

Characterization of Extracellular Dopamine Clearance in the Medial Prefrontal Cortex: Role of Monoamine Uptake and Monoamine Oxidase Inhibition

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In vitro rotating disk electrode (RDE) voltammetry and *in vivo* microdialysis were used to characterize dopamine clearance in the rat medial prefrontal cortex (mPFC). RDE studies indicate that inhibition by cocaine, specific inhibitors of the dopamine transporter (DAT) and norepinephrine transporter (NET), and low Na⁺ produced a 50–70% decrease in the velocity of dopamine clearance. Addition of the monoamine (MAO) inhibitors, L-deprenyl, clorgyline, pargyline, or *in vivo* nialamide produced 30–50% inhibition. Combined effects of uptake inhibitors with L-deprenyl on dopamine clearance were additive (up to 99% inhibition), suggesting that at least two mechanisms may contribute to dopamine clearance. Dopamine measured extracellularly 5 min after exogenous dopamine addition to incubation mixtures revealed that most conditions of DAT/NET inhibition did not produce elevated dopamine levels above controls. Inhibition of MAO produced elevated dopamine levels only after long-term, but not short-term, incubation *in vitro*. Short-term

incubation of L-deprenyl combined with DAT and NET uptake inhibitors increased dopamine above control levels, consistent with more than one mechanism of dopamine clearance. Local infusion of pargyline (100 or 300 μM) into the mPFC or striatum via microdialysis produced more pronounced and immediate increases in mPFC dopamine levels compared with striatum. Furthermore, dopamine elevation in the mPFC was not accompanied by a decrease in the dopamine metabolites, 3,4-dihydroxyphenylacetic acid and homovanillic acid, as found in the striatum. These findings may have revealed a unique mechanism of mPFC dopamine clearance and therefore contribute to the understanding of multiple behaviors that involve mPFC dopamine transmission, such as schizophrenia, drug abuse, and working memory function.

Key words: dopamine; cocaine; dopamine transporter; medial prefrontal cortex; monoamine oxidase; rotating disk electrode voltammetry

Altered function of the medial prefrontal cortex (mPFC) has been implicated in multiple processes and behavioral disorders, including schizophrenia (Weinberger, 1995), drug abuse (Goeders and Smith, 1983; Isaac et al., 1989; Piazza et al., 1991; Schenk et al., 1991; Duvauchelle et al., 1992; McGregor and Roberts, 1995; Wolf et al., 1995; McGregor et al., 1996; Wise et al., 1996; Prasad et al., 1999), depression (Baxter et al., 1989; Tanda et al., 1994; Drevets, 1999; Juckel et al., 1999; Merriam et al., 1999; Rajkowska et al., 1999), and attention deficit hyperactivity disorder (Boix et al., 1998; Ernst et al., 1998; Puumala and Sirvio, 1998), as well as normal cognitive processes, including working memory function (Williams and Goldman-Rakic, 1995; Murphy et al., 1996; Cai and Arnsten, 1997; Jentsch et al., 1997a,b; Zahrt et al., 1997; Seamans et al., 1998; Wang, 1999) and decision making (Eslinger and Damasio, 1985; Damasio, 1995). Several of these studies have

focused in particular on altered dopaminergic functioning within the mPFC.

Despite the importance of prefrontal cortical dopamine in modulating cognition and behavior, little is known regarding processes that regulate extracellular clearance of dopamine in the mPFC. Inhibitors of dopamine transport demonstrate a weak effect on mPFC extracellular dopamine levels *in vivo*, including cocaine (Moghaddam and Bunney, 1989), amphetamine (Sorg et al., 1997; Pehek, 1999), nomifensine, and GBR 12909 (Cass and Gerhardt, 1995). The decreased responsiveness to dopamine uptake inhibitors may be explained partially by the lower terminal density and decreased number of dopamine transporters per terminal relative to striatal regions (Sesack et al., 1998). However, *in vitro* studies have shown that, in contrast to the striatum/nucleus accumbens, dopamine uptake inhibitors only partially diminish dopamine uptake in the PFC (Hadfield and Nugent, 1983; Izenwasser et al., 1990; Elsworth et al., 1993; Wheeler et al., 1993). These findings suggest that an additional mechanism may contribute importantly to regulating clearance of extracellular dopamine in the mPFC.

Few studies have focused on measuring the kinetics of dopamine clearance in the mPFC. Garris et al. (1993) and Garris and Wightman (1994) have used *in vivo* voltammetry to examine clearance within the mPFC. Their findings suggest that dopamine clearance occurs over a large tissue volume because of the more restricted distribution of dopamine transporter (DAT) in this region, and they enhance the notion of volume transmission and

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the possible paracrine function of cortical dopamine. Cass and Gerhardt (1995) also have examined dopamine clearance in different regions of the mPFC using *in vivo* voltammetry. However, no studies to date have defined the contribution of metabolism to the kinetics of clearance, and some previous mPFC clearance studies are confounded by the factor of diffusion occurring *in vivo*. The present study used rotating disk electrode (RDE) voltammetry *in vitro* to characterize the kinetics of dopamine clearance in the mPFC and to determine regulatory processes that contribute to dopamine clearance. *In vivo* microdialysis in the mPFC and striatum was also performed to examine the effect of the monoamine (MAO) inhibitor, pargyline, on extracellular dopamine and metabolite levels.

MATERIALS AND METHODS

Animals and housing. Experiments were conducted according to the *National Institutes of Health Guide for the Care and Use of Laboratory Animals*, and experimental protocols were approved by the University animal care and use committee. Male Sprague Dawley rats weighing 250–300 gm were group-housed (three to four per cage) in a temperature- and humidity-controlled environment with *ad libitum* access to food and water. Animals were maintained on a 12 hr light/dark schedule, with lights on at 7 A.M.

Drugs. Cocaine-hydrochloride was a gift from the National Institute on Drug Abuse. Dopamine, pargyline, clorgyline, desmethylimipramine (DMI), and nialamide were purchased from Sigma (St. Louis, MO), and GBR 12909, fluoxetine, and L-deprenyl were purchased from Research Biochemical Inc. (Natick, MA). All drugs used for RDE experiments were dissolved in distilled water and diluted to a final concentration in the incubation buffer. Solutions were made fresh each day and stored on ice.

RDE voltammetry and HPLC. Unanesthetized rats were decapitated, and their brains were rapidly removed. The mPFC was dissected and weighed. The tissue was immediately chopped on an ice-cold glass plate and placed into 500 μ l of physiological buffer composed of (in mM): 124 NaCl, 1.80 KCl, 1.30 MgSO₄, 1.24 KH₂PO₄, 2.50 CaCl₂, 26 NaHCO₃, 10 glucose, saturated with 95% O₂–5% CO₂ gas mixture as described (Meiergerd and Schenk, 1995; Earles et al., 1998; Earles and Schenk, 1999) and maintained at 37°C in a temperature-controlled chamber. Composition of the buffer in the low Na⁺ condition consisted of replacement of 124 mM NaCl with choline chloride. The tissue was disrupted by repetitive pipetting and washed by the repeated addition and removal of 250 μ l fresh buffer seven times. The RDE (Pine Instruments, Grove City, PA) was lowered into the chamber and rotated at 2000 rpm, and a potential of 450 mV relative to a Ag/AgCl reference electrode was applied with a Bioanalytical Systems LC4B potentiostat (W. Lafayette, IN) with a 20 msec time constant, defined as 5 \times resistance \times capacitance. The tissue was incubated for 20 min until a stable baseline was reached. After the incubation period, dopamine was added to produce a final concentration of 2.0 μ M, and its clearance was monitored on a Nicolet 310 digital oscilloscope. The concentration of dopamine used was based on a previous *in vitro* RDE study in the mPFC that demonstrated this concentration to be near V_{max} for dopamine clearance (Meiergerd et al., 1997). The initial rate of dopamine disappearance was estimated as described previously (Meiergerd and Schenk, 1995; Earles et al., 1998). The velocity of clearance was expressed as picomoles per second \times grams of wet weight. This value was determined by calculating the slope of the line, as determined by time points taken every 20 msec, and converting from current to dopamine concentration by using a standard calibration curve prepared each day. The standard curve was generated using 0.1, 0.25, 0.5, 1.0, and 2.0 μ M dopamine. At a cumulative concentration of 1.85 μ M dopamine, clearance was followed for 25 sec and is reported as the “buffer” sample throughout. The supernatant was prepared as described above for tissue preparation, but after washing seven times, the tissue was rapidly centrifuged and the supernatant removed for analysis of dopamine clearance, as described above. To determine dopamine clearance (in picomoles per second \times grams of wet weight) for the buffer and supernatant, the mean tissue weight of all samples (33.4 mg) was used.

The concentrations of DAT/norepinephrine transporter (NET) uptake inhibitors were chosen on the basis of the K_i or IC₅₀ of each inhibitor and were 10–100 times higher than the K_i or IC₅₀ for each particular neurotransmitter. For DMI, both 0.1 and 100 μ M doses were used, with the former inhibiting only NET and the latter concentration inhibiting both

NET and DAT. A 0.1 μ M concentration of fluoxetine, which inhibits serotonin uptake, was chosen. Activity for NET may have been partially inhibited at this concentration of fluoxetine, the K_i of which has been reported as \sim 0.2 μ M (Burke and Preskorn, 1995). For GBR 12909, a 3.0 μ M solution was chosen, which is \sim 50 times higher than the IC₅₀ for dopamine and approximately at the K_i for norepinephrine uptake (Heikkilä and Manzino, 1984).

It should be noted that at the concentrations of L-deprenyl and clorgyline used (100 μ M), both MAO A and B would be expected to be inhibited. However, these agents as well as pargyline were examined because of previous work demonstrating an inhibitory effect of clorgyline (Lai et al., 1980; Fang and Yu, 1994) and L-deprenyl on dopamine uptake (Knoll, 1978; Lai et al., 1980; Zsilla et al., 1986; Knoll, 1992; Okuda et al., 1992; Fang and Yu, 1994), whereas pargyline has been reported not to influence dopamine uptake in the striatum (Fang and Yu, 1994).

After most treatments, at the end of a 5 min incubation, a 100 μ l sample was removed while the electrode was still rotating. Samples were placed into 20 μ l of 0.1 M perchloric acid solution and immediately centrifuged to remove the tissue. The supernatant, which will be referred to as “aqueous phase” throughout, was collected and added to 50 μ l HPLC mobile phase containing 1×10^{-7} M isoproterenol and stored at -80° C until assayed by HPLC for dopamine and its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 3-methoxytyramine (3-MT), as well as for NE. The results are reported as concentration of dopamine in micromolar taken from the original 100 μ l sample removed at the end of the 5 min incubation. Determination of dopamine and metabolites, DOPAC, HVA, and 3-MT, and NE present in the aqueous phase was conducted by HPLC analyses according to Kalivas et al. (1988).

In vivo microdialysis. *In vivo* microdialysis was conducted in awake, unrestrained rats as described by Sorg et al. (1997). Animals were implanted with a chronic guide cannula into the mPFC \sim 1 week before microdialysis experiments [for mPFC: anteroposterior (AP) = +3.2 mm from bregma, mediolateral (ML) = 0.7 mm, dorsoventral (DV) = -1.5 mm from skull; for striatum: AP = +1.0 mm from bregma, ML = 2.2 mm, DV = -4.0 mm from skull, according to Paxinos and Watson (1998)]. Microdialysis probes with an active membrane region of 3 mm (mPFC) or 2 mm (striatum) were prepared as described (Sorg et al., 1997). Just before use, pargyline was diluted to its final concentration in artificial CSF (aCSF), which consisted of (in mM): 5.0 glucose, 5 KCl, 120 NaCl, 1.2 CaCl₂, 1.2 MgCl₂, 0.23 sodium phosphate, pH 7.4. Probes were implanted the evening before the experiment, and on the next day, a minimum of 3–4 hr was allowed for a stable baseline to be obtained. After this, baseline samples were collected, and pargyline (either 100 or 300 μ M given to separate animals) was infused for a 60 min period and then replaced with aCSF for the remainder of the experiment. HPLC analyses of dopamine, DOPAC, and HVA were conducted as described by Sorg et al. (1997). Microdialysis probe placements within the mPFC and striatum were verified by cresyl violet staining of coronal brain sections (see Fig. 1, B and C, respectively).

Data analyses and statistical testing. The percentage reduction in dopamine clearance was determined by subtracting the mean value for supernatant and buffer conditions, which were not significantly different from each other, from the values obtained for control and each treatment condition, dividing by the mean of the controls, and multiplying by 100. The differences between means of the transport velocities (see Figs. 2–4), dopamine, its metabolites, and NE measured from sampled aqueous phase (see Fig. 5) were tested using a one-way ANOVA followed by a *post hoc* Fisher's test. Each neurotransmitter and metabolite was analyzed separately with an ANOVA (see Fig. 5). The microdialysis data (see Figs. 6, 7) were analyzed using a one-way repeated measures ANOVA followed by a Fisher's test to determine significant increases above the last baseline sample. All data were considered statistically significant at $p < 0.05$.

RESULTS

Figure 1A shows the brain area that was dissected for all experiments presented in Figures 2–5. This region included all mPFC regions anterior to 2.2 mm from bregma (Paxinos and Watson, 1998). Figure 1, B and C, shows photomicrographs of coronal brain sections demonstrating typical microdialysis probe placements in the mPFC and striatum for the data shown in Figures 6 and 7. In the mPFC, probes spanned the deeper layers of the cortex within the prelimbic and infralimbic cortices.

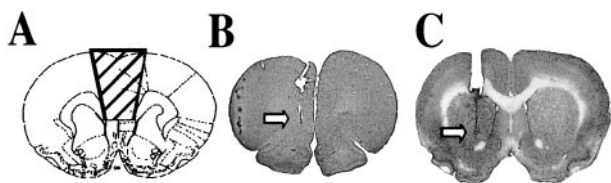


Figure 1. Coronal brain sections illustrating regions of the mPFC and striatum sampled in these studies. *A*, The portion of mPFC tissue removed for dopamine uptake studies. *Shaded area* represents the most caudal region used and includes all tissue rostral to the area shown. Diagram is taken from Paxinos and Watson (1998) at +2.2 mm from bregma. *B*, Photomicrograph of a coronal brain section demonstrating microdialysis probe placement in the mPFC. The tip of the 3 mm probe is indicated by a *white arrow*. *C*, Photomicrograph of a coronal brain section demonstrating microdialysis probe placement in the striatum. The tip of the 2 mm probe is indicated by a *white arrow*.

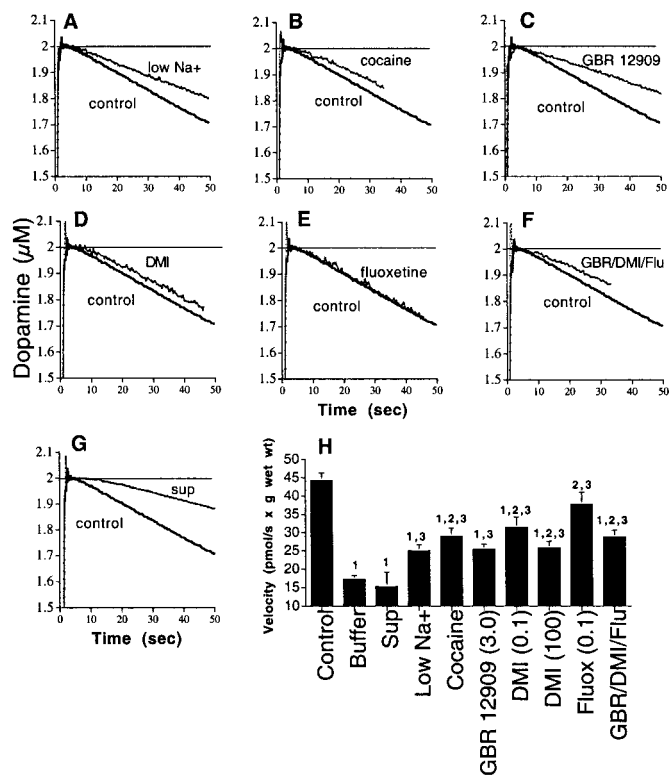


Figure 2. Velocity of 2.0 μM dopamine clearance in mPFC tissue in the presence of low Na^+ or monoamine uptake inhibitors. *A–G*, Mean raw clearance profiles for dopamine. In some cases, velocity profiles were not collected from all animals for the entire period, and the lines are truncated (*B*, *D*, *F*). *H*, Mean \pm SEM of dopamine clearance velocities shown in *A–G*. Mean values obtained in buffer and supernatant alone are shown for comparison. $n = 16$ for control; $n = 3–5$ for all other groups. $^1p < 0.05$, comparing with control condition; $^2p < 0.05$, comparing with buffer condition; $^3p < 0.05$, comparing with supernatant condition, as determined with a one-way ANOVA followed by a Fisher's test. *Sup*, Supernatant; *Low Na+*, 26 mM Na^+ ; *Cocaine*, 100 μM ; *GBR 12909*, 3.0 μM ; *DMI*, 0.1 or 100 μM (summary bar graph only); *Fluox*, 0.1 μM fluoxetine; *GBR/DMI/Flu*, combination of GBR 12909 (3.0 μM), DMI (0.1 μM), and fluoxetine (0.1 μM).

General characteristics of clearance of extracellular dopamine in the mPFC

Figures 2–4 show graphs of the mean raw data taken from control and each of the various treatment groups presented along with a summary bar graph of the mean \pm SEM of dopamine clearance

velocities. Clearance in the mPFC was found to be slower than that observed in tissues from the striatum or nucleus accumbens [compare with McElvain and Schenk (1992); Povlock and Schenk (1997), respectively] and generally consisted of a single linear phase. However, under some treatment conditions, two phases were observed and are described further in the results for Figure 4. For the summary bar graph presentation, all velocities that showed a single linear clearance phase were determined by calculating the slope from 5 to 25 sec, whereas those exhibiting biphasic profiles were calculated for the second phase (linear portion) from 20 to 30 sec. The slightly later time period of 20–30 sec was chosen for the graphs shown in Figure 4 because of the longer delay for the second phase of the velocity profile to appear. The buffer and supernatant conditions also exhibited biphasic profiles, and for these two conditions velocities were determined by calculating the slope from 15 to 25 sec.

The value of dopamine clearance in buffer (used in preparing the standard curve) was not significantly different from that observed in the supernatant preparation. By 30 sec after dopamine addition to the incubation mixture containing the supernatant, the amount of dopamine cleared was 2.5% of the total dopamine added, and this disappearance may have been caused by the sum of oxidation of dopamine by the electrode, oxidation by dissolved O_2 , auto-oxidation of dopamine, or changes in the residual current of the electrode. The dopamine clearance profile for the buffer and supernatant are shown throughout Figures 2–4 for purposes of comparison.

Monoamine uptake inhibitors

Figure 2*A–G* demonstrates the effect of low Na^+ and inhibitors of dopamine, NE, and serotonin (5-HT) uptake on the velocity of dopamine clearance in the mPFC. The data are presented in summary form (*bar graph*) in Figure 2*H*. The results indicate that ~50–70% of the dopamine clearance rate is dependent on the function of the Na^+/Cl^- -dependent transporters DAT and NET. Low Na^+ buffer (26 mM NaCl vs 150 mM for the control condition) reduced dopamine velocity by 69%. DMI added to a final concentration of 0.1 μM to specifically inhibit NET reduced dopamine clearance velocity by 46%, whereas a higher dose that inhibits both NET and DAT inhibited dopamine clearance velocity by 65%. Although the higher concentration of DMI did not significantly alter dopamine clearance velocity as compared with the 0.1 μM dose, the results suggest possible additive effects of uptake inhibitors acting at DAT and NET. The serotonin uptake inhibitor fluoxetine, given at 0.1 μM , did not significantly reduce the velocity of dopamine clearance. Addition of 3 μM GBR 12909, a DAT inhibitor, reduced dopamine clearance by ~70%. The effects of GBR 12909 and DMI were not additive, because the combination of the same concentrations of GBR 12909, DMI, and fluoxetine inhibited dopamine clearance velocity by only 55%, and this combination was equally as effective as cocaine, which inhibits all three monoamine transporters. This may have been caused partially by the concentration of GBR 12909 used, which was close to the IC_{50} reported for norepinephrine in rat cortical tissue slices (Heikkila and Manzino, 1984).

Monoamine oxidase inhibitors

Figure 3*A–F* shows the results of MAO inhibitors on the velocity of dopamine clearance in the mPFC. The propargylamines, pargyline, clorgyline, and *L*-deprenyl, all reduced dopamine clearance velocity by 30–50%. To determine whether a structurally different MAO inhibitor also attenuated the velocity of dopamine

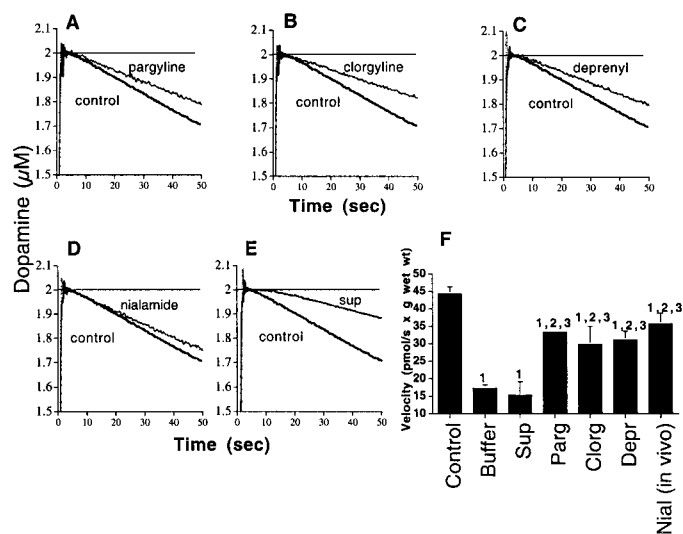


Figure 3. Velocity of $2.0 \mu\text{M}$ dopamine clearance in mPFC tissue in the presence of MAO inhibitors. *A–E*, Mean raw clearance profiles for dopamine. *D*, Nialamide (75 mg/kg , i.p.) was administered *in vivo* 5 hr before rats were killed. *F*, Mean \pm SEM of dopamine clearance velocities shown in *A–E*. Mean values obtained in buffer and supernatant alone are shown for comparison. $n = 16$ for control; $n = 3–5$ for all other groups. $^1p < 0.05$, comparing with control condition; $^2p < 0.05$, comparing with buffer condition; $^3p < 0.05$, comparing with supernatant condition, as determined with a one-way ANOVA followed by a Fisher's test. *Sup*, Supernatant; *Parg*, $100 \mu\text{M}$ pargyline; *Clorg*, $100 \mu\text{M}$ clorgyline; *Depr*, $100 \mu\text{M}$ deprenyl; *Nial*, 75 mg/kg (i.p.) nialamide *in vivo*.

clearance, nialamide was administered to rats systemically. Nialamide was administered systemically because previous *in vitro* experiments using RDE demonstrated that nialamide nonspecifically alters electrode responses (J. O. Schenk, unpublished observations). This dose of nialamide (75 mg/kg , i.p.) 5 hr before rats were killed (Hovey-Sion et al., 1989) resulted in a $\sim 30\%$ inhibition of dopamine clearance rate.

Inhibition of DAT/NET uptake and MAO activity

The time-dependent effects of DAT/NET and MAO inhibition were examined by adding these inhibitors simultaneously with dopamine to the incubation media ($t = 0 \text{ sec}$). For Figure 4, all inhibitor additions to the incubation were given simultaneously with exogenous dopamine addition at $t = 0 \text{ sec}$. Figure 4*A–F* shows that a biphasic clearance profile was observed in each case when these inhibitors were added at $t = 0 \text{ sec}$. The bar graph in Figure 4*G* summarizes the means \pm SEM of dopamine clearance occurring from 20 to 30 sec. The early component of the biphasic response demonstrated inhibition of dopamine clearance that lasted for several seconds. This initial inhibition lasted significantly longer under conditions in which cocaine + L-deprenyl were added compared with all other conditions.

Figure 4*A* demonstrates that inhibition by cocaine added at $t = 0 \text{ sec}$ was greater than that observed for cocaine when it was added 30 sec before dopamine addition, although these values were not significantly different from each other ($p = 0.23$) (compare with Fig. 2*B,H*). There was a trend for cocaine given at $t = 0 \text{ sec}$ to cause a lower rate of dopamine clearance than the buffer and supernatant conditions, but the value from cocaine at $t = 0 \text{ sec}$ shown in the summary bar graph was not statistically different from those of the buffer and supernatant conditions (Fig. 4*G*). L-deprenyl addition to the incubation at $t = 0 \text{ sec}$ also resulted in a biphasic profile and only partially inhibited the clearance veloc-

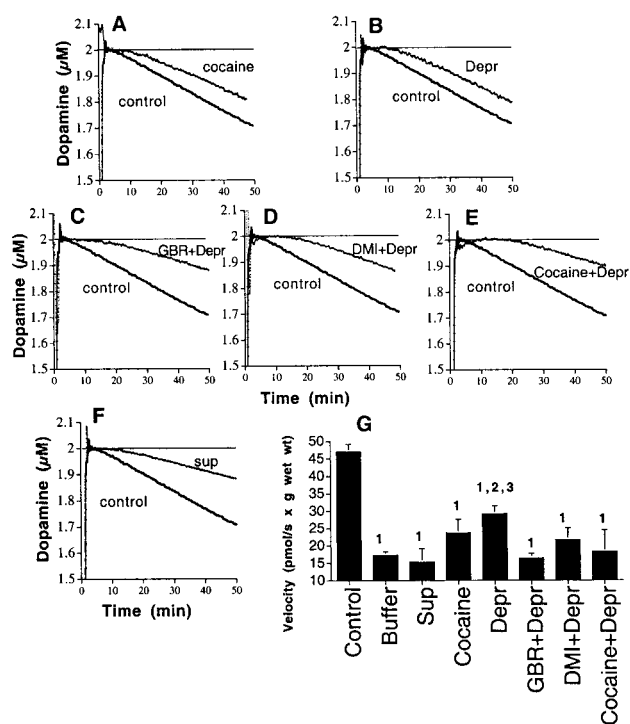


Figure 4. Velocity of $2.0 \mu\text{M}$ dopamine clearance in mPFC tissue in the presence of cocaine, L-deprenyl, or the combination of L-deprenyl with GBR 12909 ($3.0 \mu\text{M}$), DMI ($0.1 \mu\text{M}$), or cocaine ($100 \mu\text{M}$). *A–C*, Mean raw clearance profiles for dopamine. For *A–E*, all components were added simultaneously with $2.0 \mu\text{M}$ dopamine. *G*, Mean \pm SEM of dopamine clearance velocities shown in *A–F*. Mean values obtained in buffer and supernatant alone are shown for comparison. $n = 16$ for control; $n = 3–5$ for all other groups. $^1p < 0.05$, comparing with control condition; $^2p < 0.05$, comparing with buffer condition; $^3p < 0.05$, comparing with supernatant condition, as determined with a one-way ANOVA followed by a Fisher's test. *Sup*, Supernatant; *Cocaine*, $100 \mu\text{M}$; *Depr*, $0.1 \mu\text{M}$ deprenyl; *GBR+Depr*, *DMI+Depr*, and *Cocaine+Depr* were added to the incubation in the same concentrations as used singly.

ity of dopamine. This value, shown in the summary bar graph, was significantly reduced from control, although significantly higher than the values for the buffer and supernatant conditions. Inhibition by L-deprenyl added at $t = 0 \text{ sec}$ was not different from that produced by L-deprenyl present in the incubation mixture for 20 min before dopamine addition ($p = 0.61$) (compare with Fig. 3*C,F*).

Cocaine and other DAT and NET inhibitors were combined with the MAO inhibitor L-deprenyl to determine whether the partial inhibitory effects on dopamine clearance velocity described above were additive when assessed over the 20–30 sec range. Figure 4*C–E,G* demonstrates that additive effects of L-deprenyl occurred when combined with these uptake inhibitors, with percentage inhibition ranging from 83% (DMI + L-deprenyl) to 99% (GBR + L-deprenyl), with a 94% inhibition by cocaine + L-deprenyl (refer to summary bar graph in Fig. 2*H* for effects of DMI and GBR 12909 given alone).

Dopamine metabolism

For most of the RDE experiments, samples of aqueous phase from brain homogenates were collected 5 min after the addition of dopamine to determine the levels of dopamine and its metabolites, DOPAC, HVA, and 3-MT. Norepinephrine was also measured, because dopamine is taken up into NE terminals via NET. The relatively longer time point of 5 min was chosen to increase

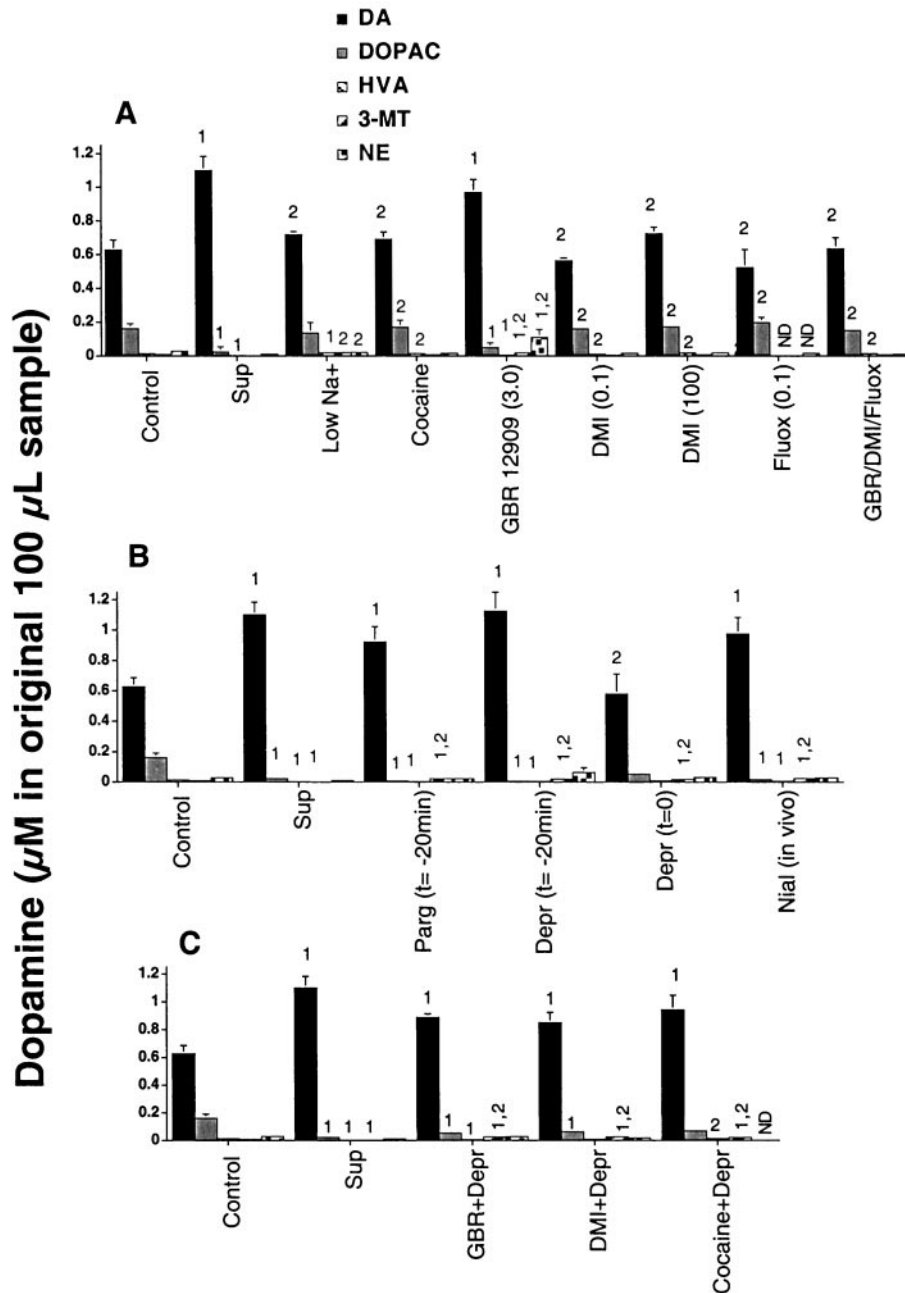


Figure 5. Concentration of dopamine, metabolites, and NE in the aqueous phase from mPFC tissue 5 min after incubation in the presence of dopamine under low Na^+ conditions or after addition of monoamine uptake/MAO inhibitors. Data represent mean \pm SEM of dopamine, DOPAC, HVA, 3-MT, and NE concentrations from the original (100 μL) sample taken from the incubation. *A*, Monoamine uptake inhibitors ($n = 15$ for control; $n = 3-5$ for all other groups). *B*, MAO inhibitors ($n = 15$ for control; $n = 3-4$ for all other groups). *C*, Combination of L-deprenyl with uptake inhibitors GBR 12909 (*GBR+Depr*), DMI (*DMI+Depr*), or cocaine (*Cocaine+Depr*) ($n = 15$ for control; $n = 3-5$ for all other groups). ¹ $p < 0.05$, comparing with control condition; ² $p < 0.05$, comparing with supernatant condition, as determined with a one-way ANOVA followed by a Fisher's test. *ND*, Not determined.

the possibility that metabolic pathways of dopamine may be traced after the various treatment conditions. Before the addition of 2.0 μM dopamine, basal concentrations from separate experiments were (in micromolar per original sample removed) as follows: dopamine, 0.0048 ± 0.0003 ; DOPAC, 0.0085 ± 0.0012 ; HVA, 0.0102 ± 0.0026 ; 3-MT, 0.0328 ± 0.0078 ; NE, 0.0058 ± 0.0008 ($n = 4$).

Figure 5*A* corresponds to the aqueous phase taken from experiments shown in Figure 2*A-G* in which dopamine uptake inhibitors were added to the incubation medium. Five minutes after the addition of dopamine, the level of dopamine on the outside (aqueous phase) of controls was $0.627 \mu\text{M}$. The level of dopamine remaining in the aqueous phase of the supernatant condition was significantly increased, whereas the metabolites, DOPAC and HVA, were significantly decreased compared with the control condition. Dopamine, metabolites, and NE values for all condi-

tions were compared with both the control and supernatant conditions, and significant differences are indicated in Figure 5. However, for purposes of clarity, comparison of these values with those from the supernatant will be discussed here only for dopamine, because several metabolites were significantly higher when the levels from tissue were compared with those from the supernatant condition.

The only uptake inhibitor that produced a significant increase in aqueous phase dopamine levels above control values after the 5 min incubation was GBR 12909, whereas no other conditions of dopamine, NE, or 5-HT uptake inhibition produced an increase in aqueous phase dopamine levels. The combination of GBR 12909 with DMI or fluoxetine also did not produce a significant elevation in aqueous phase dopamine levels. The levels of DOPAC and HVA measured in the aqueous phase after the 5 min incubation were significantly decreased with GBR 12909

addition, whereas 3-MT and NE levels were significantly elevated after GBR 12909 addition. The only other significant change from the control condition was an increase in HVA levels in the low Na^+ condition.

Figure 5B shows dopamine, its metabolites, and NE levels in the aqueous phase after the addition of MAO inhibitors corresponding to experiments shown in Figures 3A,C–E and 4B. Dopamine levels were significantly elevated after all conditions of MAO inhibition, with the exception of the condition in which L-deprenyl was added at $t = 0$ sec and incubated for the 5 min period. In all conditions, with the exception of L-deprenyl added simultaneously with dopamine ($t = 0$ sec), the MAO inhibitors significantly reduced DOPAC levels. L-deprenyl added at $t = 0$ min attenuated, but did not significantly reduce, DOPAC levels. Homovanillic acid levels were also significantly reduced after pargyline and L-deprenyl addition when these drugs were present during the 20 min baseline stabilization period. The levels of 3-MT in the supernatant were elevated above control values under all conditions of MAO inhibition. Norepinephrine levels were not significantly altered by any of the MAO inhibitors.

Figure 5C, corresponding to the aqueous phase taken from experiments shown in Figure 4C–F, demonstrates that the combination of GBR 12909 + L-deprenyl, DMI + L-deprenyl, and cocaine + L-deprenyl all significantly increased dopamine levels above control values and were not different from levels in the supernatant condition. The levels of DOPAC were significantly decreased from controls after the combination of L-deprenyl with either GBR 12909 or DMI. The combination of L-deprenyl with GBR 12909 also significantly decreased HVA levels. As with the MAO inhibitors alone, 3-MT levels were also increased when given in combination with these uptake inhibitors. Norepinephrine levels were not determined for the cocaine + L-deprenyl group, but no other treatment conditions produced significant differences in this neurotransmitter.

Microdialysis

In vivo microdialysis experiments were conducted in the mPFC and striatum to determine whether (1) local infusion of the MAO inhibitor, pargyline, would produce an increase in extracellular dopamine levels and (2) whether there were differential effects between these two brain areas in the regulation of dopamine and metabolites by local pargyline infusion. The results of this experiment are shown in Figures 6A–F (100 μM pargyline) and 7A–F (300 μM pargyline). Infusion with 100 μM pargyline produced a significant elevation in extracellular dopamine levels in the mPFC when infused at a concentration of 100 μM for a 1 hr period. This increase was $\sim 300\%$ above baseline values and occurred within 20–40 min after pargyline infusion. A significant increase in DOPAC levels was found, whereas no significant changes occurred for HVA levels. In contrast, striatal dopamine levels showed only a trend toward an increase to $\sim 200\%$ above baseline values, but the trend toward an increase above baseline was delayed by 20 min and occurred only after significant reduction in DOPAC levels, which were reduced to $\sim 50\%$ of baseline. Also in contrast to the mPFC, HVA levels in the striatum were significantly reduced to $\sim 40\%$ of baseline.

In the presence of 300 μM pargyline, dopamine levels in the mPFC increased by $\sim 5000\%$ above baseline values, and this effect occurred within 20–40 min after pargyline infusion. Again, no significant decrease in DOPAC or HVA levels was observed. Striatal dopamine increased to $\sim 1000\%$ above baseline levels, but as with the lower dose of pargyline, the effects were delayed by 20

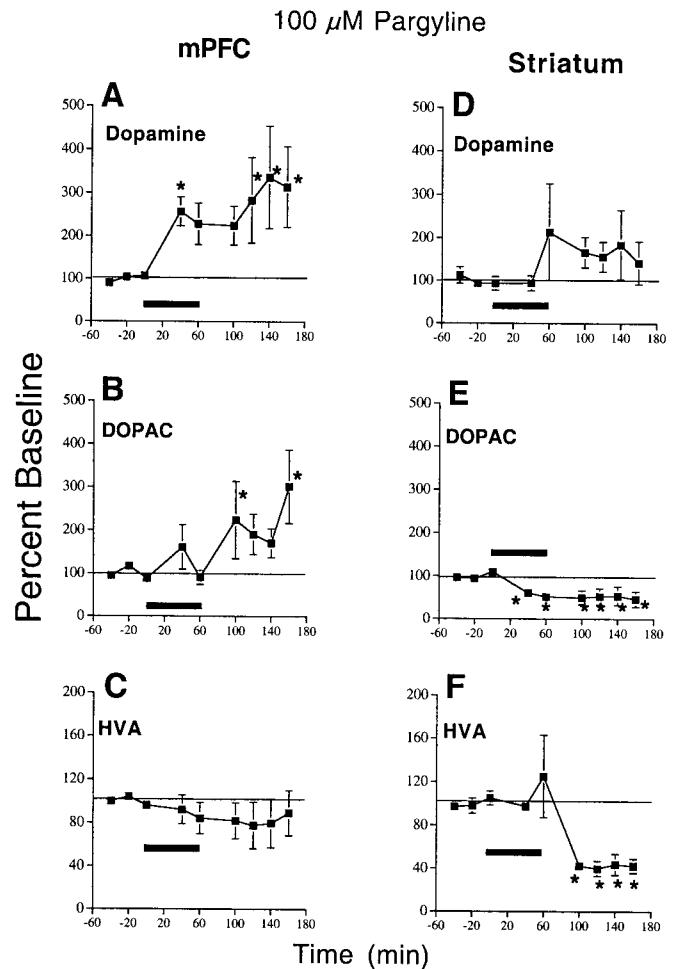


Figure 6. *In vivo* microdialysis measurements of extracellular dopamine, DOPAC, and HVA levels in the mPFC and striatum after 100 μM pargyline infusion. Data represent mean \pm SEM of dopamine, DOPAC, and HVA levels before pargyline infusion, during a 1 hr infusion with 100 μM pargyline in the mPFC (A–C) or striatum (D–F). Pargyline infusion is indicated by the black bar and was then replaced with aCSF. Samples collected for the first time point after manual switching of solutions is not shown because of alterations in aCSF flow rates. Basal levels from mPFC from all groups (including those shown in Fig. 7) were (in femtomoles per sample) as follows: for dopamine, 13.0 ± 2.4 ; for DOPAC, 247 ± 58 ; for HVA, 404 ± 57 . Basal levels from striatum from all groups (including those shown in Fig. 7) were (in femtomoles per sample) as follows: for dopamine, 48.8 ± 9.9 ; for DOPAC, 7219 ± 1286 ; for HVA, 6087 ± 1009 . Sample sizes were as follows: mPFC dopamine, $n = 9$; DOPAC and HVA, $n = 7$; striatal dopamine, DOPAC, and HVA, $n = 6$. * $p < 0.05$ compared with last baseline sample, as determined with a one-way, repeated-measures ANOVA followed by a Fisher's test.

min as compared with the early increase in mPFC dopamine levels. The levels of DOPAC, although reduced to $\sim 40\%$ of baseline, did not reach statistical significance because all values were compared with the last baseline sample, which was already decreased by 20% before pargyline infusion. HVA levels were significantly reduced to 30% of baseline levels.

DISCUSSION

The main findings from these studies are as follows. (1) DAT and NET inhibitors account for only 50–70% of the velocity of dopamine clearance in the mPFC; (2) MAO inhibitors attenuate the velocity of dopamine clearance by $\sim 30\text{--}50\%$; (3) the effects of DAT/NET uptake inhibitors plus the MAO inhibitor, L-deprenyl,

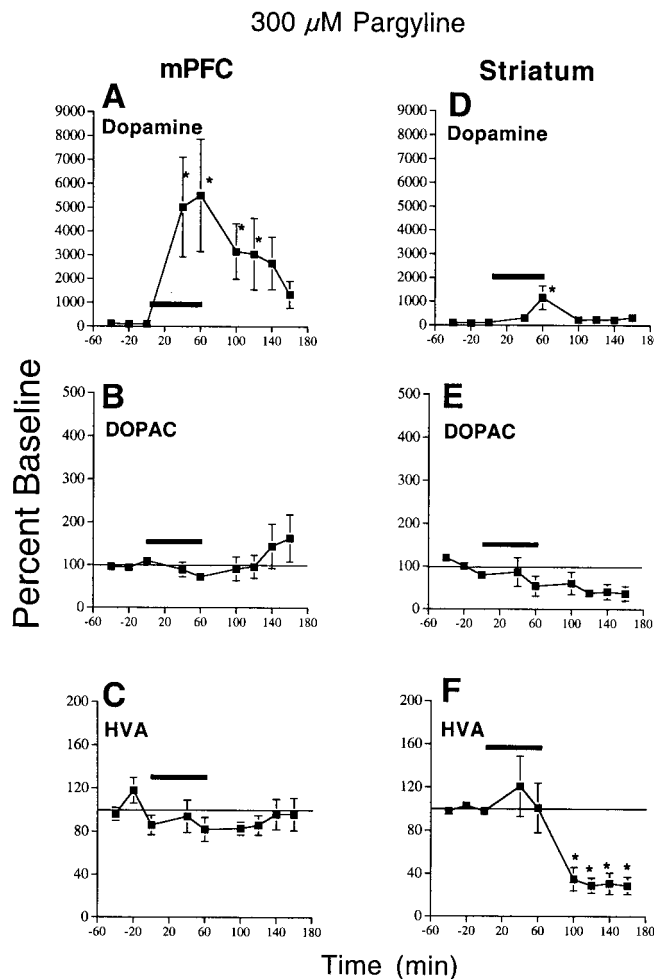


Figure 7. *In vivo* microdialysis measuring extracellular dopamine, DOPAC, and HVA levels in the mPFC and striatum after 300 μ M pargyline infusion. Data represent mean \pm SEM of dopamine, DOPAC, and HVA levels before pargyline infusion, during a 1 hr infusion with 300 μ M pargyline in the mPFC (A–C) or striatum (D–F). Pargyline infusion is indicated by the black bar and was then replaced with aCSF. Samples collected for the first time point after manual switching of solutions are not shown because of alterations in aCSF flow rates. Sample sizes were as follows: mPFC dopamine and DOPAC, $n = 9$; HVA, $n = 8$; striatal dopamine, DOPAC, and HVA, $n = 5$. * $p < 0.05$ compared with last baseline sample, as determined with a one-way, repeated-measures ANOVA followed by a Fisher's test.

on dopamine clearance appear to be additive; (4) the effect of DAT/NET inhibition on the initial rate of dopamine clearance may be compensated for by another process; and (5) local *in vivo* pargyline infusion into the mPFC via microdialysis dramatically elevates mPFC extracellular dopamine levels with no decreases in DOPAC or HVA, whereas pargyline-induced dopamine increases in the striatum occur only after infusion of the higher pargyline dose and are accompanied by decreases in DOPAC or HVA levels, or both.

Processes of mPFC dopamine clearance *in vitro*

The RDE findings are most consistent with the possibility that there are at least two major processes by which dopamine is cleared from the extracellular space. One is by a Na^+ -dependent process, presumably via DAT and NET, and the second is by a process altered by MAO inhibitors. Several previous *in vitro* studies have also reported only a partial (~40–70%) inhibition of

dopamine clearance in the mPFC after cocaine or low Na^+ conditions, or both, in contrast to the nucleus accumbens and striatum, in which cocaine inhibits dopamine clearance by 95% (Hadfield and Nugent, 1983; Izenwasser et al., 1990; Elsworth et al., 1993; Wheeler et al., 1993).

The time course of inhibition by L-deprenyl on dopamine clearance velocity suggests that there is an immediate effect of MAO inhibitors on dopamine clearance. Several possibilities for this response are considered.

One explanation for the effect of L-deprenyl is that deprenyl is converted to L-amphetamine, and this in turn inhibits dopamine uptake via DAT or NET (Karoum et al., 1982; Tetrad and Langston, 1989; Okudo et al., 1992). However, all MAO inhibitors tested in the present study decreased the dopamine clearance rate. Therefore, there may be an alternative process in the mPFC that is inhibited by all of these agents.

A second possibility is that MAO inhibitors alter the quinpirole binding site, which in turn may decrease dopamine clearance velocity. MAO inhibitors have been shown to modulate the binding of quinpirole (Levant et al., 1993, 1996). If the quinpirole binding site on D2 or D3 receptors in the mPFC is bound by MAO, modification of DAT function might be expected, given that D2 receptors have been shown to regulate DAT activity (Meiergerd et al., 1993; Cass and Gerhardt, 1994; Batchelor and Schenk, 1998). However, such an explanation is not consistent with the partial effects of low Na^+ or DAT/NET uptake inhibitors.

A third explanation is that these MAO inhibitors may inhibit other more recently described transporter systems, organic cation transporter (OCT) 2 or OCT3 (Busch et al., 1998; Wu et al., 1998; Grundemann et al., 1999). Although both of these transporters have been reported to transport dopamine and are present in the brain (Gorboulev et al., 1997), the OCT3 transporter appears much more abundant in brain tissue than OCT2 and is found in cortical regions (Wu et al., 1998). The OCT3 transporter mediates the uptake of dopamine, and amphetamine interacts with this transporter as well (Wu et al., 1998). Thus, interaction with this newly described transporter may be important in mPFC dopamine clearance.

Finally, there is the possibility that different clearance processes are present within different heterogeneous regions of the mPFC. Previous work has demonstrated regional effects of DAT and NET inhibitors (Cass and Gerhardt, 1995), and regional differences in dopamine clearance may be expected based on immunohistochemical measures of DAT location (Ciliax et al., 1995). Our studies examined the entire mPFC and therefore would not distinguish among clearance processes located within different mPFC subregions.

Biphasic effects of cocaine and MAO inhibition on dopamine clearance

Unexpectedly, when either cocaine or the MAO inhibitor, L-deprenyl, was added simultaneously with dopamine to the *in vitro* incubation mixture, there was a biphasic profile of dopamine clearance rather than a linear clearance profile. The first portion lasted on the order of seconds and was nearly or completely blocked by these agents for several seconds. The reason for observing complete inhibition of dopamine clearance for a longer period than what was observed in the supernatant condition is not clear. It should be pointed out that no alterations in baseline output were observed after cocaine or pargyline were added to mPFC tissue in the absence or presence of exogenous dopamine addition, nor did

these drugs cause release of dopamine from mPFC tissue. In addition, neither pargyline nor cocaine alters sensitivity of the electrode to dopamine (data not shown). Future studies will need to directly address the biphasic nature of dopamine clearance in this brain region.

The second phase of the biphasic profile demonstrated an additive effect for L-deprenyl with uptake inhibitors, suggesting that a second process or multiple processes may work either separately or in tandem with DAT and NET function.

Dopamine and metabolites *in vitro*

The results from the HPLC analyses of aqueous phase samples taken 5 min after exogenous dopamine addition indicated that dopamine levels were significantly elevated only after GBR 12909 addition but, unexpectedly, not in the presence of cocaine or the combination of GBR 12909, DMI, and fluoxetine. It is unclear why GBR 12909 alone would produce greater effects than with the combination of DAT/NET inhibitors. The results suggest that GBR 12909 may have alternative actions, such as direct inhibition of the MAO inhibitor-dependent process, or that binding of cocaine or DMI to NET may activate a process that is blocked by MAO inhibitors. Any effects of MAO inhibitors on dopamine clearance dependent on NET activity would not be expected to occur in brain areas that lack substantial clearance by NET, such as the nucleus accumbens or striatum. Consistent with the absence of effects of MAO inhibitors on dopamine clearance in these latter brain regions, pargyline addition does not alter dopamine clearance velocity in the nucleus accumbens or striatum when tested in the RDE system that has been used in the present studies (Meiergerd and Schenk, 1994; Povlock and Schenk, 1997).

Dopamine was elevated in the aqueous phase after all conditions of MAO inhibition, with the exception of when L-deprenyl was added at $t = 0$ sec. L-deprenyl incubated over a short-term period (added at $t = 0$ sec) produced immediate partial inhibitory effects on dopamine clearance yet did not alter extracellular dopamine levels after the 5 min incubation period, perhaps because of compensatory DAT and NET activity. However, over the longer incubation time (20 min or after *in vivo* administration), MAO inhibitors, including L-deprenyl, produced elevated dopamine levels in the aqueous phase, suggesting the possibility that uptake by DAT and NET may be impaired after longer incubation with MAO inhibitors. When L-deprenyl ($t = 0$ sec) was combined with DAT/NET uptake inhibitors, extracellular dopamine levels were significantly elevated above controls. Together, these findings support the results from the RDE studies suggesting that at least two processes contribute importantly to dopamine clearance in the mPFC.

Pargyline effects on mPFC and striatal extracellular dopamine levels *in vivo*

Microdialysis studies examining MAO inhibitor action on extracellular dopamine levels have used systemic injection of these drugs, with either increases (Sharp et al., 1986; Butcher et al., 1990; Okudo et al., 1992) or no changes reported (Kato et al., 1986; Butcher et al., 1990). In the present study, pargyline concentrations prepared for infusion through the microdialysis probe were higher than those shown to inhibit MAO activity (Cesura and Pletscher, 1992). However, it was not possible to know the concentration of pargyline reaching the surrounding tissue, and substantial increases in extracellular dopamine levels occurred in the mPFC in the absence of decreases in the levels of its metabolites, DOPAC and HVA, indicating an effect of pargyline at least

partially independent of its MAO inhibitory action. In contrast, striatal dopamine levels were elevated by pargyline infusion to a lesser degree and were accompanied by decreases in DOPAC and HVA levels. Together, these data suggest that mPFC dopamine is regulated differently from striatal dopamine and may be caused by a direct effect of pargyline on mPFC dopamine uptake processes, although contribution by long-loop feedback pathways cannot be ruled out. Future *in vivo* studies should examine whether additive effects occur for mPFC dopamine using lower concentrations of MAO inhibition combined with DAT/NET uptake inhibition.

In summary, these results demonstrate that more than one major mechanism appears responsible for dopamine clearance in the mPFC: (1) clearance occurs by DAT and NET, and (2) clearance occurs by a second component that is blocked by MAO inhibitors. The kinetics of dopamine clearance *in vitro* suggest that the effects of DAT and NET inhibitors combined with the MAO inhibitor, L-deprenyl, are additive. This is the first report describing an important contribution by MAO inhibitors for the clearance of mPFC dopamine in the kinetic domain. *In vivo* microdialysis studies infusing the MAO inhibitor, pargyline, into the mPFC suggest that pronounced increases in extracellular dopamine levels occur in the absence of decreases in extracellular DOPAC or HVA levels, whereas pargyline infusion into the striatum produces less pronounced increases that appear to be a consequence of decreased dopamine metabolism. Such findings may have implications for reinterpreting the role of MAO inhibitors in antidepressant action and offer caution against extrapolation of observations across different brain regions when examining MAO inhibitor action and monoamine transporter function. The ability to uniquely control extracellular dopamine levels within the mPFC may have importance for several psychopathological behaviors, including schizophrenia and drug abuse, as well as normal cognitive processes such as working memory.

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