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Rate of binding of various inhibitors at the dopamine transporter in vivo

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Abstract The rate of entry of drugs into brain is thought to be a factor in their abuse liability. In this investigation, we have examined the rate of entry and binding at dopamine transporters in mouse striatum for a variety of dopamine transporter inhibitors. The method utilized was based on measuring the displacement of ^{3}H-WIN 35,428 from striatal dopamine transporter sites in vivo at different times. Eleven cocaine analogs (RTI-31, RTI-32, RTI-51, RTI-55, RTI-113, RTI-114, RTI-117, RTI-120, RTI-121, WIN 35,065-2, and WIN 35,428) as well as other dopamine uptake site blockers (bupropion, nomifensine, and methylphenidate) were compared with (−) cocaine for their rates of displacement of ^{3}H-WIN 35,428 binding in vivo. The drugs that displayed the fastest occupancy rates were bupropion, (−) cocaine, nomifensine, and methylphenidate. RTI-51, RTI-121, RTI-114, RTI-117, RTI-120, RTI-32, RTI-55, and RTI-113, showed intermediate rates, whereas RTI-31, WIN 35,065-2, and WIN 35,428 exhibited the slowest rates of displacement. While many of the cocaine analogs have proven to be behaviorally and pharmacologically more potent than (−) cocaine, their rates of entry and binding site occupancy were slower than that for (−) cocaine. Earliest times of transporter occupancy by the different drugs were correlated (although weakly) with their degree of lipophilicity ($r = 0.59$; $P < 0.02$). Kinetic effects and metabolism of the compounds could complicate the interpretations of these data. There was no obvious correlation between rate of occupancy in this animal model and abuse liability in humans, which is consistent with the notion that other factors are critical as well.

Key words Dopamine transporter · Cocaine analogs · Mouse striatum

Abbreviations *RTI-31* 3β-[4-Chlorophenyl]tropane-2β-carboxylic Acid Methyl Ester Hydrochloride · *RTI-32* 3β-[4-Methylphenyl]tropane-2β-carboxylic Acid Methyl Ester Tartrate · *RTI-51* 3β-[4-Bromophenyl]tropane-2β-carboxylic Acid Methyl Ester Tartrate · *RTI-55* 3β-[4-Iodophenyl]tropane-2β-carboxylic Acid Methyl Ester Tartrate · *RTI-113* 3β-[4-Chlorophenyl]tropane-2β-carboxylic Acid Phenyl Ester Hydrochloride · *RTI-114* 3β-[4-Chlorophenyl]tropane-2β-carboxylic Acid Isopropyl Ester Hydrochloride · *RTI-117* 3β-[4-Methylphenyl]tropane-2β-carboxylic Acid Isopropyl Ester Hydrochloride · *RTI-120* 3β-[4-Methylphenyl]tropane-2β-carboxylic Acid Phenyl Ester Hydrochloride · *RTI-121* 3β-[4-Iodophenyl]tropane-2β-carboxylic Acid Isopropyl Ester Hydrochloride · *WIN-35,065-2* 3β-[Phenyl]tropane-2β-carboxylic Acid Methyl Ester Tartrate · *WIN-35,428* 3β-[4-Fluorophenyl]-tropane-2β-carboxylic Acid Methyl Ester Tartrate.

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Introduction

A neurochemical mechanism hypothesized to underlie at least some of the reinforcing effects of cocaine is an augmentation of dopaminergic transmission due to inhibition of dopamine reuptake (Wise and Bozarth

1987; Koob and Bloom 1988; Kuhar et al. 1990, 1991; Robinson and Berridge 1993). It has further been suggested that abuse liability is greater in general for those drugs that enter the brain and occupy their receptors more rapidly (see Sellers et al. 1989 for discussion). It is also known that some dopamine transporter inhibitors enter the brain and occupy transporter binding sites at different rates (Pögün et al. 1991).

Previously, the dopamine transporter has been characterized in vitro and in vivo using a variety of radioligands (Carroll et al. 1992a; Wong et al. 1993; Boja et al. 1994). Radiolabeled WIN 35,065–2 (Ritz et al. 1990) and WIN 35,428 (Madras et al. 1989) have been shown to bind in vivo in animals and humans to the dopamine transporter, and the specific binding was also shown to be higher and longer-lasting than that of the parent compound, radiolabeled cocaine (Fowler et al. 1989; Scheffel et al. 1989; Wong et al. 1993). The increased binding was presumed to be the result of higher binding affinity and less vulnerability to metabolism because of the absence of an ester linkage between the phenyl and tropane moieties (Scheffel et al. 1989). In vivo competition studies with in vivo bound ^{3}H-WIN 35,428 have shown that cocaine enters the brain more rapidly than GBR 12909, and both of these compounds enter more rapidly than mazindol (Pögün et al. 1991). Of these compounds, cocaine is most reinforcing and mazindol is least reinforcing (Chait et al. 1987). Since the rate of transporter occupancy has potential importance, we have further explored this aspect of dopamine transporter binding for 11 cocaine analogs (i.e. RTI-31, RTI-32, RTI-51, RTI-55, RTI-113, RTI-114, RTI-117, RTI-120, RTI-121, WIN-35,065-2, and WIN 35,428) and three other uptake blockers (i.e. bupropion, nomifensine, and methylphenidate) that may or may not have abuse liability (Ritz et al. 1987; Bergman et al. 1989; Garris and Ben-Jonathan 1991; Schaefer and Michael 1992).

Materials and methods

Drugs and radioactive materials

^{3}H-WIN 35,428 was purchased from DuPont NEN (Boston, Mass). The cocaine analogs, RTI-31, RTI-32, RTI-51, RTI-55, RTI-113, RTI-114, RTI-117, RTI-120, RTI-121, WIN-35,065-2, and WIN 35,428 were obtained as previously reported (Carroll et al. 1992b, 1994). Bupropion hydrochloride and methylphenidate were obtained from RBI (Natick, Mass.), nomifensine maleate from Hoechst (Germany) and (−) cocaine from Sigma (St Louis, Mo.). Prior to each experiment, all drugs were freshly dissolved in 0.9% NaCl. For complete solubilization, the solutions were sonicated. Solubilization of (−) cocaine required acidification with 25 µl glacial acetic acid for each ml of saline.

Determination of in vivo competition ED_{50} values

Male, CD-1 mice obtained from Charles River Laboratories, (Wilmington, Mass.), weighing approximately 28 g, were injected intravenously via a tail vein with 0.1 ml of the respective drug solution, ranging in concentration from 0.01 to 20 mg/kg. Controls received saline injections. Five minutes after intravenous injection of the drug, 2 µCi ^{3}H-WIN 35,428 (approximately 10.0 ng in a volume of 0.2 ml saline) was injected. Thirty minutes after tracer injection, the animals were killed by cervical dislocation. Thereafter, the brains were quickly removed and dissected on ice. The cerebellum, olfactory tubercles, corpus striata, and cerebral cortices (all cortical areas except the portion superior to the corpus striatum) were dissected, weighed, and placed into glass vials. After digestion of the tissues with Solvable (Dupont NEN Research Products), 10 ml of scintillation fluid 989 (NEN) was added to each vial, followed by measurement of the radioactivity in a Packard beta scintillation counter, keeping the counting error to 3% or less. Aliquots of the injectate were counted along with the samples. All data were expressed as percent of the injected dose per gram of tissue (% D/g). To calculate the ED_{50} of bupropion, methylphenidate, nomifensine, RTI-121, RTI-117, RTI-114, RTI-120, and RTI-113, data from the descending portion of the striatal inhibition curves were analyzed, using logit transformation methodology.

Rate of displacement of ^{3}H-WIN 35,428 in vivo by various compounds

In order to obtain an estimate of the overall rate of entry of drugs into the brain and of occupancy of the DA transporter, the displacement of maximal ^{3}H-WIN 35,428 binding from striatal and olfactory binding sites was examined at different times after injection. The experimental design was essentially the same as that used previously to compare the dopamine transporter occupancy rates of mazindol and GBR 12,909 with that of (−) cocaine (Pögün et al. 1991).

Male, CD-1 mice obtained from Charles River Laboratories, (Wilmington, Mass.), weighing approximately 28 g were injected via the tail vein with 2 µCi ^{3}H-WIN 35,428 (approximately 10.0 ng) in a volume of 0.2 ml saline. Thirty minutes after tracer injection, a time at which in vivo binding was maximal and at apparent equilibrium, the various compounds were injected IV in a volume of 0.1 ml via a tail vein over 10 s. The drugs were administered in a dose equivalent to their ED_{50} value determined in this or in previous studies. Controls received saline injections. At different times (30 s, 1, 2, 3, 5, 7, 10, 15, and 20 min) after injection of the drugs (or saline), the animals were killed by cervical dislocation, and dissections and measurements of radioactivity in the various tissue samples were performed.

For the analysis of the in vivo binding data, regional radioactivity levels were converted to percent dose per gram of tissue (%D/g). In addition, the %D/g data of each tissue region studied was divided by the %D/g of the cerebellum in order to obtain ratios of total to nonspecific binding. Data were analyzed by comparing [(tissue to cerebellum) −1] values for the various compounds. As described previously (Scheffel et al. 1989; Cline et al. 1992), these [(T/CB)−1] values provide an estimate of specific to nonspecific binding, based on the observation that dopamine uptake sites are highly concentrated in the striatum and relatively absent in the cerebellum. The time of initial occupancy of striatal DA transporters by each drug was determined as the time point at which a significant difference ($P < 0.05$) in in vivo binding from controls (saline injected) first occurred. Data were analyzed statistically by using the one-way classification of analysis of variance (ANOVA), with the post-hoc Dunnett's test.

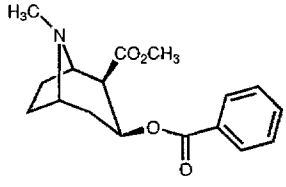
Partition coefficients

Partition coefficients were determined by the chromatographic method of Minick and co-workers (1988), using an Econosil C-8 10 µm (4.6 × 10 cm) column and mobile phases which consisted of methanol containing 0.25 v/v octanol and water containing

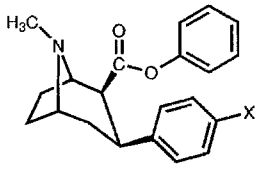
0.02 M 4-morpholinepropanesulfonic acid (MOPS) and 0.15% *n*-decylamine. Utilizing different methanol-water mixtures (75:25, 65:35, 55:45, 45:55, 35:65, and 25:75 in the instances of RTI-32, WIN 35428; WIN 35065-2) at 2 ml/min, retention times were measured in triplicate for each compound as well as the reference compounds chloronitrobenzene, naphthalene and pyrene. The column void volume, t_o, was 1.568 min as determined by the retention time of urea detected at 220 nm. The average of the retention time was then converted to k' at each solvent composition and plotted versus the percentage MeOH contained in that solvent mixture. The y-intercept derived from the linear portion of the curve was calculated, and represents the log k'_w value. The average k'_w values for each compound obtained by this HPLC method were converted to the logarithm of the partition coefficients ($\log P_{hplc}$) through substitution into the following formula: $\log P_{hplc} = 1.006 k'_w + 0.006$. This formula had been previously generated through the comparison of experimentally determined k'_w values with known $\log P_{octanol}$ values for a series of standard compounds (Musachio 1992). The value for pyrene was 4.44, which compares well with reported values of 4.89 (Braumann 1986). HPLC was performed on a Waters Associates Model 510EF pump equipped with a Model 490 UV absorbance detector.

Results

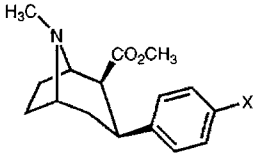
Various dopamine uptake inhibitors were selected for study. Of the available phenyltropane cocaine analogs, some methyl, isopropyl and phenyl esters (Fig. 1) as well as other uptake inhibitors including methylphenidate, bupropion and nomifensine were selected. Almost all of these compounds were more potent than cocaine in in vitro binding assays (Table 1). The potencies of the compounds in vivo in inhibiting ^{3}H WIN 35,428 binding were collected from previous publications or determined as part of this study (Table 1).



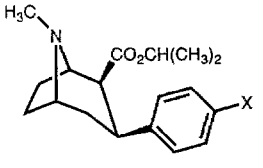
(-) Cocaine



RTI-120 X = CH_3
RTI-113 X = Cl



WIN 35,065-2 X = H
RTI-32 X = CH_3
WIN 35,428 X = F
RTI-31 X = Cl
RTI-51 X = Br
RTI-55 X = I



RTI-117 X = CH_3
RTI-114 X = Cl
RTI-121 X = I

Fig. 1 Cocaine and cocaine analogs used to displace ^{3}H-WIN 35,428 binding to the dopamine transporter

Table 1 Chemical and biological characteristics of the dopamine transporter inhibiting drugs. See materials methods for additional details. Data are mean of 3–11 determinations

Drug	In vivo ED_{50} (μmol/kg)	In vitro IC_{50} (nM)	Log P_{hplc}
WIN-35,428	0.24[b]	15.7[c]	0.79
WIN-35,065-2	0.61[b]	23.0[d]	0.81
RTI-32	0.31[c]	1.7[c]	1.00
RTI-31	0.18[c]	1.2[c]	1.08
(−) Cocaine	23.1[b]	89.0[c]	1.29
RTI-117	2.61[a]	6.45[a]	1.26
RTI-51	0.31[c]	1.8[c]	1.32
Methylphenidate	11.12[a]	59.8[a]	1.36
RTI-55	0.26[c]	1.3[c]	1.57
RTI-114	1.52[a]	1.41[d]	1.63
RTI-120	1.91[a]	3.27[d]	2.03
RTI-121	0.66[a]	0.4[d]	2.12
RTI-113	1.23[a]	1.98[d]	2.44
Bupropion	57.20[a]	199[a]	2.49
Nomifensine	9.23[a]	18.5[a]	2.77

[a] Determined during this study
[b] Scheffel et al. (1991)
[c] Boja et al. (1992)
[d] Carroll et al. (1992)

There was a significant correlation ($r = 0.77$, $P < 0.0008$) between log of in vivo binding potencies and log of in vitro binding potencies.

When determining the rate of entry, the transport inhibitors were injected into mice via the tail vein 30 min after injection of ^{3}H WIN-35,428. At that time, the striatal binding of ^{3}H WIN-35,428 in vivo is in apparent equilibrium and does not change significantly for the next 30 min or so (Fig. 2; Scheffel et al. 1991). All drugs were injected at their ED_{50} doses (Table 1) in order to inject an equieffective dose so that rate of entry rather than potency would be the major factor in our measurements. The animals were killed at varying times after drug injection in order to assess the time at which significant displacement of previously bound ^{3}H WIN-35,428 occurred.

There was a variation in the times at which the drugs showed a significant displacement of ^{3}H WIN-35,428 (Figs 2 and 3, Table 2). Some drugs such as bupropion, cocaine, methylphenidate and nomifensine showed competition with ^{3}H WIN-35,428 at early times, that is, at 1 min and 3 min. On the other hand, drugs such as WIN 35,428 and WIN 35,065–2 did not show competition until much later, that is, at the measured time point of 15 min.

When some of the compounds studied in the in vivo binding competition experiments were injected into animals and assayed for the rate of onset of locomotor activity, it was clear that different analogs had different rates of onset that were generally in agreement with our findings here. However, because of variability, it was difficult to obtain statistically significant differences at early times. Also, the maximal effects for some drugs differed from those of other drugs, making comparisons of rates of onset more difficult (not

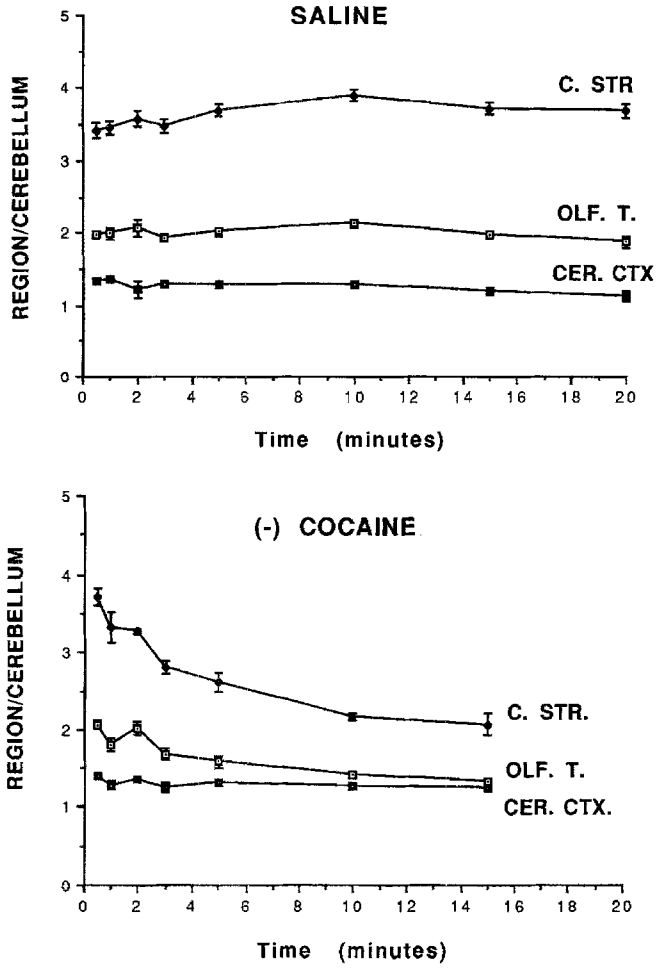


Fig. 2 Time course of ^{3}H-WIN 35,428 displacement by cocaine. Region/cerebellar ratios of ^{3}H-WIN 35,428 concentrations were determined at different times after injection of cocaine (23.07 μmol/kg in 0.1 ml saline) or saline (0.1 ml). ^{3}H WIN 35,428 (2 μCi/mouse) was injected IV 30 min before IV administration of cocaine or saline. Region/cerebellar ratios defined as % D/g tissue divided by %D/g cerebellum. Data represent means ± SEM (n = 3 – 10). Abbreviations for regions: *C. STR.* corpus striatum, *OLF. T.* olfactory tubercles, *CER. CTX.* cerebral cortex

shown). Other investigators have reported different rates of onset of behavior for different compounds (see Discussion).

Lipid solubility is an important factor for drug entry into the CNS. Accordingly, partition coefficients (P_{hplc}) were determined using an HPLC method. The log P_{hplc} varied from 0.79 to 2.77 (Table 1). When the initial occupancy time shown by these compounds as indicated in Table 2 was correlated to the log of the partition coefficient, a significant although weak relationship ($P = 0.02$, $r = 0.59$) was found (Fig. 4).

Discussion

A rapid rate of delivery of drug to receptors in brain is thought to be a significant factor in abuse liability. While this makes intuitive sense in that a rapid onset of action would be most desirable by a drug abuser, this notion has been supported experimentally as well. For example, rapid delivery of drug to brain and receptors provides optimal conditions for reinforcement, drug self-administration and human subjective response (Arendt et al. 1983; Griffiths et al. 1984a, b; Busto and Sellers 1986; Ator and Griffiths 1987; Griffiths and Wolf 1990; de Wit et al. 1992, 1993; Mumford et al. 1993). Also, prodrugs, that is, compounds which must be converted to an active form and therefore act relatively more slowly, appear to have lesser abuse liability than the ultimate products (Jaffe et al. 1983; Griffiths et al. 1984a, b). It is further postulated that routes of administration that allow for a more rapid entry of drug are more reinforcing; this is thought to be a major factor in the increased abuse liability of "crack" cocaine which is smoked as compared to insufflated cocaine (Johanson and Fischman 1989; Johanson and Schuster 1995). As rate of entry is a

Table 2 Comparison of ratios of specific to nonspecific binding [(Str/Cb) – 1] of ^{3}H-WIN 35,428 at different times after intravenous administration of dopamine transporter blockers. Ratios presented are mean ±SEM (n = 3–11). Reading from left to right, the first time point to show significance (*) is the time at which significant occupancy occurs

Drugs	0.5 min	1 min	2 min	3 min	5 min	7 min	10 min	15 min	20 min
Bupropion	2.2 ± 0.1	1.9 ± 0.1*	1.9 ± 0.1*	1.9 ± 0.1*	1.6 ± 0.1*		1.1 ± 0.1*	0.99 ± 0.1*	
Nomifensine	2.6 ± 0.1	2.2 ± 0.1	2.2 ± 0.2	2.2 ± 0.1*	1.4 ± 0.1*		1.3 ± 0.03*	1.1 ± 0.1*	
(−) Cocaine	2.7 ± 0.1	2.5 ± 0.1	2.3 ± 0.3	1.8 ± 0.1*	1.6 ± 0.1*		1.2 ± 0.04*	0.96 ± 0.1*	
Methylphenidate	2.2 ± 0.2	2.3 ± 0.2	2.3 ± 0.1	1.8 ± 0.1*	1.7 ± 0.1*		1.2 ± 0.1*	0.93 ± 0.1*	
RTI-51	2.4 ± 0.1	2.3 ± 0.2	2.0 ± 0.1	2.2 ± 0.2	1.8 ± 0.1*		1.4 ± 0.2*	1.4 ± 0.1*	
RTI-121	2.4 ± 0.1	2.4 ± 0.1	2.4 ± 0.1	2.4 ± 0.1	2.0 ± 0.1*		2.1 ± 0.1*	1.7 ± 0.1*	1.6 ± 0.1*
RTI-114	2.5 ± 0.1	2.5 ± 0.04		2.2 ± 0.2	2.3 ± 0.1	1.9 ± 0.1*	1.8 ± 0.1*	1.3 ± 0.1*	
RTI-117	2.5 ± 0.1	2.4 ± 0.04	2.1 ± 0.1	2.0 ± 0.1	2.1 ± 0.1	1.8 ± 0.02*		0.92 ± 0.1*	
RTI-120	2.5 ± 0.1	2.4 ± 0.1	2.4 ± 0.1	2.3 ± 0.3	2.4 ± 0.1	1.9 ± 0.2*	2.0 ± 0.1*	1.6 ± 0.1*	
RTI-32	2.3 ± 0.1	2.5 ± 0.1	2.4 ± 0.1	2.6 ± 0.04	2.4 ± 0.04		1.3 ± 0.2*	0.86 ± 0.1*	
RTI-55	2.5 ± 0.1		2.6 ± 0.1	2.7 ± 0.1	2.2 ± 0.1		1.9 ± 0.2*	2.0 ± 0.2*	
RTI-113	2.5 ± 0.1	2.6 ± 0.2	2.2 ± 0.3	2.4 ± 0.2	2.4 ± 0.1	2.7 ± 0.1	1.7 ± 0.2*	1.4 ± 0.1*	
RTI-31	2.3 ± 0.1	2.4 ± 0.1	2.6 ± 0.1	2.3 ± 0.2	2.4 ± 0.1		2.3 ± 0.03	1.6 ± 0.1*	
WIN-3065-2	2.4 ± 0.1	2.5 ± 0.1	2.6 ± 0.1	2.5 ± 0.1	2.1 ± 0.01		2.1 ± 0.1	1.4 ± 0.02*	1.7 ± 0.2*
WIN-35,428	2.6 ± 0.1	2.6 ± 0.1	2.6 ± 0.1	2.6 ± 0.1	2.4 ± 0.2		2.6 ± 0.1	2.1 ± 0.2*	1.8 ± 0.1*

*Values indicate at least a $P < 0.05$ value of significance compared to saline injected controls (n = 201, 2.6 ± 0.34)

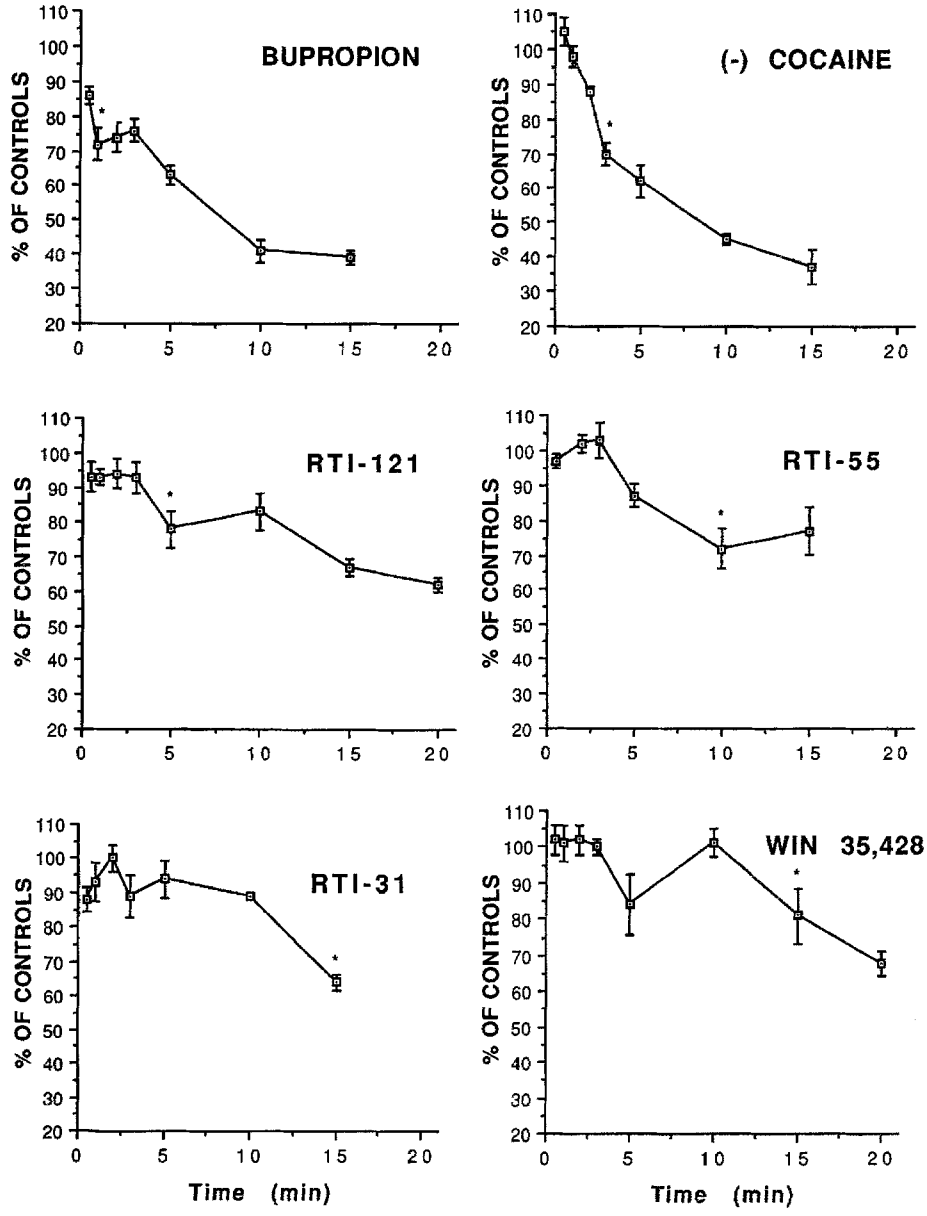


Fig. 3 Rate of inhibition of in vivo transporter binding by various drugs that display different occupancy rates. Bupropion and (−) cocaine are two of the drugs that displayed the fastest occupancy times, i.e. 1 min and 3 min, respectively. RTI-121 and RTI-55 are two of the inhibitors that displayed intermediate occupancy times, i.e. 5 min and 10 min, respectively. RTI-31 and WIN 35,428 are two of the uptake blockers that displayed slowest occupancy times, i.e. 15 min for both. Data are means ± SEM ($n = 3 - 11$) of (striatum/cerebellum − 1). The *asterisks* show the earliest time point at which a significant difference occurred ($P < 0.05$)

significant factor in the abuse liability of drugs, we have attempted to develop a way of quantifying how quickly drugs enter the brain and occupy their relevant receptors. Recently based on in vivo receptor binding competition, we showed that cocaine enters the brain more rapidly than GBR 12909 which in turn enters more rapidly than mazindol (Pögün et al 1991).

What are potential errors or effects that would affect the interpretation of our data? One factor is that the kinetics of the tracer (^{3}H WIN35428) is different from that of most of the other compounds. In other words, WIN35428 has slower entry (and presumably exit) than cocaine and most of the other compounds tested. The net result of this would be that the kinetics of agents with rapid entry would appear to be slower than they actually are. Thus, cocaine's occupancy time could be faster than 3 min, the time at which whole tissue ^{3}H WIN35428 was reduced below control. This would not be an issue with compounds entering at same rate or more slowly than that of the tracer. Additional factors that could confound our interpretation include competition with endogenous dopamine, and rapid production of metabolites of competing drugs which are also competitors and have different potencies and rates of entry. Corrections for these factors can be made in future studies as we learn to assess their significance.

The notion that drug action can be based on the rate of drug-receptor combination has been proposed by Paton (1961). The theory has been successful in explaining many but not all pharmacological observations (Goldstein et al. 1974 pp. 104–106). A potential rationale why rapid rate is important has to do with desensitization of receptor by agonist; a rapid rate of occupancy would stimulate a maximal number of

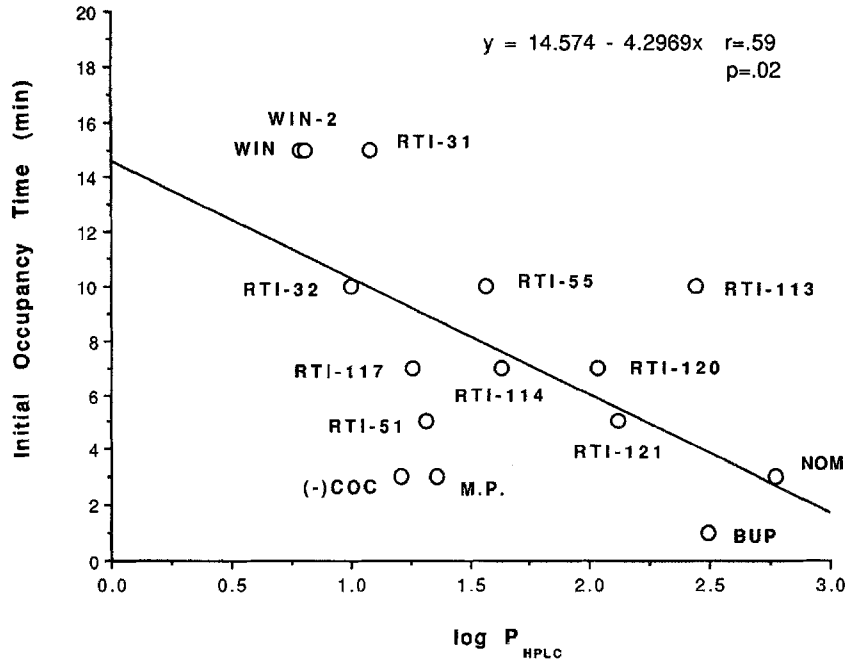


Fig. 4 Correlation between initial occupancy time of the dopamine transporter blocking drug and its lipophilicity as expressed by the log of the partition coefficient (log P_{hplc}). *M.P.* methylphenidate, *BUP* bupropion, *NOM* nomifensine, *WIN* WIN 35428, *WIN-2* WIN 35065–2

receptors before their response was lessened by desensitization. Several investigators have shown that dopamine receptors are desensitized by exposure to agonists (Memo et al. 1982; Roseboom and Gnegy 1989; Barton and Sibley 1990; Barton et al. 1991). An additional mechanism that could be involved is (slow acting?) feedback regulatory systems. As dopamine in the synapse rises, autoreceptor actions and inhibitory circuits tend to return dopamine to lower levels. Fast entering compounds might cause higher levels of dopamine in the synapse before feedback mechanisms could take effect. Whether or not such mechanisms have validity, or what additional factors might be important, will require more investigation.

As in our previous study (Pögün et al. 1991), cocaine displacement of ^{3}H WIN 35,428 was significant at 3 min after intravenous injection. In human studies with tracer doses of ^{11}C-cocaine, specific binding (i.e. striatal radioactivity greater than cerebellar radioactivity) was not detected until about 2 1/2 min after injection (Fowler et al. 1989; J. Fowler personal communication); these findings are in good agreement with those presented here. But cocaine was not the fastest entering drug; bupropion was effective in producing significant displacement at 1 min after injection. Nomifensine and methylphenidate were as fast as cocaine in displacing in vivo ^{3}H WIN 35428 binding. Of the compounds examined, WIN 35428 and WIN 35065–2 occupied receptors at the slowest rate, i.e., 15 min. The slower entry time of these latter two compounds compared to cocaine has a behavioral counterpart in that onset of behavior was slower than that of cocaine (Spealman et al. 1977, 1989). The remaining compounds entered at intermediate rates, i.e. between 5 and 15 min. According to our previous data, GBR 12,909 and mazindol appear to occupy transporters at about 5–10 min after IV injection (Pögün et al. 1991).

Are these findings compatible with other data on abuse liability? Not obviously so. Bupropion, nomifensine and methylphenidate enter and occupy drug binding sites as rapidly as or faster than cocaine yet are not abused like cocaine. Bupropion and nomifensine are self-administered by sub-human primates and occasion cocaine-appropriate responding by rats (Spyraki and Fibiger 1981; Bergman et al. 1989; Lamb and Griffiths 1990). However, there is no evidence of abuse of these compounds by humans (Griffith et al. 1983; Miller and Griffith 1983). Methylphenidate also shows potential for abuse in animal studies (Bergman et al. 1989), and in this case, there is some evidence for abuse by humans, although it is not as big a problem as cocaine (Haglund and Howerton 1982; Parran and Jasinski 1991; Chait 1994). Thus, for the drugs producing the fastest displacement, there is little evidence of abuse liability in human populations, although some evidence for abuse liability does exist. This suggests that rate of entry is not the only significant factor in abuse liability which is obviously the case.

The compounds displacing ^{3}H WIN 35428 most slowly, nonradioactive WIN 35428 and WIN 35065–2, also may have abuse liability in that they are self-administered in animals (Spealman et al. 1977; Spealman and Kelleher 1981). This suggests that all of the other compounds studied would be self-administered in that they inhibit dopamine transport and enter the brain more rapidly than WIN 35,428 or WIN 35,065–2. Thus a much slower entry time (longer than 15 min) may be needed to preclude abuse liability on the basis of rate of entry alone. Also, a confounding factor in existing studies is that animals are trained to self-administer

cocaine before being switched to other drugs (Lamb and Griffiths 1990); this may facilitate the self-administration of the other compounds and make a valid comparison of abuse liability between cocaine and the other compounds more difficult. The lack of abuse of some of these compounds in humans may be due to several factors, such as lack of drug availability or the presence of aversive effects of the drugs, such as those reported for mazindol in monkeys (Bergman et al. 1989). Obviously, lack of abuse by humans at present does not mean that abuse will not occur in the future.

After IV injections of cocaine, anecdotal reports by human subjects suggest that a "rush" occurs in typically less than a minute, which is followed by a period of mainly euphoria and arousal. Our findings that injected cocaine takes 3 min to occupy significant levels of transporters suggest that dopamine transporter inhibition mediates the later effects of cocaine rather than the earlier effects. This is supported by direct evidence where the dopamine receptor blocker, haloperidol, reduced drug liking scores elicited by cocaine (Sherer et al. 1989) but had no effect on "rush", Thus, the "rush" may be (partly) peripherally mediated or may be due to action at sites in brain where very low levels of cocaine are effective. However, the finding that cocaine appeared to occupy significant sites at 3 min might be difficult to interpret because of differences in kinetics between tracer and competitor as described above.

It has also been proposed that dopamine transporter inhibiting drugs with a slow rate of entry into brain and a slow onset of action may be useful as treatment medications (Rothman 1990). Balster and Schuster (1973) showed that increasing the duration of a cocaine infusion decreased the response rate of animals. If slow entry drugs are indeed useful, then RTI-31, WIN 35428 and WIN35065–2, which were the slowest entering drugs, may be candidates as treatment medications. Other compounds in the RTI series may be candidates as well. We have not explored the duration of action in these studies. However, it is feasible to do this by similar techniques. For example, we have recently published data regarding the occupancy of serotonin transporters by in vivo competition (Scheffel et al. 1994).

As lipid solubility is one factor which affects entry of a drug into brain, the relative lipophilicity of the drugs under study were determined by a high performance liquid chromatographic method (Minick et al. 1988) (Table 1). As expected, values found for the structurally related cocaine analogs followed two internally consistent trends. First, the log P_{hplc} increased as the alkoxy portion of the ester moiety changed from methyl to isopropyl to phenyl substitution, reflecting the donation of the additional hydrocarbon fragment. Secondly, the log P_{hplc} further increased as the substituent on the phenyl ring changed from hydrogen to halogen (Table 1; Fig. 1). Because the other drugs studied come from diverse chemical classes, direct comparisons of the values obtained with these analogs could not be made. The weak correlation ($r = 0.59$) between rate of entry and log P_{hplc} suggests that other or additional pharmacodynamic factors may be involved as well.

In conclusion, our results show that closely related compounds can have quite different rates of entry into brain and occupancy of transporter binding sites. Compounds that enter as fast as or faster than cocaine do not show the same level of abuse as cocaine in humans. This could be due to the fact that these data with mice are not applicable to humans because of pharmacokinetic or other differences. Nevertheless, rates of entry into brain must be at least a partial factor in abuse liability. Rate of entry is also a factor in consideration of developing a substitute or surrogate agonist as a medication for cocaine abuse. The approach used here provides quantitative data and may suggest some candidates for treatment medications.

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