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Model for Estimating Dopamine Transporter Occupancy and Subsequent Increases in Synaptic Dopamine Using Positron Emission Tomography and Carbon-11-Labeled Cocaine

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ABSTRACT. Although increases in dopamine secondary to the inhibition of the dopamine transporter appear to underlie the reinforcing properties of cocaine, there is presently no model that relates the elevation of synaptic dopamine to the transporter occupancy by cocaine. We propose such a model based on positron emission tomographic (PET) measurements of the brain concentration of cocaine and the assumption of rapid equilibrium between free cocaine and cocaine bound to the dopamine transporter. A euphorigenic dose of cocaine (about 40 mg) is predicted to occupy 80–90% of the transporters, while a perceptible dose (about 5 mg) occupies about 40% of the transporters. If reuptake of dopamine is reduced in proportion to the fraction of transporters occupied by cocaine, our model indicates that synaptic dopamine rises supra-linearly with occupancy, so that 5 and 40 mg doses of cocaine give about 2- and 10-fold increases, respectively. A consequence is that a given dose of cocaine produces a similar degree of elevation of dopamine regardless of the prior level of occupation of the transporters by cocaine. This prediction is supported by recent PET/neuropsychological studies in our laboratory where dopamine transporter occupancy was measured after giving methylphenidate intravenously to volunteers; similarly intense "highs" were reported whether the initial occupancy was zero or 75–85%. It could also explain why attempts to block the psychostimulant-induced "high" by pretreating subjects with drugs that block the dopamine transporter have been unsuccessful, and why the use of methylphenidate to treat cocaine addicts led to increased cocaine consumption. *Copyright © 1996 Elsevier Science Inc.*, BIOCHEM PHARMACOL 53;1:43–52, 1997.

KEY WORDS. cocaine; dopamine transport; drug abuse; methylphenidate; positron emission tomography (PET); pharmacotherapy

Intravenous injection of cocaine and inhalation of cocaine vapor are forms of drug abuse associated with serious medical and social problems. The results of clinical and animal research suggest that the rapid delivery of cocaine to the brain by these routes may be uniquely reinforcing [1–3]. Cocaine inhibits the reuptake of dopamine, serotonin, and norepinephrine [4], and also exhibits a range of interactions with other pharmacological systems [5–7]. However, a considerable body of evidence implicates inhibition of dopamine reuptake in the striatum and especially the nucleus accumbens as the first step in the chain of processes leading to reinforcement in animals and addiction in humans. The evidence includes the good correlation between affinity for the dopamine transporter and reinforcing potency of a wide range of psychostimulant drugs [8, 9], elevation by the same group of drugs of extracellular dopamine in terminal dopaminergic fields including the nucleus accumbens [10, 11], where iontophoretic application of dopamine [12] or reuptake blockers [13] also activates reward circuits, and the recent demonstration that dopamine transporter deficient mice are insensitive to cocaine [14]. The dopamine hypothesis of cocaine abuse, however, is at present only a qualitative model. Occupancies of the dopamine transporter by cocaine required to elicit a "high," to abolish craving, or to alter other parameters involved in cocaine dependency are unknown, as are the resulting occupancies of the relevant population(s) of dopamine receptors by dopamine.

The availability from PET† experiments of drug pharma-

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† *Abbreviations:* PET, positron emission tomography, B_{max}, tissue concentration of dopamine transporter (nM or pmol/g); B, concentration of cocaine in tissue bound to dopamine transporter (nM); K_d', dissociation constant *in vivo* for binding of cocaine to dopamine transporter (nM); V_{max}, Michaelis-Menten parameter for dopamine transporter (unit not defined here); K_{da}, (K_m for dopamine) Michaelis-Menten parameter for dopamine transporter (nM); [DA], concentration of synaptic dopamine (nM); SPECT, single photon emission computed tomography; WIN 35,428, 2β-carbomethoxy-3β-(4-fluorophenyl)tropane; RTI-55, 2β-carbomethoxy-3β-(4-iodophenyl)tropane.

cokinetics in human tissues provides new opportunities for relating binding site occupancies of drugs to their behavioral and physiological effects. PET experiments have demonstrated selective binding of [^{11}C]cocaine to dopamine transporters in the basal ganglia of human and baboon brains [15]. Furthermore, the time-course of binding of [^{11}C]cocaine in these regions of the human brain (peak uptake at 5 min, and a clearance half-time of 20 min) is very similar to that of the "high" reported by cocaine abusers [16]. This observation is a further indication that cocaine binding to the dopamine transporter may be the initial event in reinforcement. In contrast to PET scans where only tracer amounts of [^{11}C]cocaine (<5 μg/kg) were administered, scans where doses of cocaine known to induce euphoria in humans (0.5 mg/kg) were also given did not show preferential uptake of C-11 in the basal ganglia of baboons [17]. Peak uptake in striatum, expressed as a fraction of injected activity, was similar, but occurred earlier than with tracer doses of [^{11}C]cocaine, and both early uptake and clearance were almost identical in all brain regions ([17], see Table 1).

Modelling of the local brain kinetics of [^{11}C]cocaine has yielded estimates of its binding potential (B_{max}/K_d'; the ratio of striatal dopamine transporter concentration to the apparent dissociation constant) [18]. Recent PET experiments in baboons, using [^{11}C]cocaine at different specific activities, suggest that pharmacological doses of cocaine achieve occupancies of >70% [19]. One aim of the present study was to explore a more theoretical approach to calculating occupancies, which was prompted by the observation that the fraction of [^{11}C]cocaine taken up by the basal ganglia showed little dependence on the mass of cocaine injected (Table 1). If free and bound [^{11}C]cocaine are near equilibrium at the time of peak tissue concentration, than an estimate of occupancy at any dose can be made from this concentration, together with measurements of B_{max} for the dopamine transporter from post-mortem tissues, and PET measurements of B_{max}/K_d'. A second aim was to relate occupancy of the dopamine transporter to increases in synaptic dopamine, making simple assumptions about the kinetics of the transporter *in vivo*, and initially assuming no compensating alterations in the rate of release of dopamine. These two approaches together allowed us to relate *in vivo* cocaine binding to changes in synaptic dopamine. Although we anticipated that our calculations would provide a very simplified description of the actions of cocaine, we hoped that they might stimulate the design of critical experiments and provide a basis for more informed speculations.

METHODS AND ASSUMPTIONS

We have assumed a situation close to that of the "classical" synapse where the "high" is mediated by a population of postsynaptic dopamine receptors, and dopamine transporters are located in close proximity to the site of exocytotic release of dopamine. There is no direct evidence for this picture, but a "synaptic" rather than "extracellular" location for the relevant receptors is consistent with the rapidity of induction of the "high," since microdialysis experiments indicate that a maximum value of extracellular dopamine occurs >20 min after administration of cocaine [20]. Dopamine may be more likely to diffuse away from any given synapse than to undergo reuptake at that synapse [21, 22], but nearly all the exocytotically released dopamine is eventually cleared by reuptake [14]. Grace [23] recently proposed a model emphasizing the importance of distinct synaptic and extracellular dopamine pools.

TABLE 1. Fractional uptake of C-11 in striatum following administration of labeled cocaine to baboons and humans

Species	Dose	Peak (% IA/cc*) at (min)	B_{max}/K_d'
Human†	<5 μg/kg	0.008 (4.5)	0.67
Baboon†	<5 μg/kg	0.045 (4.5)	0.67
	0.5 mg/kg	0.053 (2.5)	

* Percent injected radioactivity per cc.
† Values from Refs 15, 17, and 18.
‡ Not determined.

Estimation of Occupancy

Cocaine binds quite weakly to the dopamine transporter, and dissociates quickly *in vitro* [8] so that rapid equilibration of free and bound cocaine is expected. This notion is supported by the short striatal retention time measured in PET experiments [15]. We assume that the fractional delivery of cocaine to the brain is independent of the dose (see Results and Table 1), and that at the time of maximum tissue radioactivity, a state of equilibrium exists between free transporter ($B_{max} - B$), free cocaine ($^{11}C - B$), and cocaine bound to transporter (B).

$$K_d' = \frac{(B_{max} - B) \times (^{11}C - B)}{B} \quad (1)$$

After solving Eqn (1) for B, fractional occupancy is given by

$$\text{Occupancy} = B/B_{max} \quad (2)$$

This analysis assumes that the elevated synaptic dopamine *does not* compete with cocaine for binding to the transporter, thus reducing the transporter occupancy indicated by equations (1) and (2). Many *in vitro* studies have shown that high concentrations of dopamine can competitively inhibit the binding of cocaine and other pyschostimulant drugs to the dopamine transporter [4, 24, 25], although the binding sites for cocaine and dopamine are not believed to be identical [26, 27]. We previously found that dopamine depletion increased striatal [^{11}C]cocaine binding in baboons, although the increase was only about one-third that seen with the dopamine D_2 receptor radioligand [^{11}C]ra-

clopride [28]. On the other hand, we were unable to document an effect of dopamine depletion on the striatal binding of [^{11}C]*d-threo*-methylphenidate in similar PET experiments [29]. Nor could we demonstrate effects of electrically stimulated dopamine release on the binding of the cocaine analogs WIN 35,428 and RTI-55 to superfused striatal slices [30]. Thus, the extent and mode of competition between cocaine and dopamine at the dopamine transporter are at present unclear.

The occupancy calculations require values for the tissue concentration of dopamine transporters (B_{max}) and the apparent equilibrium constant *in vivo* (K_d'), in addition to the concentration of [^{11}C]cocaine from the PET data. Estimates of B_{max} are available in the literature (Table 2), and PET studies using tracer [^{11}C]cocaine provide values of about 0.67 for B_{max}/K_d' from tracer kinetic analysis [18]. We chose a value of 800 nM (strictly, pmol/g) for B_{max}, near the mid-point of the values in Table 2, for most of our calculations. This implies a K_d of about 1200 nM, about 10-fold greater than most literature values [25]. Reasons for observing higher K_d values *in vivo* than *in vitro* have been discussed [38].

Equation (1) carries the implicit assumption that binding of cocaine to the dopamine transporter can be explained in terms of a single binding site. Many *in vitro* binding studies of the dopamine transporter using cocaine or a cocaine analog (WIN 35,428 or RTI-55) as radioligand report high and low affinity sites (e.g. Ref. 8), although this is controversial (e.g. Ref. 39). However, whether these sites exist *in vivo*, and reflect alternative states of the transporter with different kinetic parameters for dopamine transport, or whether they are artifacts of tissue disruption, is unknown.

Estimation of Changes in Synaptic Dopamine

We assume that striatum is regionally homogeneous with respect to dopamine release and reuptake, that all the dopamine released is recovered by reuptake, and that the average synaptic dopamine concentration is determined only by the rates of release and reuptake, which must be equal at equilibrium. If the degree of occupancy of the transporter by cocaine proportionately decreases the maximal velocity of dopamine reuptake, then a new equilibrium synaptic dopamine concentration will be established. Assuming Michaelis-Menten kinetics, which is supported by *in vivo* voltammetry data [20], and that binding of cocaine to the transporter does not result in an altered rate of release of dopamine, the new dopamine concentration can be calculated without knowing the baseline rate of dopamine reuptake. Thus,

$$v = V_{max}[DA]/(K_{da} + [DA]), \text{ and} \quad (3a)$$
$$v = (1 - \text{Occupancy})\ V_{max}[DA']/(K_{da} + [DA']) \quad (3b)$$

where V_{max} and K_{da} are the kinetic parameters of the transporter, and $[DA]$ and $[DA']$ are the equilibrium dopamine concentrations under baseline and inhibited conditions. It follows that

$$(1 - \text{Occupancy}) = (1 + K_{da}/[DA'])/(1 + K_{da}/[DA]) \quad (4)$$

This equation allows calculation of the increase in dopamine, $[DA']/[DA]$, corresponding to any occupancy level if values for $[DA]$ and K_{da} are known. For K_{da} values we chose the recent literature estimates of 150 nM [40] and 1 μM [26]. For $[DA]$, we used 50 nM, an estimate made by Ross [41] from the striatal binding of [^{3}H]raclopride in control and dopamine-depleted mice, and 10 nM, which is at the upper limit of the range of extracellular dopamine measured during *in vivo* microdialysis experiments [42].

TABLE 2. Dopamine transporter B_{max} values

Species	Tissue	Tracer	B_{max} (pmol/g tissue)
Monkey*	Striatum	Cocaine	340
Monkey†	Striatum	WIN 35,428	460
Baboon‡	Striatum	RTI-55	480
Human§	Striatum	RTI-55	61
Human‖	Caudate	GBR 12935	1360
Human¶	Putamen	Cocaine	1470
Human**	Caudate	GBR 12935	770
Human**	Putamen	GBR 12935	600

* Ref. 31.
† Ref. 32.
‡ Ref. 33.
§ Ref. 34.
‖ Ref. 35
¶ Ref. 36.
** Ref. 37.

RESULTS

Brain Concentrations of C-11 Cocaine

Table 1 gives peak values for the fractional uptakes of C-11 in striatum following administration of labeled cocaine to baboons and humans [15, 17, 18]. For baboons, data are given for high (<5 μg/kg) and low (0.5 mg/kg) specific activity. A slightly higher fraction of the pharmacological dose of cocaine (carrier added) appears to be taken up by the striatum than for a tracer (no carrier added) dose, but these differences are not marked. Human data are currently available only for high specific activity tracer.

Estimation of Occupancy as a Function of Dose

Figure 1 shows calculated values for dopamine transporter occupancy and fraction of C-11 corresponding to free cocaine as a function of cocaine dose, calculated according to equations (1) and (2) assuming peak striatal concentrations of 0.06% of injected cocaine per cc for baboons, and 0.008% of injected cocaine per cc for humans. A dose of 0.5

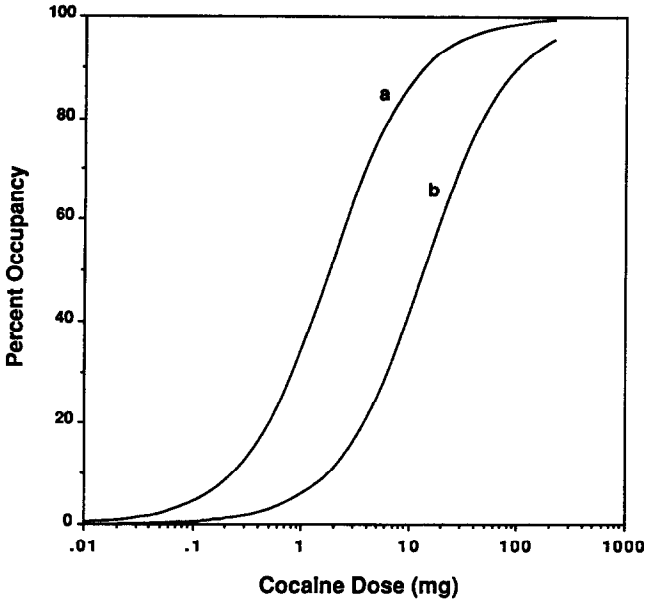

FIG. 1. Occupancy as a function of dose: Model predictions in baboon and human. Percent occupancy versus dose of cocaine for baboon (a), assuming uptake of 0.06% injected radioactivity per cc, and human (b), assuming uptake of 0.008% injected radioactivity per cc. Calculations assume a B_{max} value of 800 pmol/g of tissue and a B_{max}/K_d' of 0.67.

mg/kg of cocaine, corresponding to about 8 mg in a baboon and 40 mg in a human, gives a transporter occupancy of 80–90%. The model predicts an occupancy of about 40% for injection of 5 mg into a human, a dose that is just detectable by a naive subject.

Changes in Synaptic Dopamine as a Function of Occupancy

Figure 2 (left-hand panel) presents simulations relating the percent increase in baseline dopamine to the transporter occupancy, calculated according to equation (4). Regardless of the values chosen for baseline dopamine and K_{da}, the shape of the curve indicates a progressively increasing synaptic dopamine at an occupancy above 40%, where baseline dopamine is approximately doubled. The increase is steeper when smaller K_{da} values and higher baseline dopamine concentrations are assumed. An interesting feature of these simulations is that the increase in DA for an increase in occupancy keeps on rising. When this ratio is plotted versus occupancy, it is almost constant until quite high occupancy for three of the four sets of K_d' values and dopamine concentrations chosen (Fig. 2, right-hand panel).

Increase in Baseline Dopamine as a Function of Cocaine Dose

A combination of equations (1), (2), and (4) allows the calculated changes in dopamine in a human subject to be plotted against the dose of cocaine injected (Fig. 3). If the higher estimates of baseline dopamine concentration (50 nM) and affinity (150 nM) are chosen, a greater than 20-fold increase in synaptic dopamine is predicted for administration of even 10 mg cocaine. If 10 and 1000 nM, respectively, are chosen for these parameters, an almost linear relationship between increased dopamine and cocaine dose is obtained over the range 0 to 40 mg, where an approximately 10-fold increase is predicted. Values for transporter occupancies and increased synaptic dopamine achieved by 5 and 40 mg doses of cocaine are shown in Table 3.

Compensatory Changes in the Rate of Dopamine Release

Dopamine transporter blockade provokes compensatory decreases in dopamine release via autoreceptor activation at both nerve terminals and cell bodies (see Discussion). This can be taken account of in the model by adjusting the value

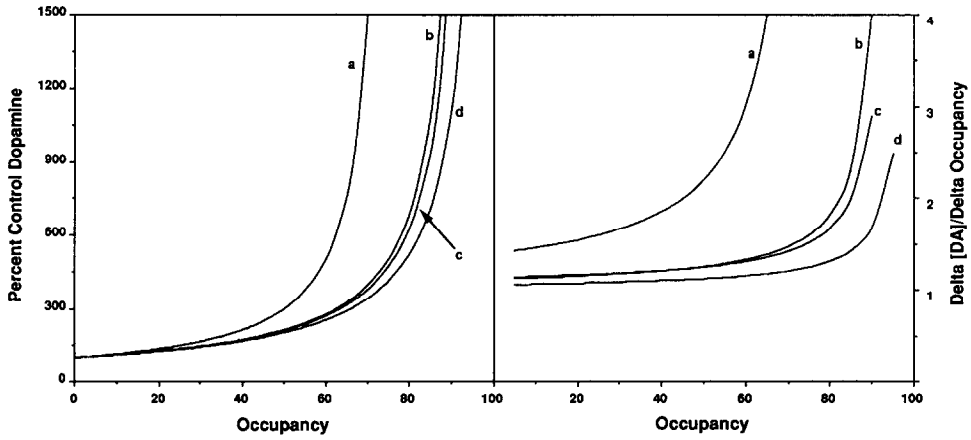

FIG. 2. Synaptic dopamine as a function of transporter occupancy. (Left-hand panel) Simulations were performed assuming values for the equilibrium constant for dopamine, and for the baseline concentration of synaptic dopamine, respectively, of: (a) 150 and 50 nM; (b) 150 and 10 nM; (c) 1000 and 50 nM; and (d) 1000 and 10 nM. (Right-hand panel) Curves are the first derivatives of those in the left-hand panel.

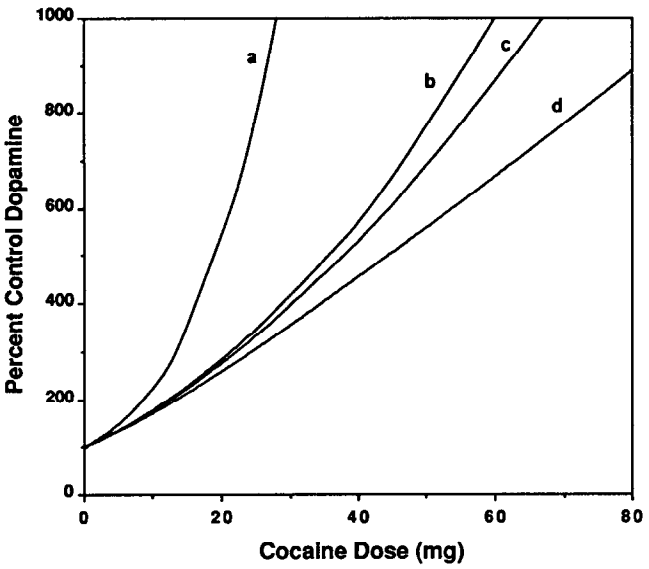

FIG. 3. Effect of dose of cocaine on synaptic dopamine. Dopamine increases in humans were estimated by combining the calculation of occupancy as a function of cocaine dose (equations (1) and (2)) and the calculation of synaptic dopamine as a function of occupancy (equation (4)). A dopamine transporter B_{max} value of 1600 pmol/g was assumed. The curves (left to right) assume values for the equilibrium constant for dopamine, and for the baseline concentration of synaptic dopamine, respectively, of: (a) 150 and 50 nM; (b) 150 and 10 nM; (c) 1000 and 50 nM; and (d) 1000 and 10 nM.

of V_{max} on the left-hand side of equation (3a). Figure 4 gives plots of the increase in dopamine predicted for a transporter occupancy of 85% with various degrees of compensatory decreases in the rate of dopamine release. For a reduction in dopamine release to 50% of control values, the increase in dopamine is 3- to 4-fold.

Cocaine in the Presence of a High Affinity Reuptake Blocker

Figure 5 shows model predictions for administration of cocaine in the situation where the dopamine transporter has been blocked by previous administration of a high affinity inhibitor, but compensatory mechanisms have restored synaptic dopamine to its original level. Simulations are shown for blockade which reduces dopamine transport over the range 10–50%. Dopamine rises faster with cocaine dose (curves b–f) than in the absence of high affinity inhibitor. This simulation assumes that cocaine is unable to displace the high affinity drug from the transporter (see Ref. 43).

DISCUSSION

Model Predictions and Implications for Pharmacotherapy of Drug Abuse

When the models for transporter occupancy and synaptic dopamine are combined (Fig. 3 and Table 3), they give estimates of the changes in dopamine as a function of cocaine dose. An intravenous injection of 40 mg of cocaine, which is typical for an abuser [2], and is reported to be euphorigenic, is predicted to raise synaptic dopamine by a factor of about 10, while a dose of 5 mg, which is about what a naive subject can detect [2], is predicted to about double synaptic dopamine, from our assumed baseline value of 10–50 nM. Although the model has many uncertainties, these predictions are intuitively reasonable.

Regardless of the actual occupancy achieved by cocaine, our model (Fig. 2, right-hand panel) predicts that an injection of a euphorigenic dose of cocaine will evoke a similar increase in dopamine, irrespective of the degree of transporter occupation remaining from previous drug administrations. Since neural reward circuits may be sensitive to changes in dopamine concentration, rather than the absolute dopamine concentration, this raises the possibility that the transporter would not have to be completely free of cocaine before another dose could evoke a "high." This behavior could contribute to the maintenance of binge drug administration. The predicted behavior of an indirect dopaminergic agonist in this regard differs from that intuitively expected from a direct agonist, where successive increments in drug dose should cause progressively smaller increments in occupancy, and thus presumably smaller increments in response.

The predictions of our model also have important implications for the pharmacotherapy of cocaine abuse. A considerable effort in recent years has gone into developing cocaine-like compounds of high affinity which might prevent cocaine binding *in vivo* [44, 45], but have little abuse potential themselves. Such compounds might be used analogously to methadone in heroin abusers. Our results suggest that such a drug would fail to inhibit cocaine-induced increases in synaptic dopamine. This could explain why attempts to treat cocaine abusers with other dopamine transporter blockers have not met with success [46–50]. For example, mazindol did not differ in efficacy from placebo in a recent 6-week long double-blind study [46]. Furthermore, pretreatment with 2 mg mazindol increased the intensity of the "rush" induced by 25 mg cocaine to that induced by 50 mg cocaine in the absence of mazindol [47]. Similarly, methylphenidate was found to lack therapeutic efficacy for treating cocaine abuse and, in fact, led to increased craving and cocaine consumption [48].

Studies with Methylphenidate

Recent studies in our laboratory with methylphenidate, which is similar to cocaine in affinity for the dopamine transporter [4, 51] and is also available in C-11 labeled form for PET scanning [28, 52, 53], have supported the predictions of our model. Volunteers received two intravenous injections of methylphenidate 60 min apart. The "high" from the second injection was perceived as identical to that from the first injection despite a residual occupancy of >75% determined by PET [54], even though subjects differ considerably in terms of liking or not liking intravenous

TABLE 3. Occupancy of dopamine transporter by cocaine, and consequent increase in dopamine

	Occupancy (%)				Increase in dopamine (%)	
	Cocaine dose		Baseline dopamine	K_d (dopamine)	Cocaine dose	
B_{max} (nM)	5 mg	40 mg	(nM)	(nM)	5 mg	40 mg
400	61	94	10	150	290	>2000
			10	1000	260	1900
			50	1000	280	>2000
800	41	88	10	150	180	1600
			10	1000	170	890
			50	1000	180	1300
1600	25	77	10	150	140	570
			10	1000	130	460
			50	1000	140	530

Calculations of occupancy (columns 2 and 3) were made from equations (1) and (2) assuming peak uptake of 0.008% injected radioactivity per cc, a B_{max}/K_d' value of 0.67, and the values of dopamine transporter B_{max} given in column 1. Increases in synaptic dopamine (columns 5, 6 and 7) were then estimated from the occupancies using equations (3a and 3b) and assuming the sets of values for baseline dopamine and K_d (dopamine) shown in columns 4 and 5.

injection of methylphenidate [55, 56]. Furthermore, a greater occupancy was not uniformly associated with a more intense "high" [54]. One possible explanation for these results is that despite the considerable evidence implicating dopamine as the relevant neurotransmitter, the "high" is induced by another compound whose release is imperfectly associated with increased synaptic dopamine. Alternative explanations are that very high transporter occupancies result in stimulation of a dopamine receptor population which mediates aversive subjective effects, or that "downstream" mechanisms may modulate the "high."

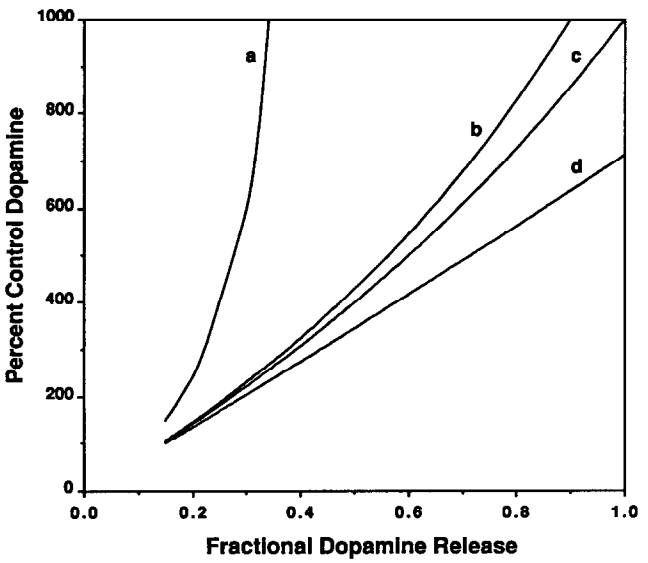

FIG. 4. Effects of decreased rates of dopamine release on cocaine-induced increases in synaptic dopamine. Calculations were conducted to examine the effects of a reduced rate of dopamine release at the synapse, to simulate the effects of increased stimulation of release regulating autoreceptors due to higher concentrations of synaptic dopamine. A transporter occupancy of 85% was assumed. The curves (left to right) assume values for the equilibrium constant for dopamine, and for the baseline concentration of synaptic dopamine, respectively, of: (a) 150 and 50 nM; (b) 150 and 10 nM; (c) 1000 and 50 nM; and (d) 1000 and 10 nM.

Other Attempts to Relate Dopamine Transporter Occupancy to Behavioral Effects

Several groups have estimated the occupancy of the dopamine transporter achieved by psychostimulant drugs from the ability of the drugs to inhibit or displace the *in vivo* binding of a dopamine transporter radioligand (Table 4). These studies have used a variety of radiotracers, test drugs, and measures of occupancy. In some rodent experiments, locomotor activity has been used as an index of dopamine

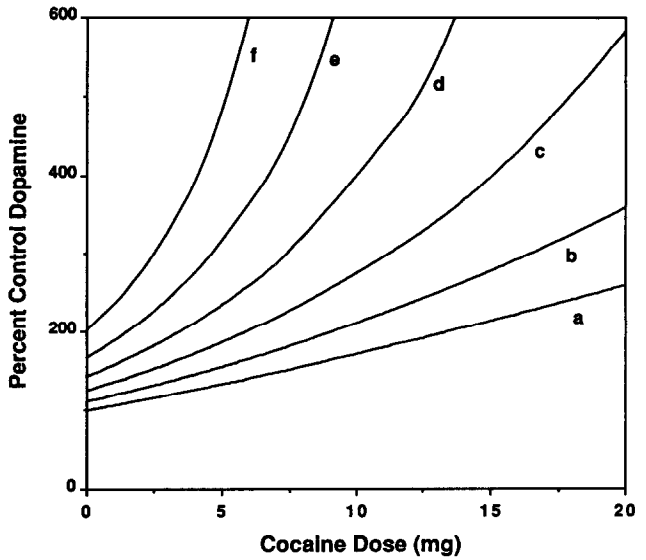

FIG. 5. Cocaine in the presence of a high affinity reuptake blocker. Calculations were conducted to examine the effects of prior blockade of transporters on the rise in synaptic dopamine induced by various doses of cocaine. Key: (a) control; (b) 10% blockade; (c) 20% blockade; (d) 30% blockade; (e) 40% blockade; (f) 50% blockade. The assumptions were made that compensatory mechanisms had allowed synaptic dopamine to restabilize to its normal baseline value (10 nM) before administration of cocaine, and that cocaine did not displace any of the high affinity drug. A transporter B_{max} of 1600 pmol/g and a K_m for dopamine of 1000 nM were assumed.

TABLE 4. Studies of dopamine transporter occupancy

Species	Radioligand	Measure	Tracer uptake period (min)	Drug uptake period	Conclusions
Rodent					
Mouse*	WIN 35,428	St/Cb† - 1	25	5 min	Occupancy and locomotion poorly correlated (8 inhibitors)
Mouse‡	GBR 12783	St - Cb	60	~1–3 hr	Occupancy and locomotion poorly correlated (5 inhibitors)
Rat§	BTCP	St (filtered)	15	15 min	Max. stimulation of locomotion at 60% occupancy (cocaine, WIN 35,065, nomifensine) or 100% occupancy (GBR 12909)
Mouse‖	RTI-55	St/Cb - 1	30	4 hr	Zero occupancy by cocaine or RTI-55
	Cocaine	St/Cb - 1	5	4 hr	30% Occupancy by cocaine; 60% occupancy by RTI-55
Primate					
Cynom.¶	RTI-55	SPECT	n/a**	n/a	7 mg/kg Cocaine causes 50% occupancy
Human††	RTI-55	SPECT	n/a**	n/a	Abused dose of cocaine causes 20% occupancy
Baboons‡‡	Cocaine	PET	54	0–13 days	0.5 mg/kg RTI-55 causes 90% occupancy
Human§§	dtMP	PET	60	7–60 min	Euphoric dose of methylphenidate causes 77% occupancy; prior blockade does not inhibit response to second dose

* Ref. 57.
† St and Cb are the concentrations of radioactivity in striatum and cerebellum, respectively.
‡ Ref. 58.
§ Ref. 59.
‖ Ref. 43.
¶ Ref. 60.
** Displacement paradigm, cocaine administered after [^{123}I]RTI-55.
†† Ref. 61.
‡‡ Ref. 62.
§§ Ref. 54.

receptor activation, and thus of elevated dopamine. Two studies in mice [57, 58] and one in rats [59] concluded that for particular panels of psychostimulant drugs calculated occupancies were not correlated with increased locomotor activity. For example, Rothman *et al.* [59] found that cocaine, WIN 35,428, or nomifensine gave maximum locomotor activities with occupancies of 60%, whereas for GBR 12909 maximum locomotor activity required total occupancy. Because the animal must be killed to measure the striatal radioactivity levels, rodent experiments give only a single time-point for both drug and radioligand administrations. The degree of displacement of radioligand binding by the test drug depends on several factors including the pharmacokinetics of the test drug, the binding kinetics of the radioligand, and the time the animal is killed. If a drug such as cocaine which clears the brain rapidly is used to displace a radioligand with slow dissociation kinetics, such as RTI-55, then the "displacement" observed (and therefore the occupancy calculated) may be less than the real occupancy, because the maximal displacement is governed by how much radioligand dissociates while drug is available to replace it. This underestimation of occupancy could be exacerbated if enough radioligand remains in the circulation for brain binding to increase significantly during the experimental period. These considerations presumably explain recent results of mouse experiments in our laboratory where non-radioactive cocaine and RTI-55 were used as test drugs [43]. When [^{123}I]RTI-55 was used as the radioligand, both cocaine and RTI-55 failed to produce a significant displacement of [^{123}I]RTI-55, but produced displacements of 40 and 70%, respectively, when [^{3}H]cocaine was used as radioligand. In recent SPECT studies, Malison *et al.* [61] have shown that a bolus injection of cocaine which induces euphoria in humans (40 mg) displaces about 20% of an equilibrium striatal concentration of [^{123}I]RTI-55. This displacement is considerably less than the occupancy of the dopamine transporter indicated by our own work [17, 19]. Clearly, these results show that the "occupancy" experiments in the literature must be interpreted with great caution, and suggest that the radioligand used to probe the availability of binding sites should not have a higher affinity than the test drug [43, 61].

Regulation of Dopamine Release and Reuptake

The assumption that transporter occupancy proportionately decreases flux through the transporter is reasonable unless there are interactions between individual transporter molecules (e.g. Ref. 63). If flux through transporters is enhanced by stimulation of dopamine D_2 receptors located on the same terminals [64, 65], then the degree of upward curvature of Figs. 2–5 would be reduced. It is also possible that the transporter V_{max} and its affinity for dopamine and/

or drugs are affected by other factors. For example, ATP has been reported to decrease the affinity of the transporter for dopamine [66].

In addition to possible direct modulation of the transporter by dopamine, adaptive responses are known to occur which should mitigate the rise in DA after systemic administration of cocaine [67]. Activation of autoreceptors at the cell body after cocaine administration reduces the firing frequency of dopamine cells by about 50% [68, 69], and activation of terminal autoreceptors further reduces dopamine release [70]. These as well as indirect mechanisms mediated by other neurotransmitter systems will attenuate the rise in synaptic DA caused by cocaine.

Dopamine During Withdrawal

Our calculations may be relevant to the suggestion that the dysphoric state induced by withdrawal of dependent individuals from chronic exposure to cocaine results from decreased synaptic dopamine [71]. Equation (4) predicts that doubling of transporter activity would very nearly halve dopamine.

CONCLUSIONS

We have presented a model to relate the cocaine-induced increase in synaptic dopamine to the degree of dopamine transporter occupancy by cocaine, as measured using PET. Our model has two components. First, we estimate dopamine transporter occupancy by assuming rapid equilibrium between free cocaine and cocaine bound to transporters. Second, we estimate the rise in synaptic dopamine by assuming that this parameter is determined by the balance between dopamine release and reuptake, that reuptake is reduced in proportion to transporter occupancy, and that reuptake is the only mechanism by which dopamine is removed from the environment of the receptors. Our model predicts that synaptic dopamine rises in an accelerating fashion with increasing occupancy. The exact enhancement will depend on the normal concentration of synaptic dopamine, the K_m of the transporter for dopamine, the degree to which exocytotic dopamine release is reduced to compensate for the increased synaptic dopamine levels, and the extent to which elevated dopamine competes with cocaine for binding to the transporters. Nevertheless, this behavior provides a possible explanation of why volunteers given intravenous methylphenidate in our laboratory reported identical subjective responses to each of two injections given 1 hr apart, in spite of a 75% residual occupancy from the first injection at the time of the second injection. Our model also suggests that blocking the dopamine transporter with a high affinity cocaine-like compound may enhance the increase in synaptic dopamine caused by a subsequent administration of cocaine, even if compensatory mechanisms restore normal baseline dopamine. This could explain why previous attempts to treat cocaine abusers with other dopamine transporter blockers have failed, and bring into question the rationale for cocaine abuse pharmacotherapy with potential drugs such as high affinity cocaine analogs, which may in fact increase the "high."

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