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Displacement of RTI-55 from the dopamine transporter by cocaine

S. John Gatley [a,*], Nora D. Volkow [a], Ruoyan Chen [b], Joanna S. Fowler [b], F. Ivy Carroll [c], Michael J. Kuhar [d]

[a] *Medical Department, Brookhaven National Laboratory, Upton, NY 11973, USA*
[b] *Chemistry Department, Brookhaven National Laboratory, Upton, NY 11973, USA*
[c] *Research Triangle Institute, Research Triangle Park, NC 27709, USA*
[d] *Neurosciences Branch, Addiction Research Center NIDA, Baltimore, MD 21224, USA*

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Abstract

The cocaine analog 3β-(4-iodophenyl)tropane-2β-carboxylic acid methyl ester (RTI-55 or βCIT) has a higher affinity for the dopamine transporter and may be potentially useful in interfering with cocaine's actions in brain. However, imaging studies have demonstrated displacement of tracer doses of [^{123}I]RTI-55 by a subsequent dose of cocaine. Similar displacement of pharmacological doses of RTI-55 might compromize therapy with RTI-55 in cocaine abuse. The reduction in dopamine transporter availability, assessed in vivo in mouse striatum using [^{3}H]cocaine, caused by pretreatment with RTI-55 was significantly mitigated by subsequent administration of cocaine. In a similar experiment using a tracer dose of [^{123}I]RTI-55 significant reductions of striatal radioligand binding by pretreatment with cocaine or RTI-55 were not observed. These results suggest that: (1) cocaine can displace pharmacological doses of RTI-55 from striatum, and (2) radioligands used to assess binding site occupancy should have a lower affinity than the occupying drug.

Keywords: [^{123}I]RTI-55; Cocaine analog; Psychostimulant abuse; (Mouse)

1. Introduction

The reinforcing and addictive properties of cocaine are associated with its inhibition of the dopamine transporter (Calligaro and Eldefrawi, 1988; Ritz et al., 1987). RTI-55 (3β-(4′-iodophenyl)tropane-2β-carboxylic acid methyl ester, also known as β-CIT) is a cocaine analog possessing much higher affinity for the dopamine transporter than cocaine itself (Boja et al., 1991, 1992; Carroll et al., 1992; Laruelle et al., 1993, 1994a,b). Long-lasting binding of [^{123}I]RTI-55 (at tracer doses) in striatum of humans and non-human primates has been demonstrated (Innis et al., 1993; Laruelle et al., 1993; Muller et al., 1993), which contrasts with the rapid clearance of radiolabeled cocaine from the human striatum (Fowler et al., 1989). However, the administration of cocaine has been previously demonstrated to displace no carrier added [^{123}I]RTI-55 and [^{11}C]RTI-55 from striatum of non-human primates (Laruelle et al., 1993; Muller et al., 1993). We recently made use of repeated serial administrations of [^{11}C]cocaine over a period of 2 weeks to probe the kinetics of dopamine transporter availability in striatum of baboons following administration of a quantity of RTI-55 calculated to be sufficient to saturate the dopamine transporters (Volkow et al., 1995b). A half-life of occupancy of several days was calculated, similar to that observed with no carrier added [^{123}I]RTI-55, which suggested that pharmacological amounts of RTI-55 or a similar drug might prevent the repeated 'high' associated with binge administration of cocaine, and thus find a role in the treatment of cocaine abuse (Rothman, 1990). Parallel experiments using [^{3}H]-cocaine were conducted in mice and revealed an occu-

* Corresponding author. Medical Department, 490 Bell Avenue, Brookhaven National Laboratory, Upton, NY 11973, USA. Tel.: (516) 282-4394; fax: (516) 282-5311; e-mail: gatley@brain.med.bnl.gov.

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pancy half-life for striatal RTI-55 of several hours (Volkow et al., 1995b).

The purpose of the present studies was to examine the ability of a single administration of cocaine to release non-tracer RTI-55 from mouse striatum, using [^{3}H]cocaine to probe dopamine transporter availability. We also conducted parallel experiments using [^{123}I]RTI-55 as the probe. If pharmacological quantities of RTI-55 can be displaced from the dopamine transporter by cocaine then the rationale for prevention of binge abuse of cocaine with RTI-55 may be compromised.

2. Materials and methods

2.1. Materials

Male Swiss-Webster mice (nominally 25 g) were purchased from Taconic Farms and maintained in the BNL animal facility with free access to food and water until use. RTI-55 and its trimethyltin congener were synthesized at Research Triangle Institute. Cocaine was obtained from the National Institute on Drug Abuse. [^{3}H]Cocaine was purchased from New England Nuclear Dupont, and [^{123}I]I$^-$ from Nordion International. [^{123}I]RTI-55 was prepared by reaction of [^{123}I]I$^-$ with its trimethyltin starting material essentially as described in the literature (Boja et al., 1991; Pan et al., in press).

2.2. Uptake of radioligands in mouse brain

For each experiment, animals from an individual batch were used. All mice received two intraperitoneal injections, which were given 1 h apart as indicated in Fig. 1. Injection 1 was either RTI-55 (2 mg/kg) dissolved in 0.2 ml of 0.9% saline, or vehicle alone. Injection 2 was either cocaine (20 mg/kg) dissolved in 0.2 ml of 0.9% saline, or vehicle alone; 3 h after the second injection, either [^{3}H]cocaine (1 μCi) or [^{123}I]RTI-55 (1 μCi) was injected in 0.2 ml of 0.9% saline via a tail vein. The animals were killed by cervical dislocation followed by immediate decapitation after a further 5 min, for [^{3}H]cocaine, or after a further 30 min, for [^{123}I]RTI-55. Brains were immediately dissected on ice-cooled moistened filter paper, and regions were weighed and counted for ^{123}I on a gamma counter where appropriate. For assay of tritium, tissues were dissolved in tissue solubilizer. Following addition of scintillation fluid, samples were assayed at 35–45% efficiency using a liquid scintillation counter, and converted to d.p.m. using an external standard system. In a representative experiment with control mice, weights of tissue samples (means ± S.D. for $n = 4$) were 12.5 ± 1.1, 5.5 ± 0.2 and 63.5 ± 3.7 mg for striatum, olfactory tubercle and cerebellum, respectively, and the tritium contents were 1577 ± 211, 546 ± 45 and 5517 ± 196 d.p.m., respectively.

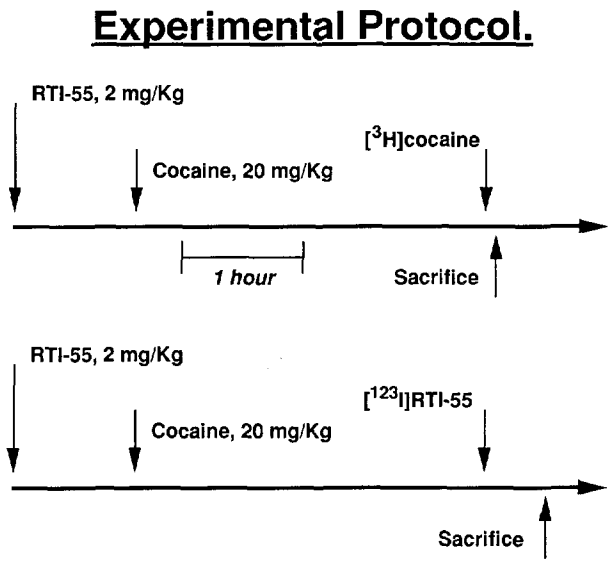

Fig. 1. Sequence of injections. Mice received two intraperitoneal injections 1 h apart. Injection 1 was either RTI-55 (2 mg/kg) dissolved in 0.2 ml of 0.9% saline, or vehicle alone. Injection 2 was either cocaine (20 mg/kg) dissolved in 0.2 ml of 0.9% saline, or vehicle alone. 3 h after the second injection, either [^{3}H]cocaine (1 μCi, upper panel) or [^{123}I]RTI-55 (1 μCi, lower panel) was injected in 0.2 ml of 0.9% saline via a tail vein. The animals were killed by cervical dislocation followed by immediate decapitation after a further 5 min, for [^{3}H]cocaine, or after a further 30 min, for [^{123}I]RTI-55.

2.3. Data analysis

Results were expressed as radioactivity concentrations (% injected radioactivity per gram) in brain regions, and as tissue-to-cerebellum ratios. The striatum-to-cerebellum ratio for [^{3}H]cocaine at 5 min was taken as an index of dopamine transporter availability, based on our earlier work (Volkow et al., 1995b) and other studies of the time-course of [^{3}H]cocaine in mouse brain tissues (Scheffel et al., 1989). In order to evaluate the ability of pharmacological doses of cocaine to displace RTI-55, comparisons were made between striatum-to-cerebellum ratio for [^{3}H]cocaine when animals were treated with either cocaine or saline after RTI-55. The effects on the striatum-to-cerebellum ratio of prior cocaine, and of vehicle injections alone, were evaluated in control groups of mice. Groups of mice were compared using an unpaired, two-tailed t-test. A P-value of less than 0.05 was considered significant.

3. Results

3.1. Experiments with [^{3}H]cocaine

Pretreatment with 2 mg/kg RTI-55 4 h before administration of radiotracer lowered the striatum-to-

Table 1
Effect of pretreatment with RTI-55 and/or cocaine on regional brain uptake of [^{3}H]cocaine

Group	n	Inj. 1	Inj. 2	Tissue uptake				
				St (%ID/g)	OT (%ID/g)	Cb (%ID/g)	St/Cb	OT/Cb
A	9	Saline	Saline	5.68 ± 0.71	4.73 ± 0.57	3.81 ± 0.46	1.49 ± 0.08	1.25 ± 0.13
B	10	Saline	Cocaine	4.67 ± 0.79	3.89 ± 0.62	3.50 ± 0.43	1.33 ± 0.13	1.11 ± 0.08
		Sig vs. Group A (P value)		< 0.01	< 0.01	n.s.	< 0.01	< 0.01
C	14	RTI-55	Saline	4.37 ± 0.65	4.01 ± 0.62	3.76 ± 0.43	1.17 ± 0.15	1.07 ± 0.14
		Sig vs. Group A (P value)		< 0.01	< 0.01	n.s.	< 0.01	< 0.01
D	24	RTI-55	Cocaine	4.41 ± 0.86	3.85 ± 0.81	3.52 ± 0.70	1.26 ± 0.07	1.10 ± 0.08
		Sig vs. Group A (P value)		< 0.01	< 0.01	n.s.	< 0.01	< 0.01
		Sig vs. Group B (P value)		n.s.	n.s.	n.s.	n.s.	n.s.
		Sig vs. Group C (P value)		n.s.	n.s.	n.s.	< 0.05	n.s.

Mice were injected intraperitoneally with RTI-55 (2 mg/kg in 0.2 ml saline) or with vehicle alone at time zero (Injection 1). After 1 h they were injected intraperitoneally with cocaine (20 mg/kg in 0.2 ml saline) or with vehicle alone (Injection 2). At 4 h they were injected intravenously with [^{3}H]cocaine (1 μCi in 0.2 ml saline) and then killed 5 min later. Values are the means ± S.D. for the indicated number of animals in each group. n.s. = not significant ($P > 0.05$). St, OT, Cb = striatum, olfactory tubercle and cerebellum, respectively. St/Cb and OT/Cb = striatum-to cerebellum ratio and olfactory tubercle-to-cerebellum ratio, respectively. %ID/g = percentage of injected radioactivity per gram of tissue.

cerebellum ratio (striatum-to-cerebellum ratio) obtained with [^{3}H]cocaine from 1.49 ± 0.08 to 1.17 ± 0.15 (Table 1, groups A and C). When 20 mg/kg cocaine was administered 1 h after RTI-55, a striatum-to-cerebellum ratio of 1.26 ± 0.07 was obtained (Table 1, group D). This corresponded to an 8% increase which was significant ($P < 0.05$). Treatment with 20 mg/kg cocaine alone 3 h before [^{3}H]cocaine also decreased the striatum-to-cerebellum ratio (1.33 ± 0.13; Table 1, group B) significantly compared with the control mice (group A). There were no significant differences between the groups of mice in Table 1 in terms of cerebellar concentration of radioactivity. Striatal radioactivity concentrations for the groups treated with RTI-55 and/or cocaine were significantly different ($P < 0.01$) from the saline/saline control group (Table 1). Binding of [^{3}H]cocaine in the olfactory tubercle was also decreased by pretreatment with RTI-55 or cocaine, but there were no significant differences between the groups given RTI-55 and RTI-55 plus cocaine.

3.2. Experiments with [^{123}I]RTI-55

A similar experiment was done using the radiolabeled cocaine analog [^{123}I]RTI-55 instead of [^{3}H]-cocaine to probe transporter availability after pretreatment with RTI-55 and cocaine. The regional brain uptake of [^{123}I]RTI-55 was not significantly affected by cocaine pretreatment (Table 2). In addition, pretreatment with RTI-55 did not reduce binding of [^{123}I]RTI-55 in the striatum. However, binding in the olfactory tubercles was significantly reduced ($P < 0.02$ for the percentage of injected radioactivity per g of tissue, and

Table 2
Effect of pretreatment with RTI-55 and/or cocaine on regional brain uptake of [^{123}I]RTI-55

Group	n	Inj. 1	Inj. 2	Tissue uptake				
				St (%ID/g)	OT (%ID/g)	Cb (%ID/g)	St/Cb	OT/Cb
A	5	Saline	Saline	12.6 ± 2.1	12.8 ± 2.1	4.3 ± 0.38	2.9 ± 0.03	3.0 ± 0.32
B	4	Saline	Cocaine	13.7 ± 1.96	13.8 ± 1.95	4.80 ± 0.83	2.9 ± 0.11	2.9 ± 0.14
		Sig vs. Group A (P value)		n.s.	n.s.	n.s.	n.s.	n.s.
C	5	RTI-55	Saline	12.0 ± 1.61	8.5 ± 1.0	4.8 ± 0.83	2.7 ± 0.47	1.9 ± 0.32
		Sig vs. Group A (P value)		n.s.	< 0.02	n.s.	n.s.	< 0.002
D	5	RTI-55	Cocaine	10.0 ± 1.1	7.73 ± 0.76	4.72 ± 0.23	2.1 ± 0.16	1.6 ± 0.13
		Sig vs. Group A (P value)		< 0.02	< 0.001	n.s.	< 0.002	< 0.001
		Sig vs. Group B (P value)		< 0.05	< 0.01	n.s.	< 0.001	< 0.001
		Sig vs. Group C (P value)		n.s.	n.s.	n.s.	n.s.	n.s.

Mice were injected intraperitoneally with RTI-55 (2 mg/kg in 0.2 ml saline) or with vehicle alone at time zero (Injection 1). After 1 h they were injected intraperitoneally with cocaine (20 mg/kg in 0.2 ml saline) or with vehicle alone (Injection 2). At 4 h they were injected intravenously with [^{123}I[RTI-55 (1 μCi in 0.2 ml saline) and then killed 30 min later. Values are the means ± S.D. for the indicated number of animals in each group. n.s. = not significant ($P > 0.05$). St, OT, Cb = striatum, olfactory tubercle and cerebellum, respectively. St/Cb and OT/Cb = striatum-to-cerebellum ratio and olfactory tubercle-to-cerebellum ratio, respectively. %ID/g = percentage of injected radioactivity per gram of tissue.

$P < 0.002$ for olfactory tubercle to cerebellum ratio). In contrast to the experiment with [^{3}H]cocaine, the striatum-to-cerebellum ratio for [^{123}I]RTI-55 was *decreased* rather than increased by RTI-55 plus cocaine (2.1 ± 0.16) compared with RTI-55 alone (2.7 ± 0.47). However, the decrease was not significant ($P < 0.08$).

4. Discussion

4.1. Effects of cocaine and RTI-55 on [^{3}H]cocaine binding

The striatal binding of [^{3}H]cocaine was significantly decreased by pharmacological doses of either cocaine given 3 h previously or RTI-55 given 4 h previously (Table 1). However, effects of administration of both cocaine and RTI-55 were not additive, as would be expected for two drugs which both compete with a radioligand for the same binding site (s). In fact, combined administration of these drugs caused a significantly smaller decrease in striatum to cerebellum ratio for [^{3}H]cocaine than did RTI-55 alone (Table 1). These observations support the notion that cocaine can displace pharmacological quantities of RTI-55 from the dopamine transporter, in addition to its previously documented ability to displace tracer doses of [^{123}I]RTI-55 (Laruelle et al., 1993; Muller et al., 1993). It should be noted that we examined only one combination of the five experimental variables: doses of cocaine; dose of RTI-55; interval between RTI-55 and cocaine; between cocaine and [^{3}H]cocaine, and between [^{3}H]cocaine and death. Almost certainly, this combination was not optimum to observe displacement of RTI-55 by cocaine.

4.2. Effects of cocaine and RTI-55 on [^{123}I]RTI-55 binding

In contrast to the results with [^{3}H]cocaine (Table 1), binding of the radiolabeled cocaine analog [^{123}I]RTI-55 in striatum and olfactory tubercle was not sensitive to administration of cocaine 3 h before radiotracer (Tables 2). The lack of effect of cocaine was initially surprising since cocaine given *after* [^{123}I]RTI-55 has approached equilibrium partially displaces the radiotracer (Laruelle et al. 1993). However, our results can be rationalized as follows. There may be a minor contribution from the fact that tissue concentrations of [^{123}I]RTI-55 were measured 30 min after administration, whereas [^{3}H]cocaine was measured at 5 min. Additional clearance of cocaine would be expected in the experiment with [^{123}I]RTI-55 during the extra 25 min. A more important factor may be the very different affinities for the dopamine transporter of cocaine and RTI-55, which have in vitro IC_{50} values of about 1 nM and 100 nM, respectively (Carroll et al., 1992; Seeman, 1993). Correspondingly, while striatal uptake of [^{3}H]cocaine peaks at about 5 min, and then rapidly dissociates from the dopamine transporter (Scheffel and Hartig, 1989), [^{123}I]RTI-55 continues to accumulate in rodent striatum for about 120 min after administration (Boja et al., 1992; Boja et al., 1991; Cline et al., 1992). Thus binding of the radiolabeled, high affinity cocaine analog is expected on the basis of prior in vitro and in vivo studies to be much less affected than that of [^{3}H]cocaine by the prior administration of cocaine. In addition to having a lower binding affinity, cocaine is also more susceptible to metabolism than RTI-55, which lacks the enzymatically labile benzoyl ester bond of cocaine (Gatley, 1991). This may contribute to the greater persistence of RTI-55 than cocaine.

The striatal binding of [^{123}I]RTI-55 was not reduced significantly by prior administration of a pharmacological dose of RTI-55 (Table 2). However, significant reduction became apparent after pretreatment with both RTI-55 and cocaine. RTI-55 alone did significantly reduce binding of [^{123}I]RTI-55 in the olfactory tubercle. This may be related to the lower concentration of dopamine transporters in olfactory tubercle than in striatum (Kaufman et al., 1991). The different responses of striatal binding of [^{123}I]RTI-55 and [^{3}H]cocaine to pretreatment with RTI-55 are consistent with the much higher affinity of [^{123}I]RTI-55 (Carroll et al., 1992; Seeman, 1993). We previously observed full recovery of murine striatal [^{3}H]cocaine binding by 6–12 h after 2 mg/kg RTI-55 (Volkow et al., 1995b). Thus after 4.5 h a large fraction of the administered RTI-55 has probably been excreted, and a state of equilibrium achieved between free RTI-55 in plasma and tissues and RTI-55 bound to striatal dopamine transporters. Dissociation of RTI-55 from the dopamine transporter in vitro is over 90% complete within 30 min (Laruelle et al., 1994a; Little et al., 1993). This is much faster than in vivo clearance and could be explained if free RTI-55 in striatum has a much higher probability of (re)binding than of clearing the tissue. Thus the no-carrier added [^{123}I]RTI-55 could be retained by striatum by competing for binding with unlabeled RTI-55. In contrast, [^{3}H]cocaine which has a 100-fold weaker affinity for the transporter is not expected to compete effectively with RTI-55 for (re)binding. If this explanation for our results is correct, it implies that PET or SPECT experiments designed to probe the occupancy of a binding site with a drug may generally require that the radioligand should have a lower affinity than the drug for the binding site.

4.3. Pharmacotherapy of cocaine abuse

The reinforcing effects of drugs of abuse are well known to depend on their speed of entry into the brain

(Balster and Schuster, 1973). Recent data suggest that the intense euphoria induced by dopamine transporter inhibitors such as cocaine is associated with the initial fast uptake of drug into the brain, rather than with their persistence in brain (Fowler et al., 1989; Stathis et al., 1995). Thus the peculiarly destructive binge pattern of cocaine abuse may be facilitated by the drug's rapid dissociation from the dopamine transporter in vivo, which permits repeated administrations (Volkow et al., 1995a). A 'cocaine antagonist' able to block its euphorigenic actions would thus be a potentially valuable aid in the treatment of cocaine abuse (Rothman, 1990). Although cocaine analogs with higher affinity than cocaine are reinforcing in animal models (Spealman et al., 1983), their slower net dissociation from the dopamine transporter may allow adaptive neurochemical changes which cause behavioral tolerance to dopamine transporter occupancy. While occupancy remains high, it would be expected that cocaine would not be able to induce further 'highs', but this strategy would fail if cocaine could displace the high affinity reuptake blocker. Replacing one uptake inhibitor with another (i.e. RTI-55 with cocaine) would not be expected to immediately change the synaptic dopamine concentration. However, cocaine, unlike RTI-55, would dissociate rapidly, leaving unblocked transporters and a lower synaptic dopamine concentration which might re-initiate craving.

RTI-55 which binds to the dopamine transporter with about 100 times higher affinity than cocaine would seem to be a possible lead compound for development of cocaine antagonist drugs, as suggested by our previous documentation of its long lasting inhibition of striatal [^{3}H]cocaine binding (Volkow et al., 1995b). However, the present results support the idea that a single administration of a pharmacological dose of cocaine is able to displace a fraction of the previously bound RTI-55 from the dopamine transporter, and partially restore the ability to bind [^{3}H]cocaine (Table 1). Thus it is possible that RTI-55 would not in fact prevent the 'highs' associated with repeated self-administration of cocaine, if one considers dopamine transporter occupation as the pertinent variable. However, one has to also consider differences in adaptation responses secondary to administration of dopamine transporter inhibitors. For example, it is well documented that cocaine administration induces short lasting inhibition of mesencephalic dopamine cell firing, via activation of impulse regulating autoreceptors (Chiodo, 1988). It is possible that a drug with a slower dissociation rate than cocaine, such as RTI-55, may induce a longer lasting inhibition of dopamine cells making the animals refractory to further dopaminergic stimulation, even if the putatively therapeutic dopamine transporter ligand were to be displaced by cocaine.

RTI-55 is believed to bind to the dopamine transporter at or very near to the binding site (s) of cocaine, to which it is structurally closely related (Boja et al., 1991; Carroll et al., 1992). There has been considerable discussion of the possibility of developing compounds which inhibit the binding of cocaine to a greater extent than the reuptake of dopamine (Carroll et al., 1995; Deutsch and Schweri, 1994). This concept is supported by several studies which indicate that stimulant and neurotransmitter binding sites are not identical (Carroll et al., 1995; McElvain and Schenk, 1992; Simoni et al., 1993). The present studies suggest that the mode of inhibition of *cocaine binding* by potential cocaine antagonists is as important as the mode of inhibition of *dopamine binding*. Even dopamine transporter ligands of very high affinity such as RTI-55 may not prevent binge-type abuse if they can be competitively displaced by a single high dose of cocaine, and if they are found to not affect the responses of dopamine cells to cocaine administration. It is also not yet clear whether after non-toxic doses of RTI-55, a high enough plasma level of the drug could be maintained to allow rapid re-occupation of dopamine transporters which had been 'cleared' by cocaine. Dopamine transporter ligands which are irreversible or non-competitive, rather than competitive, inhibitors of cocaine binding may have more promise for therapy of cocaine abuse (Deutsch et al., 1992).

To some extent, a parallel may be drawn between the potential use of high affinity dopamine transporter blockers for the treatment of cocaine abuse, and the use of methadone to treat opioid addiction. Methadone would be expected to be displaced from receptors by heroin (or derived morphine), and yet it is a useful drug (Goodman-Gilman et al., 1993). However, there are important differences between heroin and cocaine pharmacologies which may decrease the importance of therapeutic drug displacement for the opioids. Firstly, opioid drugs are direct postsynaptic agonists, whereas cocaine is an indirect agonist which acts presynaptically. Secondly, methadone has a similar binding affinity and potency to morphine, whereas putative dopamine transporter blockers such as RTI-55 have higher affinity than cocaine (Raynor et al., 1995). Thirdly, the success of methadone has been ascribed to its long plasma half-life, and the behavior of tissue stores as a drug 'depot', so that a very constant level of receptor occupancy is maintained which prevents withdrawal symptoms but allows addicts to lead normal lives (Dole, 1988). Fourthly, adaptive responses may differ after long-term self-administration of opioids and psychostimulants, and these differences are expected to affect the responses of the various therapeutic interventions. In the development of treatments for preventing cocaine bingeing behavior, adaptation responses of mesencephalic dopamine cells to the intervention must be considered. A search for drugs which

induce very long lasting and rather constant inhibition of dopamine cell firing may represent a productive research strategy.

The idea that RTI-55 may therapeutically prevent cocaine binding (and thus putatively euphoria) for long periods is supported by its low net rate constant for dissociation from striatal binding sites in vivo, which is about 0.005–0.02 h^{-1} in baboons and humans (Laruelle et al., 1993, 1994b; Volkow et al., 1995b). However, as discussed above, dissociation rate constants of RTI-55 from baboon and human striatal homogenates are at least 100 times higher than in vivo clearance rate constants (Laruelle et al., 1994a,b; Little et al., 1993). Presumably, the discrepancy is due to a high probability in vivo of re-binding following a dissociation event, combined with continued uptake of RTI-55 from plasma. When cocaine is available to occupy dopamine transporter binding sites vacated by RTI-55, the net dissociation rate is expected to be increased, both for radiotracer RTI-55 and for pharmacological quantities of the drug (Table 1).

4.5. Conclusions

Our results indicate that cocaine can displace pharmacological dose of RTI-55 from striatum. They also suggest that a PET or SPECT radioligand used to assess the occupancy of a tissue binding site should have a lower affinity for the binding site than the occupying drug, so that it has a lower probability of capture by a transiently free binding site, which could result in underestimation of transporter availability.

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