ELSEVIER

European Journal of Pharmacology 311 (1996) 109–114

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Highly potent cocaine analogs cause long-lasting increases in locomotor activity

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Received 22 January 1996; revised 28 May 1996; accepted 31 May 1996

Abstract

Three cocaine analogs were compared with cocaine for the capacity to affect: (1) dopamine transporter binding and function; and (2) locomotor activity. RTI-55 (3β-[4-iodophenyl]tropane-2β-carboxylic acid methyl ester tartrate), RTI-121 (3β-[4-iodophenyl]tropan-2β-carboxylic acid isopropyl ester hydrochloride) and RTI-130 (3β-[4-chlorophenyl-2β-[1,2,4-oxadiazol-3-phenyl-5-yl]tropane hydrochloride) competed for [^{3}H]WIN 35428 binding in rat striatum in vitro, with IC_{50} values at least 50-fold less than that of cocaine. These analogs inhibited [^{3}H]dopamine transport into rat striatal synaptosomes, with IC_{50} values again less (at least 100-fold) than that for cocaine. Intravenous RTI-55, RTI-121 or RTI-130 injection effected dose-related increases in locomotor activity in mice, with estimated relative potencies at least 10-fold greater than that of cocaine. These increases were long lasting: whereas increased activity ceased within 2 h after cocaine administration, increased locomotion was observed at least 10 h after RTI-55, RTI-121, or RTI-130 administration. Parallel line analysis indicated that the slopes of the ascending portion of the RTI-121 and RTI-130 dose-response curves differed from that of cocaine, suggesting the involvement of mechanisms different from that of cocaine.

Keywords: Cocaine analog; Behavior; Dopamine transporter

1. Introduction

Cocaine has several sites of action in the central nervous system, although it is the site associated with the dopamine transporter that has been implicated most frequently in causing the reinforcing properties of cocaine (Ritz et al., 1987; Koob and Bloom, 1988; Bergman et al., 1989). Cocaine exerts its behavioral effects, at least in part, by binding to the dopamine transporter, blocking synaptic dopamine reuptake, and thereby potentiating dopaminergic (particularly mesolimbocortical) neurotransmission (for review, see Kuhar et al., 1991). Considerable effort has been directed towards developing cocaine analogs with high affinity for the dopamine transporter since such may be of benefit not only as neurochemical tools for studying cocaine reinforcement, but also as probes for dopamine transporter imaging (Innis et al., 1991, 1993; Shaya et al., 1992), and perhaps as medications for treatment of cocaine dependence and abuse (Kuhar, 1992; Stathis et al., 1996).

Important properties for medications designed to treat cocaine abuse might include potency, specificity, slow entry into the brain and a long duration of action. Numerous compounds fulfilling the first two criteria, high potency and selectivity, have been developed to target the dopamine transporter, many having affinity for the transporter far greater than cocaine itself (Carroll et al., 1992; Clarke et al., 1973; Goodman et al., 1994; Innis et al., 1993; Kung et al., 1995; Meltzer et al., 1994; Neumeyer et al., 1994; Newman et al., 1994). RTI-130 (3β-[4-chlorophenyl-2β-[1,2,4-oxadiazol-3-phenyl-5-yl]tropane hydrochloride) (Fig. 1), for example, has greater than 50 times the affinity for the dopamine transporter than does cocaine, as defined by its IC_{50} for [^{3}H]WIN-35428 binding in rat striatum (Carroll et al., 1993). RTI-55 (3β-[4-

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PII S0014-2999(96)00423-2

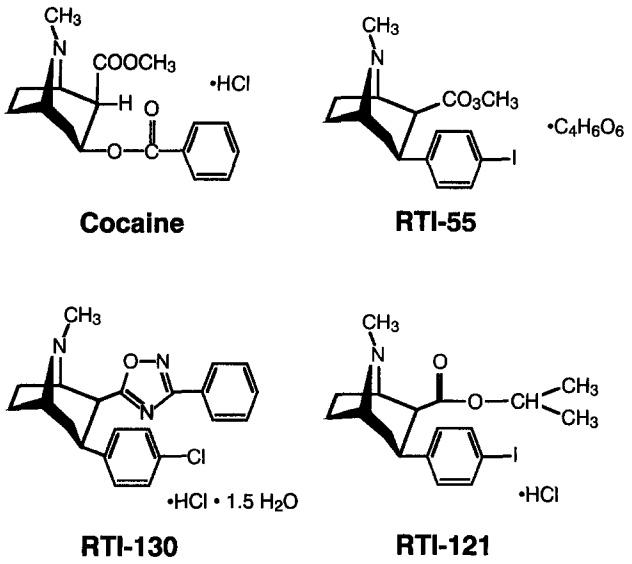

Fig. 1. Chemical structures of (−)-cocaine, RTI-55, RTI-121 and RTI-130.

iodophenyl]tropane-2β-carboxylic acid methyl ester tartrate) (also known as β-CIT; Fig. 1) and RTI-121 (3β[4-iodophenyl]tropan-2β-carboxylic acid isopropyl ester hydrochloride) (Fig. 1) likewise have high affinity for dopamine transporter (Boja et al., 1992, 1995; Carroll et al., 1995). RTI-55 and RTI-121 also fit the third criterion for a putative medication since both enter the brain more slowly than does cocaine itself (Stathis et al., 1996). Although binding characteristics of these and other cocaine analogs have been well described, behavioral effects have been less so. In the present study, effects of RTI-55, RTI-121, and RTI-130 on locomotor activity were assessed and compared with that of cocaine. Effects of these compounds on [^{3}H]WIN-35428 binding and [^{3}H]dopamine uptake were also determined. The results reveal that, consistent with a role for the dopamine transporter in mediating psychostimulant-affected behavior, these three analogs which profoundly affect dopamine transporter binding and function greatly affect locomotor activity. The long duration of effect caused by these compounds may make them reasonable prototypes in the search for medications to treat cocaine abuse, or long acting probes for the study of cocaine's action.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (Harlan, Indianapolis, IN, USA; 200–300 g) or male CD-1 mice (Charles River Laboratories, Wilmington, DE, USA; 25–40 g) were maintained under standard conditions (12 h light-dark cycle) with food and water provided ad libitum. Maintenance of animals and experimental procedures were carried out in accordance with approved National Institutes of Health guidelines.

2.2. Materials

3β-[4-Iodophenyl]tropan-2β-carboxylic acid methyl ester tartrate (RTI-55), 3β-(4-iodophenyl)tropan-2β-carboxylic acid isopropyl ester hydrochloride (RTI-121), 3β-(4-chlorophenyl-2β-(1,2,4-oxadiazol-3-phenyl-5-yl)tropane hydrochloride (RTI-130), 3β-[4-fluorophenyl]tropan-2β-carboxylic acid methyl ester tartrate (WIN 35428) and (−)-cocaine hydrochloride were obtained from the Research Triangle Institute (Research Triangle Park, NC, USA). [^{3}H]WIN 35428 (80 Ci/mmol) was purchased from DuPont NEN (Boston, MA, USA) and [7,8-^{3}H]dopamine (48 Ci/mmol) was purchased from Amersham (Buckinghamshire, UK). Pargyline hydrochloride was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Drugs to be administered in vivo were dissolved in 0.9% sterile saline; compounds used in in vitro assays were dissolved in appropriate assay buffer.

2.3. Locomotor activity testing

In the early morning on the day of observation, mice were brought to the testing room and kept in this quiet environment until behavioral monitoring began. Testing took place with individual animals placed within a transparent Plexiglas box (40 × 40 × 30 cm) framed by an 8 × 8 beam photocell array (Omnitech Electronics, Columbus, OH, USA). Cages were connected to an analyzer programmed to print mean distance traveled (cm) in 10-min intervals. Each mouse was habituated to the text box for 30 min prior to drug/saline administration. Mice were then removed from the box, injected once via the tail vein with saline or drug, and returned immediately to the box; testing began each day at approximately 13:00 h. Each mouse was used only once. Basal activity was defined as the activity measured over the 10 min interval immediately prior to drug administration, and was subtracted from drug-induced activity to correct for variability of individual mice.

2.4. [^{3}H]WIN 35428 binding in vitro

For determination of [^{3}H]WIN 35428 binding in vitro, frozen rat striata were homogenized in 80 volumes (w/v) of ice-cold buffer (0.3 M sucrose, 7.3 mM $Na_2HPO_4 \cdot 7H_2O$, 2.1 mM NaH_2PO_4; pH 7.4 at 25°C) using a Polytron homogenizer (setting 6 of 10) and centrifuged at 50 000 × g (2–4°C) for 10 min. The resulting pellet was resuspended in fresh buffer, and the centrifugation procedure repeated. The final pellet was resuspended in fresh buffer to a concentration of 10 mg original tissue wet weight/ml. Incubations were initiated by adding 100 μl tissue suspension (i.e., 1 mg wet weight tissue/tube) to triplicate tubes containing a fixed concentration (0.5 nM

final concentration) of [^{3}H]WIN 35428 and increasing concentrations of unlabeled test compound to yield a 0.5 ml final assay volume; nonspecific binding of [^{3}H]WIN-35428 was defined in the presence of 30 μM (−)-cocaine HCl. All test tubes were incubated on ice for 120 min and filtered under vacuum through Whatman GF/B filtered soaked previously in 0.05% polyethylenimine. Filters were washed rapidly 3 times with 5 ml ice-cold buffer using a Brandel M48R filtering manifold (Brandel Instruments, Gaithersburg, MD, USA). Radioactivity trapped in filters was counted using a Beckman LS 3801 liquid scintillation counter at an efficiency of approximately 50%.

2.5. [^{3}H]Dopamine transport

Fresh rat striata were homogenized in approximately 80 volumes (w/v) of ice-cold 0.3 M sucrose using a hand-held glass and teflon homogenizer and centrifuged at 800 × *g* (2–4°C) for 10 min. The resulting supernatant was centrifuged at 20 000 × *g* (2–4°C) for 10 min. The subsequent pellet was resuspended in ice cold 0.3 M sucrose to a concentration of 15 mg original tissue wet weight/ml and left undisturbed on ice for 15 min. 100 μl tissue suspension (i.e., 1.5 mg wet weight tissue/tube) was then added to quadruplicate tubes and incubated at 30°C for 10 min in the presence of assay buffer (126 mM NaCl, 4.8 mM KCl, 1.3 mM $CaCl_2$, 16 mM sodium phosphate, 1.4 mM $MgSO_4$, 11 mM dextrose, 1 mM ascorbic acid; pH 7.4) and 1 μM pargyline. The assay was initiated by adding 100 μl of 5 nM [^{3}H]dopamine to each tube (i.e., 0.5 nM [^{3}H]dopamine/tube) to yield a 1 ml final assay volume. Nonspecific values were determined in the presence of 50 μM (−)-cocaine HCl. Assay tubes were then incubated at 30°C for 3 min and filtered under vacuum through Whatman GF/B filtered soaked previously in 0.05% polyethylenimine. Filters were washed rapidly 3 times with 5 ml ice-cold 0.3 M sucrose using a Brandel M48R filtering manifold. Radioactivity trapped in filters was counted using a Beckman LS 6000 liquid scintillation counter at an efficiency of approximately 50%.

2.6. Data analysis

IC_{50} values were determined using EBDA and LIGAND computer software. In order to assess relative potency of drugs, ascending portions of dose-response curves were analyzed by standard parallel line bioassay technique (Finney, 1964) using a statistical analysis program (v3.3) developed for the Neuroimaging and Drug Action Section, NIDA Addiction Research Center, Baltimore, MD, USA. This technique employs a one-way analysis of variance (ANOVA) to determine whether the slopes of dose-response curves differ from that of cocaine. It also compares doses required to elicit a given response and provides a value for relative potency. All other analyses were conducted using analysis of variance followed by Tukey's test. Differences among groups were considered significant if the probability of error was less than 5%.

3. Results

Table 1 summarizes dopamine transporter binding and uptake data from present and previous studies, and demonstrates that RTI-55, RTI-121 and RTI-130 (for structures, see Fig. 1) are each more potent than cocaine in binding to the dopamine transporter and inhibiting its function. Specifically, RTI-55, RTI-121 and RTI-130 each competed for [^{3}H]WIN 35428 binding in rat striatal homogenates in vitro, with IC_{50} values that were far less than that of cocaine. The ED_{50} for inhibition of [^{3}H]WIN35428 binding in mice in vivo for these compounds was similarly less than that of cocaine. RTI-55, RTI-121 and RTI-130 inhibited the transport of [^{3}H]dopamine into rat striatal synaptosomes, with IC_{50} values that were less than the IC_{50} values for cocaine (Table 1).

Results presented in Fig. 2 and Fig. 3 reveal that a single intravenous injection of RTI-55, RTI-121 or RTI-130 each caused increases in locomotor activity in mice that were longer lasting than that of cocaine. In this experiment, independent ANOVA was performed for each compound using data collected over a 10 min interval at 5, 10, 15 or 20 h following drug or saline administration; comparisons were made among doses with data from time-matched saline-treated controls. As shown in Fig. 2 (upper panel), increased locomotor activity ceased within 2 h after cocaine administration. In contrast, significant increases in locomotor activity were observed 5 and 10 h after injection of 1 and 3 μmol/kg RTI-55, respectively (Fig. 2; bottom panel). Increases were observed similarly at 5 and 10 h after administration of 30 μmol/kg RTI-121 (Fig. 3; upper panel). Five, 10 and 15 h after administration of 1 μmol/kg RTI-130, significant increases in locomotor activity were likewise detected (Fig. 3; lower panel). The RTI-55-, RTI-121- and RTI-130-induced increases in ambulatory activity appeared dose-related during the first h after administration except at the highest doses at which activity was increased less. Marked stereotypy following administration of these high doses, behaviors consisting of

Table 1
Average inhibitory concentrations for cocaine and analogs at the dopamine transporter. See text for experimental details

	In vitro binding IC_{50} (nM)	In vivo binding ED_{50} (μmol/kg)	In vitro uptake IC_{50} (nM)
(−)-Cocaine	89.1 ±5.2	40.93 [a]	153.2 ±7.9
RTI-55	1.26±0.06 [a]	0.26 [a]	0.79±0.03
RTI-121	0.43±0.08	0.66 [b]	1.50±0.12
RTI-130	1.62±0.03	0.72 [c]	0.59±0.07

[a] From Boja et al. (1992) and Cline et al. (1992). [b] From Stathis et al. (1996). [c] Personal communication, U. Scheffel. Standard errors were less than 15% of the determined values.

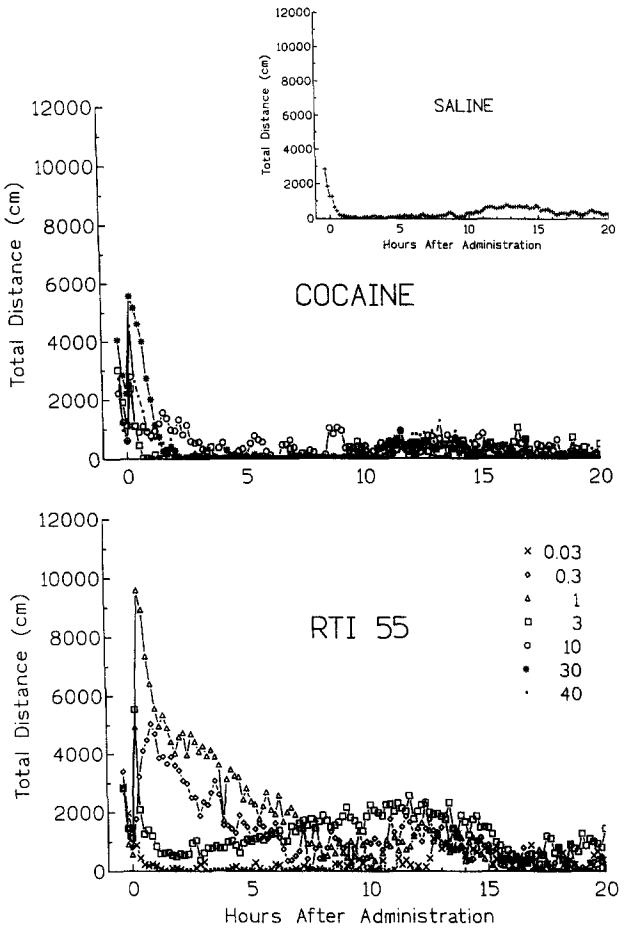

Fig. 2. Time course of effects of saline, cocaine and RTI-55 on locomotor activity in male mice. Mice were placed in behavioral monitors and locomotor activity was recorded for 3 consecutive 10 min intervals. Mice then received drug (i.v., doses indicated in figures) or saline vehicle (i.v., 5 ml/kg) at time = 0, and locomotor activity was determined for 10 min intervals for 20 h thereafter. Values represent mean determinations from 4–6 drug-treated or 31 saline-treated mice.

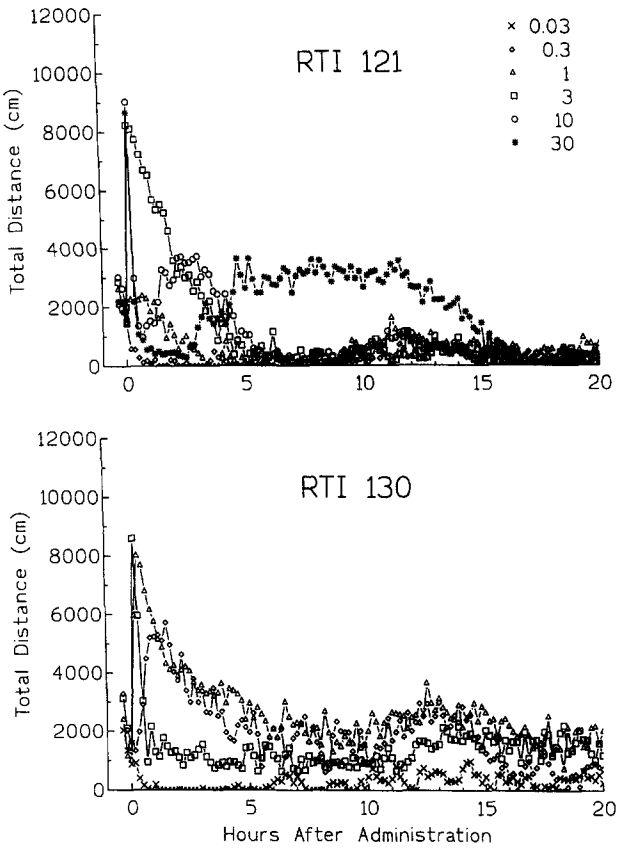

Fig. 3. Time course of the effects of RTI-121 and RTI-130 on locomotor activity in male mice. Mice were placed in behavioral monitors and locomotor activity was recorded for 3 consecutive 10 min intervals. Mice then received drug (i.v., doses indicated in figures) or saline vehicle (i.v., 5 ml/kg; see Fig. 2) at time = 0, and locomotor activity was determined over 10 min intervals for 20 h thereafter. Values represent mean determinations from 4–6 mice.

repetitive grooming and gnawing on the Plexiglas cage floor, were noted upon visual inspection.

Dose-response data compiled from data obtained during the initial 20 min post-drug administration (a time by which peak drug effects had been reached) from the same experiment presented in Fig. 2 and Fig. 3 are depicted in Fig. 4. These data are presented as mean area under time-action curves for each dose corrected for individual variability among animals' basal locomotor activities, with basal activity considered to be the activity measured over the 10 min interval immediately prior to drug or saline administration. Cocaine effected a dose-related increase in locomotor activity at doses up to 30 μmol/kg; doses greater than 40 μmol/kg were not employed since preliminary data indicated that these induced significant mortality. RTI-55, RTI-121 and RTI-130 likewise increased locomotor activity in mice with estimated relative potencies that were 55-, 13-, and 31-fold greater, respectively, than that of cocaine. Parallel line analysis (for description, see Methods section) indicated that the slopes for RTI-121 ($P < 0.027$) and RTI-130 ($P < 0.004$), but not RTI-55 ($P < 0.28$), differed significantly from that of cocaine.

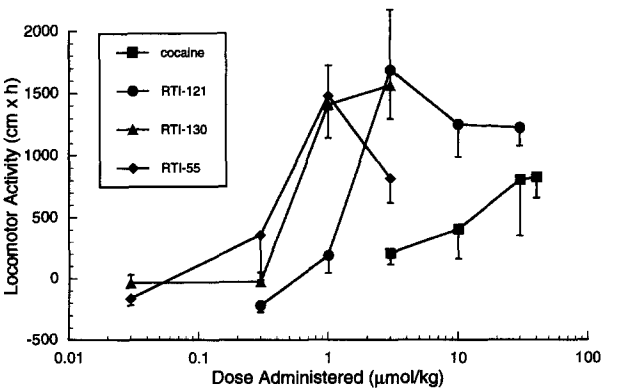

Fig. 4. Dose-response effects of cocaine, RTI-55, RTI-121 and RTI-130 on locomotor activity in male mice. Values represent mean area under the locomotor activity curve corresponding to the 20 min period immediately following drug administration (see text for details) ±1 S.E.M. for determinations in 4–6 mice.

4. Discussion

Considerable evidence indicates that the psychostimulant and locomotor properties of cocaine are related to its ability to inhibit dopamine reuptake by binding to a specific site on the dopamine transporter and thereby potentiating dopaminergic neurotransmission. It would be anticipated, therefore, that administration of cocaine analogs that bind to the dopamine transporter and block [^{3}H]dopamine uptake would likewise increase locomotor activity. Consistent with this supposition, the results from the present study reveal that the analogs RTI-55, RTI-121 and RTI-130, compounds which bind avidly to the dopamine transporter both in vitro and in vivo, and which inhibit dopamine uptake into synaptosomal preparations, profoundly increase locomotor behavior. Interestingly, these compounds which were more potent than cocaine in binding to the dopamine transporter and inhibiting dopamine uptake, were more potent in increasing locomotor activity.

The increased locomotor activity observed following administration of RTI-55, RTI-121 or RTI-130 was dose-related except at the highest doses where activity either plateaued or decreased. These plateaus or decreases corresponded with observed increases in stereotypic behaviors such as repetitive gnawing and grooming. Similarly, stereotypy following high dose administration of cocaine or other psychostimulants has been described (Cline et al., 1992; Costall and Naylor, 1977). These behaviors likely interfered with ambulatory activity, thereby accounting for sub-maximal locomotor responses, although some unknown factors might also have contributed to the reduced activity.

RTI-55, RTI-121 and RTI-130 are distinctly different from cocaine in that these compounds effect long-lasting increases in locomotor activity after only one injection. Unlike cocaine with behavioral effects that persisted less than 2 h, increased locomotor activity was observed in the present study at least 10 h after administration of each of the analogs. The mechanism responsible for this long duration is unknown, but is likely related to the compounds' reduced susceptibility to metabolic degradation. The effects of cocaine are short-lived because it is cleaved in vivo at the 2β and 3β positions on the tropane ring (Baselt, 1982). In contrast, RTI-55 and RTI-121, although structurally similar to cocaine in that they retain the ester moiety at the 2β position, lack the esteric link between the phenyl and tropane rings (Fig. 1); this difference likely makes these compounds less susceptible in vivo to degradation. RTI-130 lacks esteric links at both the 2β and 3β positions perhaps rendering it even less susceptible to degradation by esterases. Consistent with this presumed enhanced resistance to degradation, RTI-130 caused the longest duration of effect among the compounds tested. We cannot rule out that other synaptic effects, such as altering of storage pools (Juorio, 1982; Miller and Shore, 1982) contributes to the behavior observed here.

The mechanisms whereby cocaine, RTI-55, RTI-121 and RTI-130 affect locomotor activity may be related not only to resistance to degradation, but also to their affinity for transporters other than the dopamine transporter. Although highly selective for dopamine transporter, each of the analogs tested has appreciable affinity for serotonin and norepinephrine transporters (Carroll et al., 1993, 1995), and binding to these transporters is well established to affect behavior. Interestingly, the slopes of the dose-response curves for RTI-121 and RTI-130 differ from that of cocaine, suggesting that RTI-121 and RTI-130 increase locomotor activity through mechanisms somewhat different or less complex than that of cocaine. It is interesting to speculate that the enhanced selectivity of these compounds for the dopamine transporter relative to the serotonin and norepinephrine transporters may be related to their prolonged duration of effect. More data would, however, be necessary before such a correlation could be established.

Compounds with long durations of action, if used as medications, would facilitate a convenient and economical dosing schedule. For example, oral methadone has a longer half-life than injected heroin and this is advantageous because once a day rather than multiple daily dosing with methadone is feasible. Treatment can therefore be carried out under medical supervision (Jaffe, 1990). However, agonist substitution therapy with long-acting cocaine-like compounds has yet to be proven effective. RTI compounds may be suitable candidates for such therapy.

In conclusion, the present data demonstrate that RTI-55, RTI-121 and RTI-130 cause dose-related, long-lasting increases in ambulatory activity in mice. Although a role for the dopamine transporter in mediating the effects of each of these cocaine analogs appears certain, overall mechanisms whereby RTI-121 and RTI-130 increase locomotor activity may be somewhat dissimilar to that of cocaine. Factors related to degradation or affinity for other transporters are likely involved in the ability of these compounds to increase locomotor activity.

Acknowledgements

The authors gratefully acknowledge the excellent advice and assistance of Dr Lawrence Sharpe.

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