM), a mixed dopamine D_2/D_1 receptor antagonist, attenuated basal and agonist-stimulated uptake in both groups (approximately 40% decrease in uptake, $P < 0.05$). While the dopamine D_2 receptor antagonist sulpiride (10 μM) caused a slight decrease in basal dopamine uptake in synaptosomes from ovariectomized and estrogen-primed animals, this decrease did not reach statistical significance. However, potentiated uptake was significantly reduced in both groups ($P < 0.05$). In contrast, the dopamine D_1 receptor antagonist SCH-23390 (1 μM) had no effect on basal or quinpirole-stimulated dopamine uptake.

Table 1
Quinpirole potentiation of [^{3}H]dopamine uptake

Quinpirole (μM)	20 s		40 s		60 s	
	Control	Estrogen	Control	Estrogen	Control	Estrogen
0	1593 ± 140	1300 ± 136	2468 ± 289	2226 ± 247	3370 ± 356	2700 ± 142
1	2484 ± 167	1885 ± 311	3231 ± 203	2541 ± 338	3248 ± 333	2219 ± 199[d]
10	3693 ± 322[a,b]	3270 ± 363[a,b,c]	3969 ± 226[a]	3124 ± 294[d]	4570 ± 134[a,b]	3173 ± 227[d]
100	3078 ± 431[a]	1935 ± 115[d]	3539 ± 153[a]	2855 ± 119[d]	4405 ± 129[a]	3330 ± 164[a,b,d]

Synaptosomes prepared from ovariectomized or estrogen-primed animals were preincubated in the presence or absence of quinpirole for 5 min prior to incubation with [^{3}H]dopamine as described in Methods. Uptake was terminated after 20, 40 or 60 s by filtration through glass fiber filters followed by three 4 ml rinses with ice-cold buffer. Data are presented as c.p.m./mg original wet tissue weight and are expressed as the means ± S.E.M. obtained from four to nine experiments. Statistical significance was determined by ANOVA followed by suitable multiple comparison tests or Student's *t*-test as described in methods. $P < 0.05$: (a) vs. no drug, time and tissue matched; (b) vs. 1 μM quinpirole, time and tissue matched; (c) vs. 100 μM quinpirole, time- and tissue-matched; (d) vs. drug- and time-matched control.

3.4. Effect of protein phosphatase inhibition on quinpirole potentiation of dopamine uptake in vitro

The ability of selective protein phosphatase inhibitors to modulate basal and quinpirole potentiation of [^{3}H]dopamine uptake was examined (Fig. 3). In these experiments, tissue from ovariectomized and estrogen-primed rats was pretreated with a phosphatase inhibitor or vehicle (aliquot of 50% ethanol) for 5 min prior to incubation with quinpirole. [^{3}H]dopamine uptake was allowed to proceed for 1 min as before. Data are presented as percent change from vehicle control and are the means of at least three experiments performed in quadruplicate. In tissue from ovariectomized animals, pretreatment with either of the protein phosphatase 2B inhibitors, deltamethrin or cypermethrin, produced an attenuation of basal and quinpirole-potentiated dopamine uptake ($P < 0.05$). This effect was dose-dependent, 1 μM, but not 0.1 μM, was effective (data not shown). In contrast, inhibition of protein phosphatase 2B has no effect on basal or stimulated dopamine uptake in tissue prepared from estrogen-primed animals. Tautomycin, a selective protein phosphatase 1 inhibitor produced a slight, but significant, attenuation of dopamine uptake in response to quinpirole in ovariectomized, but not estrogen-primed tissue. This effect was also dose-dependent; 0.75 μM, but not 0.1 μM, tautomycin was effective.

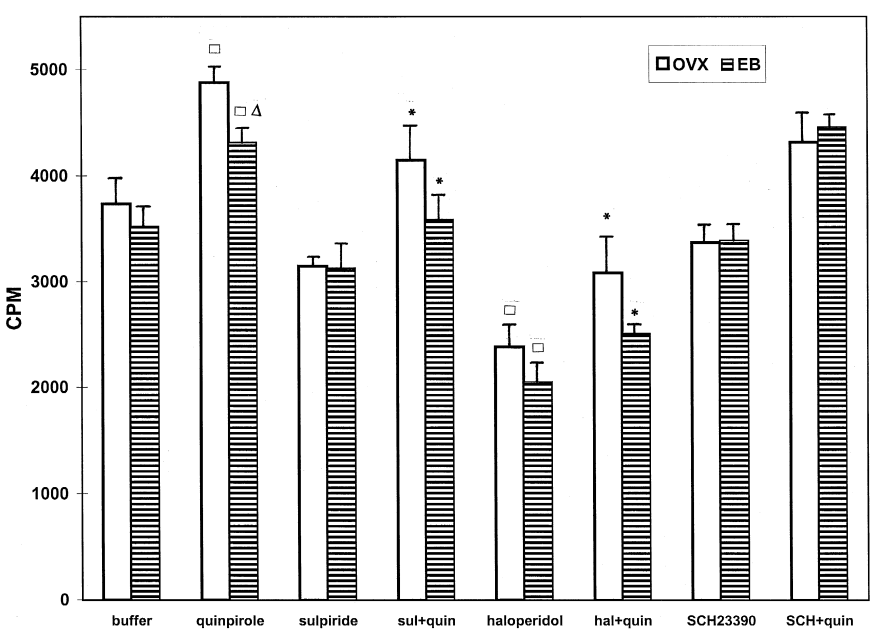

Fig. 2. Effect of agonist and antagonist treatment on [^{3}H]dopamine uptake in synaptosomes from ovariectomized and estrogen-primed animals. [^{3}H]dopamine uptake was measured in synaptosomal suspensions prepared from ovariectomized or estrogen-primed animals. Tissue was preincubated with quinpirole (10 μM), sulpiride (10 μM), haloperidol (10 μM) or SCH-23390 (1 μM) as described. Results are expressed as the mean ± S.E.M. of 4–14 experiments done in quadruplicate. $P < 0.05$: # vs. buffer; * vs. quinpirole; Δ vs. ovariectomized.

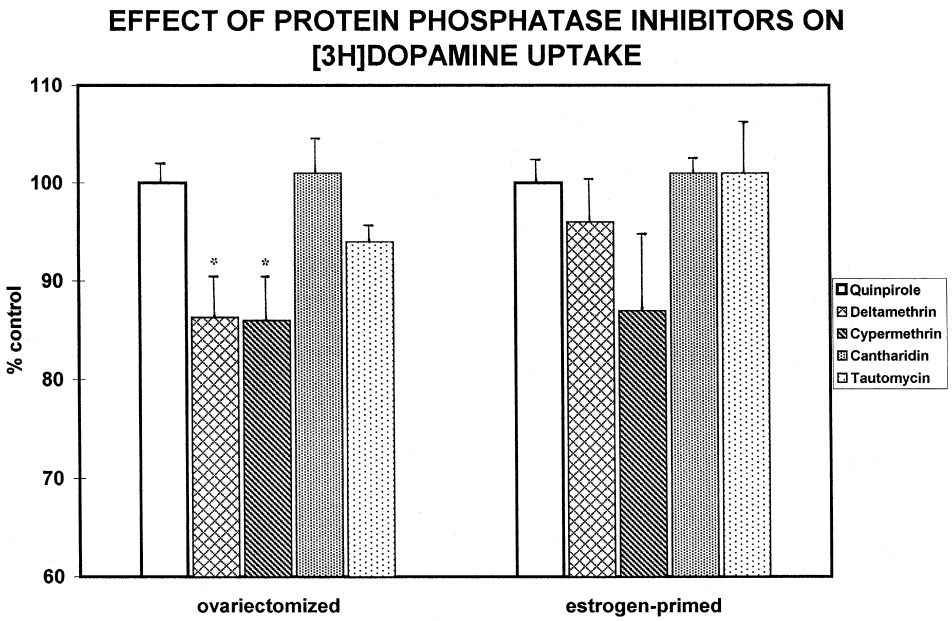

Fig. 3. Effect of protein phosphatase inhibitors on [^{3}H]dopamine uptake. Synaptosomes were preincubated with phosphatase inhibitors 5 min prior to quinpirole addition. [^{3}H]dopamine uptake was measured as described. Protein phosphatase 2b inhibitors = deltamethrin and cypermethrin (1 μM); protein phosphatase 2a inhibitors = cantharidin (1 μM); protein phosphatase 1 inhibitor = tautomycin (0.75 μM). Data are presented as percent uptake in the presence of quinpirole (10 μM). $N = 3–5$ experiments per condition. * $P < 0.05$ with respect to quinpirole.

Cantharidin, a selective protein phosphatase 2A inhibitor, had no effect on basal or quinpirole-potentiated uptake in either group.

4. Discussion

Rapid and controlled removal of neurotransmitters from the synaptic cleft is essential for effective neuronal communication. Dopamine clearance proceeds primarily by high affinity uptake into the presynaptic terminal by action of the dopamine transporter and to a lesser extent by diffusion and extracellular metabolism (Horn, 1990; Garris and Wightman, 1995). The cellular mechanisms responsible for the regulation of the dopamine transporter are unknown, long-term regulation may involve changes in protein synthesis and/or turnover, while rapid regulation may be mediated by posttranslational modification of the protein (Reith et al., 1997). In the current study, the ability of estrogen to modulate the activity of mesolimbic dopamine transporter activity was investigated. It was hypothesized that estrogen priming may modulate dopamine reuptake through an alteration in dopamine autoreceptor activation. This hypothesis was tested by measuring changes in [^{3}H]dopamine uptake in response to pharmacological manipulation in a synaptosomal preparation prepared from the nucleus accumbens of ovariectomized and estrogen-primed animals.

The results of this study demonstrate, for the first time, that autoreceptor-mediated potentiation of dopamine uptake is altered following estrogen priming and that this effect is achieved with a physiologically relevant dose of estrogen. While no apparent change in basal [^{3}H]dopamine uptake was observed following estrogen priming, a decrease in quinpirole-potentiated uptake was observed in these animals. The fact that no change in basal uptake was detected suggests that estrogen priming did not have a direct effect on either the density or affinity of the dopamine transporter. This is consistent with results reported by Morisette and DiPaolo (1993b). In that study, no change in [^{3}H]-GBR-12935 binding was detected in the accumbens of ovariectomized animals primed with estrogen. In the current report, steroid treatment resulted in an attenuation of autoreceptor-mediated dopamine uptake. Changes in striatal dopamine D_2 receptor affinity have been reported over the estrous cycle (Di Paolo et al. 1988) and Levesque and DiPaolo (1993) have reported apparent alterations in dopamine D_2 receptor/G-protein coupling following estrogen treatment. Together, these data suggest that the estrogen-priming conditions utilized in the current study may have been sufficient to produce an alteration in dopamine D_2 receptor activity, as measured by an attenuation of agonist-mediated uptake, which is consistent with a functional downregulation of the presynaptic autoreceptor.

Haloperidol (and to a lesser extent sulpiride) was found to be very effective in inhibiting basal [^{3}H]dopamine uptake in both ovariectomized and estrogen-primed animals. Inhibition of quinpirole-stimulated dopamine uptake by sulpiride has been reported in striatal suspensions prepared from male rats (Batchelor and Schenk, 1998). In the current study, inhibition of potentiated uptake was found to be receptor subtype specific in that the dopamine D_1

receptor antagonist SCH23390 was without effect. No apparent difference was detected in response to antagonist treatment between ovariectomized and estrogen-primed animals as might be expected if estrogen priming produced a functional downregulation of the dopamine D_2 receptor. However, this negative finding may be due to the fact that only one antagonist concentration was used. It is conceivable that a lower concentration of the antagonist would be sufficient to block the quinpirole effect and at the same time allow for the detection of subtle differences in receptor sensitivity. This possibility is currently being investigated.

The ability of dopamine D_2 receptor antagonists to attenuate basal uptake independent of steroid priming is particularly interesting. While one generally assumes that antagonists block the action of agonists without producing an independent effect, this is frequently not the case (Milligan et al., 1995; Griffon et al., 1996). The observation that dopamine D_2 receptor antagonists inhibited dopamine uptake reinforces the significance of the association between receptor activity and dopamine transport. It suggests that intracellular processes that normally mediate the activity of the dopamine transporter can be inhibited simply by preventing autoreceptor activation. The dopamine D_2 autoreceptor is a G-protein linked receptor, which is negatively coupled to adenylate cyclase (Jarvie et al., 1994). Dopamine binding to the autoreceptor results in a decrease in cAMP production and therefore a decrease in protein kinase A activation. Autoreceptor-mediated suppression of protein kinase C has also been reported (Iannazzo et al., 1997). In addition, incorporation of [^{32}Pi] into striatal tyrosine hydroxylase is reduced following treatment with low concentration of pergolide, a dopamine D_2 autoreceptor agonist (Salah et al, 1989). Together, these data suggest that activation of the dopamine D_2 presynaptic receptor results in conditions favorable to the dephosphorylation of cellular proteins. Because conditions that promote protein phosphorylation have been shown to alter dopamine transporter activity and autoreceptor activation has been shown to increase dopamine transporter activity; we hypothesized that the dopamine D_2 receptor may regulate dopamine-transporter activity through a potentiation of protein phosphatase activity (Kitayama et al., 1994; Jarvie et al., 1994; Huff et al, 1997; Reith et al., 1997; Batchelor and Schenk, 1998). To investigate this possibility, the ability of three classes of protein phosphatase inhibitors to modulate basal and quinpirole potentiation of [^{3}H]dopamine uptake was examined using tissue from both ovariectomized and estrogen-primed animals.

Deltamethrin, cypermethrin and tautomycin were effective in preventing agonist potentiation of dopamine uptake in ovariectomized animals. These chemicals have been shown to be potent and selective inhibitors of protein phosphatase 2B and protein phosphatase 1 in a number of cell systems (Gong et al., 1992; Enan and Matsumura, 1992; Fischer et al., 1998). In addition, treatment with the protein phosphatase 2B inhibitors also produced a slight but significant attenuation of basal uptake, which is consistent with the decrease in basal uptake observed in response to the dopamine D_2 receptor antagonists. These data strongly suggest that the dopamine D_2 autoreceptor modulates dopamine transporter activity through a regulation of a protein phosphorylation cascade, which involves, at least in part, activation of protein phosphatase 2B and protein phosphatase 1. This response was selective, the inhibition of phosphatases whose activity is directly linked to dopamine receptor activation was effective in blocking quinpirole potentiation of uptake while the inhibition of protein phosphatase 2A was ineffective. Whether the dopamine transporter is a direct substrate for protein phosphatase 2B and/or protein phosphatase 1 remains to be determined. Interestingly, in estrogen-primed tissue none of these compounds were effective in altering [^{3}H]dopamine uptake. Assuming estrogen priming results in a functional downregulation of the dopamine D_2 receptor and agonist potentiation of dopamine uptake requires the activation of a dephosphorylation cascade, autoreceptor-mediated changes in protein dephosphorylation would be expected to be reduced in estrogen-primed animals. Under these conditions, the effect of protein phosphatase inhibition would be attenuated. This is what was observed in the current study.

In summary, autoreceptor-mediated potentiation of dopamine uptake in the nucleus accumbens can be attenuated by estrogen priming. This attenuation is consistent with a functional downregulation of the presynaptic dopamine D_2 autoreceptor. While the cellular mechanisms responsible for this attenuation have not been elucidated, the current results suggest that autoreceptor-mediated regulation of dopamine transport can be modulated by protein phosphorylation-dependent mechanisms. Estrogen priming may impair the ability of the dopamine autoreceptor to be effectively coupled with cellular messengers responsible for regulating the activity of the dopamine transporter.

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References

Attali, G., Weizman, A., Gil-Ad, I., Rehavi, M., 1997. Opposite modulatory effects of ovarian hormones on rat brain dopamine and serotonin transporters. Brain Res. 756, 153–159.

Batchelor, M., Schenk, J.O., 1998. Protein kinase A activity may kinetically upregulate the striatal transporter for dopamine. J. Neurosci. 18, 10304–10309.

Cass, W.A., Gerhardt, G.A., 1994. Direct in vivo evidence that D_2

dopamine receptors can modulate dopamine uptake. Neurosci. Lett. 176, 259–263.

Di Paolo, T., 1994. Modulation of brain dopamine transmission by sex steroids. Rev. Neurosci. 5, 27–42.

Di Paolo, T., Falardeau, P., Morissette, M., 1988. Striatal dopamine agonist binding sites fluctuate during the rat estrous cycle. Life Sci. 43, 665–672.

Enan, E., Matsumura, F., 1992. Specific inhibition of calcineurin by type II synthetic pyrethroid insecticides. Biochem. Pharmacol. 43, 1777–1784.

Fischer, H., Illek, B., Machen, T., 1998. Regulation of CFTR by protein phosphatase 2B and protein kinase C. Eur. J. Physiol. 436, 175–181.

Garris, P., Wightman, R.M., 1995. Regional differences in dopamine release, uptake and diffusion measured by fast-scan cyclic voltammetry. In: Boulton, A.A., Baker, G.B., Adams, R.N. (Eds.), Neuromethods: Voltammetric Methods in Brain Systems. Humana Press, Totowa, NY, pp. 179–220.

Giros, B., Caron, M.G., 1993. Molecular characterization of the dopamine transporter. Trends Pharmacol. Sci. 14, 43–49.

Gong, M.C., Cohen, P., Kitazawa, T., Ikebe, M., Masuo, M., Somlyo, A.P., Somiyo, A.V., 1992. Myosin light chain phosphatase activities and the effects of phosphatase inhibitors in tonic and phasic smooth muscle. J. Biol. Chem. 267, 14552–14668.

Griffon, N., Pilon, C., Sautel, F., Schwartz, J-C., Sokoloff, P., 1996. Antipsychotics with inverse agonist activity at the dopamine D_3 receptor. J. Neural Transm. 103, 1163–1175.

Hitri, A., Hurd, Y.L., Wyatt, R.J., Deutsch, S.I., 1994. Molecular, functional and biochemical characteristics of the dopamine transporter: regional differences and clinical relevance. Clin. Neuropharmacol. 17, 1–22.

Horn, A.S., 1990. Dopamine uptake: a review of progress in the last decade. Prog. Neurobiol. 43, 387–400.

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

Iannazzo, L., Sathananthan, S., Majewski, H., 1997. Modulation of dopamine release from rat striatum by protein kinase C: interaction with presynaptic D_2-dopamine-autoreceptors. Br. J. Pharmacol. 122, 1561–1566.

Jarvie, K.R., Tiberi, M., Caron, M.G., 1994. Dopamine receptors and transporters. In: Niznik, H.B. (Ed.), Pharmacology, Structure and Function. Marcel Dekker, New York, pp. 133–150.

Kitayama, S., Dohi, T., Uhl, G., 1994. Phorbol esters alter functions of the expressed dopamine transporter. Eur. J. Pharmacol. 268, 115–119.

Levesque, D., Di Paolo, T., 1993. Modulation by estradiol and progesterone of the GTP effect on striatal D-2 dopamine receptors. Biochem. Pharmacol. 45, 723–733.

Meiergerd, S.M., Patterson, T.A., Schenk, J.O., 1993. D_2 receptors may modulate the function of the striatal transporter for dopamine: kinetic evidence from studies in vitro and in vivo. J. Neurochem. 61, 764–767.

Milligan, G., Bond, R.A., Lee, M., 1995. Inverse agonism: pharmacological curiosity or potential therapeutic strategy? TIPS 16, 10–13.

Morissette, M., Di Paolo, T., 1993a. Effect of chronic estradiol and progesterone treatments of ovariectomized rats on brain dopamine uptake sites. J. Neurochem. 60, 1876–1883.

Morissette, M., Di Paolo, T., 1993b. Sex and estrous cycle variations of rat striatal dopamine uptake sites. Neuroendocrinology 58, 16–22.

Parsons, L.H., Schad, C.A., Justice, J.B., 1993. Co-administration of the D_2 antagonist pimozide inhibits up-regulation of dopamine release and uptake induced by repeated cocaine. J. Neurochem. 60, 376–379.

Reith, M.E.A., Xu, C., Chen, N-H., 1997. Pharmacology and regulation of the neuronal dopamine transporter. Eur. J. Pharmacol. 324, 1–10.

Salah, R.S., Kuhn, D.M., Galloway, M.P., 1989. Dopamine autoreceptors modulate the phosphorylation of tyrosine hydroxylase in rat striatal slices. J. Neurochem. 52, 1517–1522.

Thompson, T.L., 1999. Attenuation of dopamine uptake in vivo following priming with estradiol benzoate. Brain Res. 834, 164–167.

Thompson, T.L., Moss, R.L., 1994. Estrogen regulation of dopamine release in the nucleus accumbens: genomic- and nongenomic-mediated effects. J. Neurochem. 62, 1750–1756.

Thompson, T.L., Moss, R.L., 1997. Modulation of mesolimbic dopaminergic activity over the estrous cycle. Neurosci. Lett. 229, 145–148.

Zhang, L., Coffey, L.L., Reith, M., 1997. Regulation of the functional activity of the human dopamine transporter by protein kinase C. Biochem. Pharmacol. 53, 677–688.

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.