# Transport of Dopamine at the Blood-Brain Barrier of the Guinea Pig: Inhibition by Psychotropic Drugs and Nicotine

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***Purpose.*** Transport of dopamine (DA) across the blood-brain barrier (BBB) was examined in guinea pigs.

***Methods.*** In situ brain perfusion (1–10 min), capillary depletion, and high pressure liquid chromatography (HPLC) were used.

***Results.*** There was a saturable DA influx into the brain with a $K_M$ of 389 ± 55 nM, and a $V_{MAX}$ of 1.95 ± 0.25 pmol/min/g of brain. The diffusion constant, $K_D$, was not significantly different from zero. About 0.5% of DA remained tightly bound to cerebral microvessels isolated from the perfused brain. DA influx into the brain was not altered by the monoamine oxidase-B (MAO-B) inhibitor pargyline (50 μM). HPLC analysis of perfused brain confirmed transport of intact DA, and no detectable increases in DA metabolites were observed. At perfusate concentrations of 500 nM, several dopaminergic receptor antagonists inhibited [$^3$H]-DA (21 nM) influx; the percent inhibitions for the mixed $D_1$ and $D_2$ antagonists haloperidol and chlorpromazine, the $D_1$ antagonist SCH-23390, and the $D_2$ antagonist spiperone were 90%, 68%, 77%, and 50%, respectively. Brain perfusion with nicotine (500 nM) inhibited DA uptake by 86%. This nicotine effect was not altered by mecamylamine, but was partially prevented by the nicotinic receptor antagonist hexamethonium.

***Conclusions.*** a) A significant cerebrovascular permeability to intact DA is mediated by a MAO-B independent specific transport system at the BBB, b) this system could be inhibited by $D_1$ and $D_2$ DA receptor antagonists, and c) DA blood-to-brain transport was inhibited by nicotine.

**KEY WORDS:** dopamine; transport; blood-brain barrier; guinea pigs; nicotine; psychotropic drugs.

## INTRODUCTION

Dopamine (DA) is a monoamine transmitter with important central and peripheral functions. Its central actions involve motor control and cognitive functioning (1,2). The loss of dopaminergic activity has been related to Parkinson's disease, depression and Huntington's disease, while an overactivity of DA pathways is implicated in the pathogenesis of schizophrenia and mania (1,2). It is generally believed that the peripheral and central pools of DA are separated by the blood-brain barrier (BBB), and that circulating DA is not critical in either pathogenesis or treatment of DA-related central nervous system disorders (3,4).

Based on short-term arterial infusion studies (1 sec) in rats, it has been suggested that DA is prevented from crossing the BBB by its high polarity and an absence of a specific transport mechanism (3), as well as by breakdown by the enzyme monoamine oxidase-B (MAO-B), which serves as part of a monoamine enzymatic barrier in rats (4). Striatal DA depletion by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which causes a Parkinsonian syndrome in humans and primates, is prevented by breakdown of MPTP by MAO-B in rats (4). In contrast to previous studies, we recently demonstrated moderate BBB permeability to DA by conducting longer term arterial infusion studies (up to several minutes) in guinea pigs (5). This species contains low levels of MAO-B at the BBB, similar to humans and other primates (4), and consequently suffers striatal DA depletion with the administration of MPTP as humans and primates do (4).

An analysis of DA uptake at the BBB was performed in guinea pigs using an *in situ* brain perfusion technique (6). The length of exposure of DA to the BBB was 60 to 600 times longer than that used by Oldendorf (3). As $D_1$ and $D_2$ receptors have recently been characterized in cerebral microvessels (7), the effects of several psychotropic drugs which influence DA receptors were examined. The effect of nicotine, which causes the release of DA form dopaminergic terminals in the brain (8), and inhibits specific DA transporters (9), was also studied. Our preliminary results have been reported (5).

## METHODS

### Perfusion Technique for Guinea Pig Brain

This method has been described previously (6). Adult Hartley guinea pigs of either sex, weighing 250–350 g, were used. They were anesthetized intramuscularly with 6 mg/kg xylazine (Rompun, ICN) and 30 mg/kg ketamine (Ketaset, Western Medical Supply). The neck vessels were surgically exposed, and the right common carotid artery was cannulated with a fine polyethylene catheter connected to an extracorporeal brain perfusion circuit. At the beginning of the perfusion, the left common carotid artery was ligated, and both jugular veins were cut to allow free drainage of the perfusate. The perfusion was terminated by cutting the right common carotid artery and decapitating the animal.

### Perfusion Medium

Guinea pig brains were perfused with a medium consisting of washed sheep red blood cells suspended at a hematocrit of 20% in a saline medium composed of (in mM): 123 NaCl, 4 KCl, 2.5 $CaCl_2 \cdot H_2O$, 25 $NaHCO_3$, 1.2 $KH_2PO_4$, 1.8 $MgCl_2 \cdot 6H_2O$, and 5.5 D-glucose. A physiologic colloid osmotic pressure was achieved by the addition of 70,000 MW dextran at 48 g/l. The perfusate was gassed with 96% $O_2$ – 4% $CO_2$, and warmed to 37°C in a water bath. It was debubbled and filtered through polymer wool before entering the animal's circulation. Under these conditions, a $pCO_2$ of 38–40 mmHg and a pH of 7.35 can be maintained (6).

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### Perfusion System and Conditions

The perfusate was pumped from a reservoir through the water bath in low gas permeable silicon tubing by a Rainin Rabbit peristaltic pump. Perfusion pressure, respiration and heart rate were continuously monitored.

### Measurement of DA Uptake

Isotopically labeled [$^3$H]-DA (5 μCi/ml) was introduced into the perfusion circuit along with the cerebrovascular space marker [$^{14}$C]-sucrose (2 μCi/ml) by a Harvard syringe pump at the rate of 0.2 ml/min. After the perfusion, ipsilateral brain tissue which included cortex and white matter was prepared for analysis. In some experiments, cerebral microvessels were isolated from perfused brain using a capillary depletion procedure (10) to determine the fraction of radiolabel that remained sequestered or tightly bound to the BBB (6). Samples were taken in triplicate and dispensed into preweighed scintillation vials and dissolved overnight in 2 ml of Beckman Tissue Solubilizer (BTS-450). Twenty microliter aliquots of plasma perfusate were treated the same way. Before counting, 16 ml of Beckman Ready Organic scintillation cocktail were added. Radioactivity was determined in a Beckman LS-7500 liquid scintillation spectrometer.

### HPLC Analysis of DA Uptake

The levels of DA and its main metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were determined by HPLC in ipsilateral brain (including cortex and white matter) taken from guinea pigs perfused for 10 min with 10 μM unlabeled DA (introduced into the perfusion circuit at 0.2 ml/min by a Harvard syringe pump). This concentration was used so that maximum uptake would be achieved. Levels were determined according to the procedure of Kalivas (11). Brain samples were weighed, sonicated briefly in 0.5 ml of 0.1 M trichloroacetic acid (TCA) containing $2 \times 10^{-7}$ M isoproterenol (an internal standard), and then centrifuged at 5,000 g for 10 minutes. The supernatant was saved and stored at −70°C until ready for use. HPLC was performed on a 25 cm silica-filled column (Cole Scientific) in degassed mobile phase (0.1 M TCA, 0.01 M Na acetate, and 0.1 mM EDTA, adjusted to pH 3.65, and 18% methanol, v/v) at a flow rate of 1 ml/min. Oxidation of catecholamines was performed at +0.7 V. Standard curves using concentrations from $10^{-8}$ to $10^{-6}$ were constructed for all compounds measured including DA, DOPAC and HVA. The quantitative analysis was based on peak height, which our experience has shown to be a more reliable measure of the level of catecholamines in sample than peak area.

### Experimental Protocols

The time dependence of DA uptake at the BBB was determined by perfusing 16 guinea pigs with [$^3$H]-DA and [$^{14}$C]-sucrose for times varying from 1–10 min. In order to study the kinetics of DA entry into the brain, a total of 29 guinea pigs were pre-perfused for 4.5 min with various concentrations of unlabeled DA (0.005–250 μM) and then perfused simultaneously with [$^3$H]-DA for an additional 10 min. The effects on [$^3$H]-DA uptake at the BBB of the MAO-B inhibitor pargyline (studied at a concentration of 50 μM), the $D_1$ antagonist SCH-23390 (at 500 nM), the $D_2$ antagonist spiperone (at 500 nM), the mixed antagonists chlorpromazine and haloperidol, and nicotine (all at 500 nM) were examined. The effects of these compounds were determined by a 4.5 min pre-perfusion with the individual compounds followed by a 10 min simultaneous perfusion of [$^3$H]-DA with the compound. The alteration of DA brain uptake in the presence of nicotine by the nicotinic antagonists hexamethonium and mecamylamine was studied by a 4.5 min pre-perfusion with a 500 nM concentration of either hexamethonium or mecamylamine followed by a 10 min simultaneous perfusion with either [$^3$H]-DA and 500 nM nicotine and hexamethonium, or [$^3$H]-DA and 500 nM nicotine and mecamylamine. In all protocols the concentration of [$^3$H]-DA was 21 nM. During all perfusions, the perfusion pressure was recorded and the animal's respiration and heart rate were monitored; these parameters were not significantly altered by any of the experimental protocols.

### Biomathematical Modeling

Kinetic data were analyzed as described previously (6). The corrected volume of distribution, $V_D$, for radiolabeled DA (adjusted for nonspecific sucrose uptake) was calculated as follows:

$$V_D = ([^3\text{H}]\text{-DA dpm/g tissue})/([^3\text{H}]\text{-DA dpm/ml perfusate}) - ([^{14}\text{C}]\text{-sucrose dpm/g tissue})/([^{14}\text{C}]\text{-sucrose dpm/ml perfusate}) \quad (1)$$

where dpm (disintegrations per minute) is the radioactivity, measured in brain tissue, cerebral microvessels or perfusate. The initial volume of distribution, $V_D$, is the extrapolated $V_D$ at zero time. The cerebrovascular permeability surface area product, PS, is the same as the unidirectional transport rate determined during the phase of linear uptake if PS << cerebral blood flow, a condition satisfied in this study. Thus,

$$PS = (V_D - V_I)/T \quad (2)$$

When DA uptake was studied in the presence of various concentrations of unlabeled DA, the following equation was used for analysis (6):

$$PS = V_{MAX}/(K_M + C_{PL}) + K_D \quad (3)$$

where $C_{PL}$ is the total concentration of DA in the perfusate, $V_{MAX}$ is the maximal rate of saturable uptake, $K_M$ is the Michaelis constant, and $K_D$ is the diffusion constant. The values of $K_M$, $V_{MAX}$ and $K_D$ were obtained from fitting the DA uptake data to equation 3 by nonlinear regression least squares. Unidirectional DA influx ($J_{IN}$) was calculated as

$$J_{IN} = F(1 - e[-PS/F])C_{PL} \quad (4)$$

where F is the perfusion blood flow. Since F >> PS, $J_{IN} \approx PS \cdot C_{PL}$ (6), it follows that

$$J_{IN} = V_{MAX}C_{PL}/(K_M + C_{PL}) + K_DC_{PL} \quad (5)$$

### Statistical Analysis

All values were presented as mean ± SE. Means were compared using a Student's t-test. When variances between two

groups did not significantly differ according to the Bartlett test for homogeneity of variance, the t-test for samples with equal variance was used. When the variances were significantly different, Satterthwaite's approximation was used.

### Unlabeled Compounds

Dopamine, spiperone, chlorpromazine, haloperidol, pargyline, nicotine, hexamethonium and mecamylamine were all from Sigma. SCH-23390 was obtained from Research Biochemicals Incorporated.

### Radioactive Compounds

[2,5,6-$^3$H]-DA, 5–15 Ci/mmol, and [$^{14}$C]-sucrose, 0.46–0.66 Ci/mmol, were obtained from Amersham.

## RESULTS

Figure 1 is a multiple time point uptake plot obtained during perfusion of the brain with [$^3$H]-DA and [$^{14}$C]-sucrose. There is a time dependent progressive uptake of DA and to a much lesser extent of sucrose—the non-corrected volume of distribution (in μl/g) was 14.75 for DA vs. 2.06 for sucrose at 1 minute, and 61.53 for DA vs. 5.15 for sucrose at 10 minutes. The initial volumes of distribution, $V_I$ (in μl/g defined as the y-intercept value derived from linear regression analysis), however, were not significantly different for the two substances,

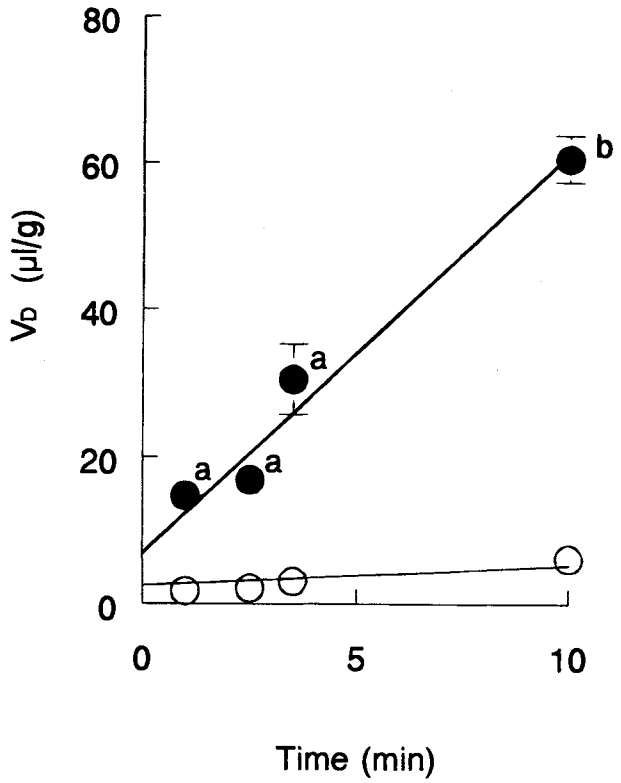



**Fig. 1.** Time dependence of [$^3$H]-DA (·) and [$^{14}$C]-sucrose (o) uptake into perfused guinea pig brain. The volume of distribution ($V_D$) is plotted against the perfusion time, T. Each point represents mean ± SE for 3–4 animals. Each value was determined in triplicate. [a]Significantly greater than sucrose uptake, $p < 0.01$; [b]$p < 0.001$; by Student's t-test.

i.e., 6.94 ± 3.01 for DA and 2.58 ± 0.46 for sucrose (mean ± SE). Table I gives the permeability surface area product, PS, and the cerebrovascular permeability constants, P, for [$^3$H]-DA and [$^{14}$C]-sucrose, determined by multiple time point graphic analysis. The PS of [$^3$H]-DA was approximately twenty times higher than that of sucrose. However, in the presence of 1 μM unlabeled DA, the uptake of [$^3$H]-DA became indistinguishable from sucrose.

HPLC analysis of brain tissue perfused for 10 min in the absence or presence of 10 μM DA (typical chromatograms are shown in Figure 2A and 2B, respectively) was performed. The DA-perfused tissue had an average of 41.8 pmol/g more DA, a difference of 59 %, while levels of DOPAC and HVA were not significantly changed (Figure 2C).

As shown in Figure 3, the fraction of [$^3$H]-DA tightly bound by brain cerebral microvessels is very small—most of the [$^3$H]-DA uptake is accounted for by uptake into brain tissue. The uptake of [$^3$H]-DA into the brain as well as the sequestration by microvessels was strongly inhibited by 1 μM unlabeled DA (Figure 3 and Table I).

Figure 4A shows the self-inhibition of [$^3$H]-DA uptake by several concentrations of unlabeled DA. The inset is an expanded section of the lower concentration region of the curve. The uptake of DA into the brain follows Michaelis-Menten kinetics. The curve was fit with the use of nonlinear weighted least squares to equation 3 and yielded a $K_M$ value of 389 ± 55 nM and a $V_{MAX}$ of 1.95 ± 0.25 pmol/min/g (Table II). $K_D$ was found to be negligible, indicating the lack of a significant passive diffusional component to this transport. Figure 4B shows the unidirectional influx of [$^3$H]-DA (calculated with equation 5) as a function of the concentration of unlabeled DA in the perfusate.

The MAO-B inhibitor pargyline, at a concentration of 50 μM, was found to have no significant effect on the cerebrovascular permeability, i.e. the PS product, of [$^3$H]-DA (Figure 5). The PS product of [$^3$H]-DA was significantly reduced, however, by several DA receptor-blocking drugs, including the $D_1$ receptor antagonist SCH-23390, the $D_2$ receptor antagonist spiperone, and the mixed antagonists chlorpromazine and haloperidol (all tested at a concentration of 500 nM) (Figure 5).

Table II shows the effect of nicotine on DA uptake in the presence and absence of the nicotinic antagonists hexamethonium and mecamylamine. Nicotine had a stronger inhibitory

**Table I.** Permeability Surface Area Products of Dopamine and Sucrose in Perfused Guinea Pig Brain

| | PS (ml/g/s × $10^6$) | P (nm/s) | Corrected PS (ml/g/s × $10^6$) |
|---|---|---|---|
| [$^3$H]-Dopamine | 90.7 ± 3.7[a] | 9.07 | 82.3 ± 3.3 |
| + 1 μM DA | 6.0 ± 0.8[ns] | 0.60 | 2.0 ± 0.3 |
| [$^{14}$C]-Sucrose | 4.5 ± 1.0 | 0.45 | |

Multiple time point graphic analysis was used to determine PS values. The cerebrovascular permeability constant, P, was determined on the assumption of a capillary surface area of 100 cm$^2$/g brain (20). Corrected PS values are adjusted for uptake of sucrose (vascular space marker). Values are mean ± SE for 3–4 perfused brains. [a]Significantly greater than PS of sucrose ($p < 0.01$); [ns], not significantly different from PS of sucrose; by Student's t-test.

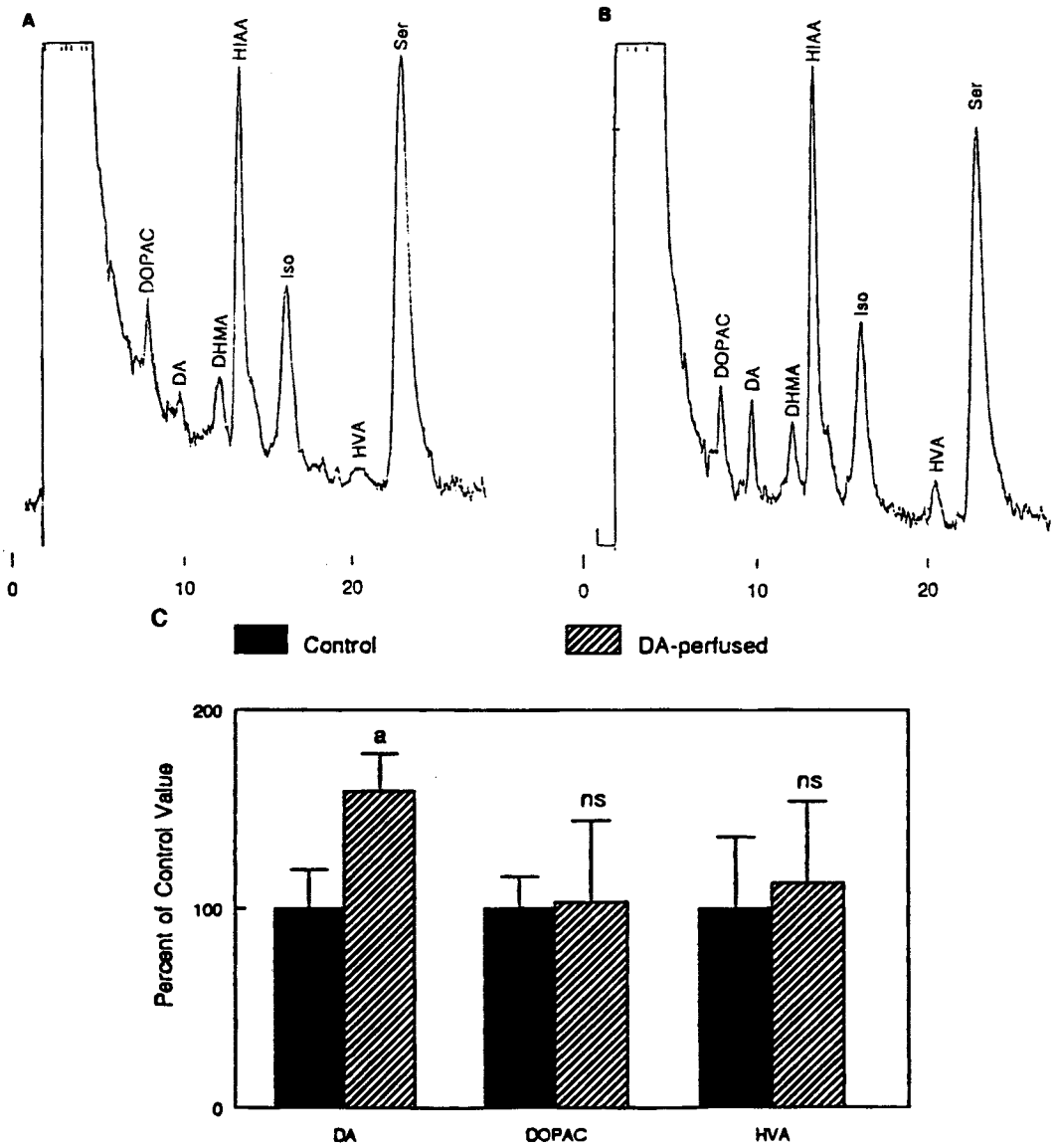



**Fig. 2.** A and B. HPLC analysis of DA and its metabolites in guinea pig brain perfused for 10 min without (A) and with (B) 10 μM DA. C. Relative levels of DA, DOPAC, and HVA in control (solid bars) and DA-perfused (cross-hatched bars) guinea pigs. Values are mean ± SE based on 7–8 animals. [a]Significantly greater than control, $p < 0.05$; [ns], not significant; by Student's t-test. DA = dopamine; DHMA = 3,4-dihydroxymandelic acid; DOPAC = 3,4-dihydroxyphenylacetic acid; HIAA = 5-hydroxyindole-3-acetic acid; HVA = homovanillic acid; Iso = isoproterenol; Ser = serotonin.

effect on dopamine transport than DA itself. Simultaneous perfusion with mecamylamine did not prevent this inhibition, but simultaneous perfusion with hexamethonium lead to a significant decrease in the inhibition of DA transport by nicotine.

## DISCUSSION

This study demonstrates the presence of a significant cerebrovascular permeability to DA mediated by a specific, MAO-B independent transport system at the BBB. This system could be inhibited by several $D_1$ and $D_2$ DA receptor antagonists. The cerebrovascular permeability of DA was also markedly reduced by nicotine.

The uptake by brain tissue of [$^3$H]-DA is time dependent and linear for at least 10 min (Figure 1), and is inhibitable by unlabeled DA (Table I), as is the capillary sequestration of [$^3$H]-DA (Figure 3). The DA BBB transport follows Michaelis-Menten kinetics (Figure 4), and has a low capacity ($V_{MAX}$ = 1.95 ± 0.25 pmol/min/g), but a high affinity ($K_M$ = 389 ± 55 nM). The fact that normal plasma levels of DA are in the nanomolar range raises the possibility that such a transport mechanism may be of physiological importance in the regulation of brain DA homeostasis. The lack of effect of MAO-B inhibitor pargyline (Figure 5) suggests that MAO-B is not involved in DA BBB uptake, which is expected given the low cerebral capillary MAO-B levels of guinea pigs.[4] The HPLC

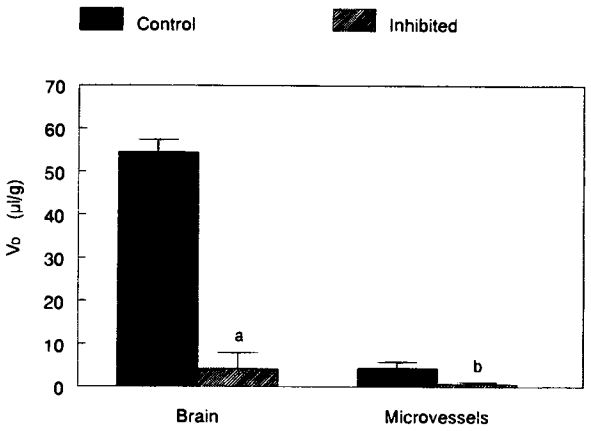
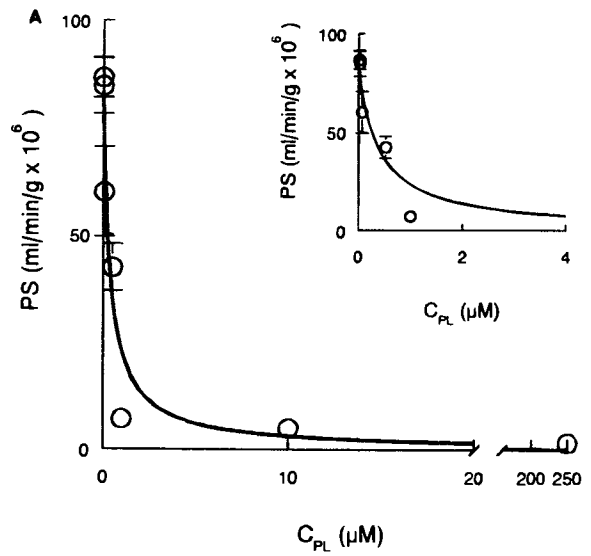



**Fig. 3.** Inhibition of [$^3$H]-DA uptake by 1 M unlabeled DA at the cerebral microvessels and in brain tissue, studied after 10 min of perfusion. Values are mean ± SE based on 3–4 animals and are corrected for sucrose. Solid bars, uninhibited uptake; hatched bars, inhibited uptake. [a]Significantly less than control, $p < 0.001$; [b]$p < 0.05$; by Student's t-test.



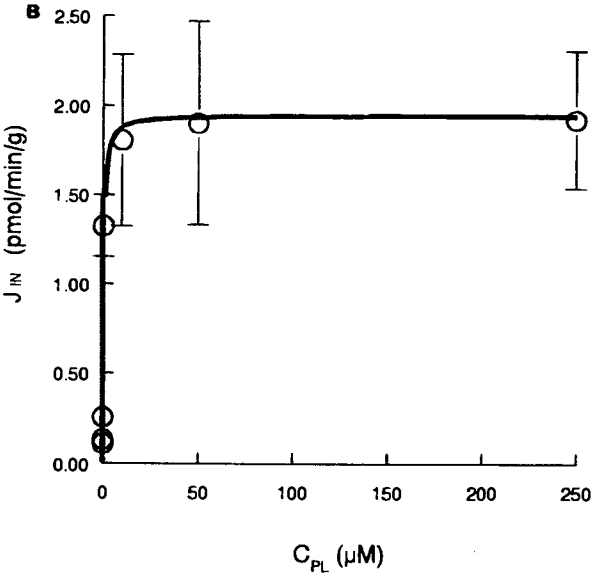




**Fig. 4.** A. Permeability surface area product, PS, for [$^3$H]-DA plotted against DA concentration in the perfusate, $C_{PL}$. Curve is nonlinear regression least squares fit of PS values to equation 3; each point is mean ± SE for 3–4 perfused brains and is corrected for sucrose. The inset illustrates the uptake at the lower concentrations. B. DA influx, $J_{IN}$, into the brain plotted against $C_{PL}$. Curve represents influx predicted by equation 5; each point is mean ± SE for 3–4 perfused brains and is corrected for sucrose.

analysis verified that the uptake measured was in fact of intact DA and not of its breakdown products (Figure 2). The levels of DA metabolites were not significantly higher in DA-perfused brains than in control brains, while there was a significant increase in the level of DA itself which can only be attributed to uptake of circulating DA. However, it is possible that some metabolism of DA may occur at concentrations that are lower than presently studied.

Several known DA receptor antagonists including SCH-23390 (a $D_1$ antagonist), spiperone (a $D_2$ antagonist), as well as mixed $D_1$ and $D_2$ antagonists chlorpromazine and haloperidol, were shown to inhibit the DA BBB transport system (Figure 5). Since both types of DA receptors are present at the BBB (7), the $D_1$ and $D_2$ antagonists may inhibit the DA brain uptake by blocking these receptors. The DA antagonists tested in the present study are lipophilic (12), so they could cross the BBB via lipid-mediated diffusion to displace circulating DA from post-BBB dopaminergic sites, as they do with endogenous DA. Whether, the DA BBB receptors are directly involved in uptake of circulating DA, or specific DA BBB transporters are modulated by these receptors remains to be resolved. The DA transporters with a role of terminating dopaminergic neurotransmission by reaccumulation of DA into presynaptic neurons have been recently cloned (see for review 13,14), but their presence at the BBB still needs to be confirmed.

The uptake of DA was strongly inhibited by nicotine (see Table II). Mecamylamine, which crosses the BBB and blocks nicotinic receptors in the brain (15), did not alter the effect of nicotine on DA transport, suggesting that nicotinic brain receptors and nicotine-induced release of endogenous DA (8), most likely do not participate in inhibition of DA BBB transport. In contrast, hexamethonium, which does not cross the BBB(15) and therefore could modulate the action of nicotine only at the level of the BBB, significantly inhibited nicotine effect on DA BBB transport. This suggests that the inhibition of DA BBB transport by nicotine may involve an indirect action of nicotinic receptor on DA transporter at the BBB, similar to recently reported nicotine-induced inhibition of DA uptake by PC12 cells (9).

The presence of a specific transport system for DA at the BBB may have many important clinical implications. This system could supply DA to the brain in addition to endogenous brain synthesis. It may be involved in the transport and mechanism of action of psychotropic DA-related drugs such as chlorpromazine and haloperidol. By interacting with this transport mechanism, nicotine may interfere with physiological transport of DA into the brain and possibly with the action of DA-related

**Table II.** Influence of Nicotine and the Nicotinic Antagonists Hexamethonium and Mecamylamine on Dopamine Uptake

| Inhibitor (500 nM) | %Inhibition of DA uptake |
|---|---|
| Nicotine | 86.4 ± 31.7[a] |
| Nicotine + Mecamylamine | 90.8 ± 4.9 |
| Nicotine + Hexamethonium | 62.8 ± 5.4[b] |

The percent inhibition was determined from the decrease in the PS (corrected for sucrose uptake) of DA uptake in the presence of the inhibitor from the control value. Relative inhibition was calculated as % inhibition by inhibitor/% inhibition by 500 nM DA. Values are mean ± SE based on 3 to 5 perfused brains. [a]$p < 0.001$; [b]significantly less inhibition of DA uptake than nicotine alone ($p < 0.01$).

drugs as well. A further characterization of this system and its potential manipulation could have important ramifications for the treatment of many DA-related disorders, such as Parkinson's disease and schizophrenia.

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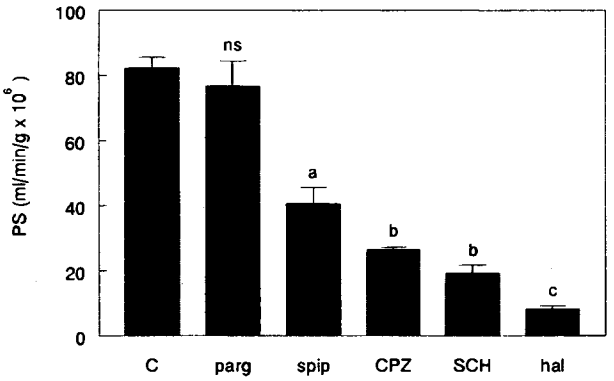


**Fig. 5.** Permeability surface area product, PS, for [$^3$H]-DA in guinea pig brain in the absence and presence of various competitors and/or inhibitors. All values are mean ± SE and are corrected for sucrose. N = 3–6 for each value. All compounds were tested at a concentration of 500 nM. C, control; parg, pargyline; spip, spiperone; CPZ, chlorpromazine; SCH, SCH-23390; hal, haloperidol. [a]Significantly less than control, $p < 0.05$; [b]$p < 0.01$; [c]$p < 0.001$; [ns], not significant; by Student's t-test.

## REFERENCES

1. M. A. Menza, L. I. Golbe, R. A. Cody, and N. E. Forman. Dopamine related personality traits in Parkinson's disease, *Neurology* **43**:505–508 (1993).
2. D. J. Diehl and S. Gershon. The role of dopamine in mood disorders, *Comprehensive Psychiatry* **33** (2):115–120 (1992).
3. W. H. Oldendorf. Brain uptake of radiolabeled amino acids, amines and hexoses after arterial injection, *Am. J. Physiol.* **221**:1629–1638 (1971).
4. S. I. Harik, J. J. Riachi, R. N. Kalaria, and J. C. Lamanna. The role of the blood-brain barrier in preventing MPTP toxicity. In B. B. Johansson, C. Owman, and H. Widner (eds), *Pathophysiology of the Blood-Brain Barrier,* Elsevier Science Publishers, Cambridge, 1990, pp. 255–267.
5. C. L. Martel, J. B. Mackic, M. H. Weiss, J. G. McComb, and B. V. Zlokovic. Transport and binding of circulating dopamine at the blood-brain barrier in guinea pigs, *Soc. Neurosci. Abstr.* **19**:695 (1993).
6. B. V. Zlokovic. Cerebrovascular permeability to peptides: manipulations of transport systems at the blood-brain barrier, *Pharmaceut. Res.* **12**:1395–1406 (1995).
7. F. Basic, S. Uematsu, R. M. McCarron, and M. Spatz. Dopaminergic receptors linked to adenylate cyclase in human cerebromicrovascular endothelium, *J. Neurochem.* **57**:1774–1780 (1991).
8. S. Grady, M. J. Marks, S. Wonnacott, and A. C. Collins. Characterization of nicotinic receptor-mediated [$^3$H]dopamine release from synaptosomes prepared from mouse striatum, *J. Neurochem.* **59**:848–856 (1992).
9. H. Yamashita, S. Kitayama, Y.-X Zhang, T. Takahashi, T. Dohi, and S. Nakamura. Effects of nicotine on dopamine uptake in COS cells possessing the rat dopamine transporter and in PC12 cells, *Biochem. Pharmacol.* **49**:742–745 (1995).
10. D. Triguero, J. Buciak, and W. M. Pardridge. Capillary depletion method for quantification of blood-brain barrier transport of circulating peptides and plasma proteins, *J. Neurochem.* **54**:1882–1888 (1990).
11. P. W. Kalivas. Sensitization to repeated enkephalin administration into the ventral tegmental area of the rat. II. Involvement of the mesolimbic dopamine system, *J. Pharmacol. Exp. Ther.* **235**:544–550 (1985).
12. T. Suhara, K. Nakayama, O. Inoue, H. Fukuda, M. Shimizu, A. Mori, and Y. Tateno. $D_1$ dopamine receptor binding in mood disorders measured by positron emission tomography, *Psychopharm.* **106**:14–18 (1992).
13. J. L. Corey, M. W. Quick, N. Davidson, H. A. Lester, and J. Guastella. A cocaine-sensitive Drosophila serotonin transporter: cloning, expression, and electrophysiological characterization, *Proc. Natl. Acad. Sci. USA* **91**: 1188–1192 (1994).
14. M. A. Hediger, Y. Kanai, G. You, and S. Nussberger. Mammalian ion-coupled solute transporters, *J. Physiol.* **482**:7S–17S (1995).
15. B. Greenwood, E. Blank, and W. J. Dodds. Nicotine stimulates esophageal peristaltic contractions in cats by a central mechanism, *Am. J. Physiol.* **262**:G567–G571 (1992).