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Novel monoamine transporter ligands reduce cocaine-induced enhancement of brain stimulation reward

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Abstract

Six novel monoamine reuptake inhibitors were screened for their intrinsic effects on brain stimulation reward (BSR), as well as for their potential to reduce cocaine-induced reward-enhancement in that paradigm. Two of the compounds, nocaine-3B and 5-ara-74A (disubstituted piperidines) significantly reduced locus of rise (LOR), threshold measure of reward, at some doses. One compound, 1-RV-96A (a hybrid of the GBR and WIN-like agents) significantly reduced reward (increased LOR), but only at the highest dose tested. No effect of dose was found for MC9-20 (a GBR-like acyclic analogue of the *N*-bisarylmethoxyethyl-*N*′-phenylpropyl piperazine), nocaine-250B or 4-ara-42C (disubstituted piperidines). When cocaine (10 mg/kg, ip) and selected, hedonically neutral doses of novel compounds were combined, the following findings were obtained: MC9-20 (2.5 mg/kg, ip) showed a significant increase in cocaine-induced reward enhancement (0.2 log units or 53%). In contrast, nocaine-250B and 1-RV-96A (both at 10 mg/kg, ip) demonstrated a significant reduction (0.13 log units or 41%) in cocaine-induced reward enhancement ($P < .01$ and $P < .05$, respectively), as measured by changes in LOR. There were no differences in the maximum behavioral output (MAX) at either dose of each of the six drugs, or when selected doses were combined with cocaine. These results indicate that nocaine-250B and 1-RV-96A constitute two potential anticocaine compounds worthy of further behavioral and biochemical evaluation.

Keywords: Anticocaine behavioral screen; Brain stimulation reward; GBR analogue; Piperidine; Piperazine; Phenyltropane; Reward threshold; WIN analogue

The 1997 National Household Survey on Drug Abuse reported that approximately 3.6 million people in the USA alone abuse cocaine regularly (SAMHSA, 1998). With such a substantial number of individuals who are either already addicted or are on a path to addiction, there is a need for the development of a medication to treat cocaine dependence that despite intensive research efforts, does not yet exist (Alterman et al., 1994; Altman et al., 1996; Dewey et al., 1998; Glowa et al., 1997; Klein, 1998; Mendelson and Mello, 1996; Smith et al., 1999).

From the behavioral perspective a potential pharmacotherapeutic agent should not exhibit abuse potential when administered alone and it should substantially reduce cocaine's reinforcing properties, even in the presence of elevated blood levels of the addictive substance (Carroll et al., 1999). In addition, to be most effective, a pharmacological treatment should also reduce craving, which is often experienced after a period of abstinence and is thought to be a major factor in relapse (Alterman et al., 1994; Altman et al., 1996; Gawin, 1991; Robinson and Berridge, 1993). It is possible that no one single agent could address both aspects of addiction. Thus, it is likely that once cocaine's reinforcing properties are pharmacologically blunted, another agent, designed to target craving alone, could be administered.

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The present report addresses the first issue of this drug discovery process, i.e., the identification of an agent that would reduce cocaine's reinforcing effects, without being either positively or negatively hedonic by itself. In addition, it also demonstrates that brain stimulation reward (BSR) paradigm is a useful and effective drug-screening behavioral model.

Cocaine inhibits the transport of serotonin (SERT), norepinephrine (NET), and dopamine (DAT) with similar potency (Woolverton and Johnson, 1992), yet it is the inhibition of DAT, particularly in the mesocorticolimbic terminals fields, that is thought to be largely responsible for the reinforcing and euphorigenic effects of cocaine (Ritz et al., 1987; Volkow et al., 1997). Since this drug's action at the DAT seems to be critical to its abuse liability, modulation of cocaine binding, specifically at the DAT, represents a conceivable strategy in the development of an effective pharmacotherapeutic agent (Johnson et al., 1992; Kitayama et al., 1992; Smith et al., 1999). However, some potent DAT inhibitors, such as benztropine used to treat Parkinson's disease, or buproprion given to treat nicotine dependence, do not possess abuse liability (Rothman, 1990). This discrepancy between the action of cocaine and other DAT inhibitors may be explained by the fact that cocaine has a rapid onset of action. In addition, experimental evidence suggests that cocaine may have an effect on behavior separate from its function at the DAT by binding to SERT and NET (e.g., Ritz et al., 1987; Smith et al., 1999; Volkow et al., 1997; Woolverton and Johnson, 1992). Accumulating evidence also points to the fact that multiple binding locations exist on the DAT itself (e.g., Boja et al., 1992; Carroll et al., 1992; Tella et al., 1996) and that cocaine affects a site that is distinct from dopamine (DA) or the amphetamine-binding location on the DAT (Johnson et al., 1992; Lin et al., 1999b; Wayment et al., 1998). In addition to the cocaine site, two other regions have been identified, one that interacts with phenyltropanes (cocaine analogs of the WIN series) and the other that interacts with diphenylmethoxyethylpiperazines or GBR analogs (Carroll et al., 1992; Newman et al., 1994; Vaughan and Kuhar, 1996), although no convincing data have yet been reported indicating that the cocaine site might be different from the WIN site.

The two most recent approaches in the discovery of successful treatments have been based on extensive structure–activity relationship studies. These investigations compare the effects of structure modification on the IC50 or K_i values (in nanomolar) for the inhibition of cocaine binding and DA uptake at the DAT. The first approach focused on agents that exhibit a higher affinity for the cocaine-binding site than for the DA-binding site. Such drugs would block the binding of cocaine on the DAT while only minimally inhibiting DA transport alone (Simoni et al., 1993). Although the DAT is not a true receptor, these agents would also be analogous to naltrexone (used to treat heroin and alcohol addiction) in that they would block the binding of cocaine to the DAT and possibly other monoaminergic transporters without greatly altering the reuptake of DA.

Since cocaine also strongly inhibits SERT and NET, the second approach focused on selective monoamine reuptake inhibitors (disubstituted piperidines), agents that are tailored to have a range of selectivities at the three monoamine transporters and also slightly reduce DA reuptake. These compounds would constitute treatment analogous to buprenorphine replacement therapy used to treat heroin addiction and would be expected to possess minimal hedonic effects. Preliminary studies have shown that these agents somewhat inhibit DA reuptake, yet they fail to produce significant locomotor activation, characteristic of psychostimulants (Kozikowski et al., 1998). In addition, in a drug-discrimination study one of these agents, nocaine-3B, failed to produce robust cocaine-like effects (Kozikowski, unpublished observations).

The present paper describes the behavioral evaluation of six of the recently synthesized monoamine reuptake inhibitors in a BSR paradigm (e.g., Backus et al., 1988; Bauco and Wise, 1997; Gallistel, 1987; Maldonado-Irizzary et al., 1994; Miliaressis et al., 1986; Stellar et al., 1983; Wise, 1996). 1-RV-96A is a hybrid of the GBR and WIN series (cocaine congeners) (Prakash et al., 1999). It is potent at binding to the mazindol site on the DAT and reducing DA uptake in vitro, while also exhibiting selectivity for DAT vs. SERT in rat synaptosomes (Prakash et al., 1999). MC9-20 is an acyclic analogue of the *N*-bisarylmethoxyethyl-*N*′-phenylpropyl piperazine, a GBR-like compound (Choi et al., 1999). In vitro binding data show that this agent strongly inhibits DA reuptake and is also fairly potent at DAT and NET, but not SERT (Johnson, unpublished observation). Nocaine-3B, nocaine-250B (Tamiz et al., 2000), 4-ara-42C, and 5-ara-74A (Kozikowski et al., 1998) are disubstituted piperidines that have a high affinity for the DAT (Dutta et al., 1998), SERT, and also NET (Tamiz et al., 2000). Nocaine-3B binds more strongly to NET than DAT or SERT. Nocaine-250B, however, binds more strongly to SERT than DAT or NET (Tamiz et al., 2000). Both 4-ara-42C and 5-ara-74A are more potent than cocaine at the cocaine site on the DAT. These compounds also strongly inhibit the reuptake of DA on that transporter, with 5-ara-74A being more effective in that respect than 4-ara-42C (Kozikowski et al., 1998).

In order to assess whether any of the above compounds would constitute an effective pharmacotherapeutic candidate against cocaine's addiction, each one was first screened for its intrinsic effect on BSR and also for its ability to alter cocaine-induced reward enhancement, as determined by this paradigm. The relationship between the rate of bar pressing and frequency of stimulation at a set current intensity was analyzed into two behavioral measures: the maximum behavioral output (MAX) and the frequency necessary to sustain half-maximal rate of responding, called the locus of rise (LOR). MAX measures performance and, to some extent, motor output of the animal, and as such is not an effective measure of

reward magnitude. Instead, LOR is a relatively performance-free measure of the reward threshold and is analogous to ED50 in pharmacology (Miliaressis et al., 1986; Volkow et al., 1997; Wise, 1996). Rightward displacements of the rate–frequency curve (increase in LOR from the baseline, no drug condition) are indicative of a decrease in reward (anhedonia) and the leftward shifts (decrease in LOR in comparison to baseline) are reflective of an increase in reward (hedonia). For example, low to intermediate doses of pimozide (an antagonist of the D2 receptor and a potent antipsychotic) shift the rate–frequency curve to the right, thus increasing LOR, high doses also decrease MAX (Stellar et al., 1983). In contrast, amphetamine (e.g., Wise, 1996) and cocaine (e.g., Bauco and Wise, 1997; Maldonado-Irizzary et al., 1994) shift the rate–frequency to the left (decrease LOR) in a dose-dependent fashion, without significantly altering MAX. Thus, the characteristic that makes this behavioral screen unique is that BSR can quantitatively assess progressive changes in rewarding efficacy of any compound alone and in combination with another agent (i.e., cocaine) of interest. Further, the changes in drug reward and performance/motor capacity can be separately evaluated, and finally the hedonically positive, negative, or neutral drug effects can be differentiated.

1. Method

1.1. Subjects and surgery

Adult 300–500 g, male Sprague–Dawley rats (Charles River, CD strain), 4–12/group, were individually housed in standard plastic cages on a 12:12-h reversed day–night cycle (light onset at 7:00 a.m.) in a temperature- (23–25°C) and humidity- (approximately 55%) controlled colony (Maldonado-Irizzary et al., 1994; Stellar et al., 1988). Testing was conducted in the light phase. Animals had free access to food and water at all times, except during the 15-min behavioral evaluation. At the time of surgery, rats were anesthetized with sodium pentobarbital (50 mg/kg, ip), given atropine sulfate (5 mg/kg, sc), and implanted with a monopolar, stainless steel intracranial electrode (Plastics One), aimed at the lateral hypothalamus. The coordinates (bregma as a reference) were: AP: −3.0 mm, ML: −1.6 mm, DV: −7.5 mm (from dura). Skull screws, serving as the electrode ground, together with the electrode were secured to the skull with dental acrylic. Animals were monitored daily and maintained on topical antibiotics (if needed) for several days until recovery was complete. All experimental procedures were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 8523), and Institutional Review Committee for the use of animal subjects (Division of Laboratory Animal Medicine at Northeastern University).

1.2. Behavioral training

Approximately 1 week following surgery, animals were placed inside a 25 × 25 × 25-cm operant chamber fitted with a lever, lever light, and a house light. All subjects were trained on a variable interval 1 (VI-1) schedule of reinforcement to self-stimulate for 0.25-s bursts of square-wave monopolar cathodal pulses at 100 Hz at the current that would give the highest rate of responding without producing any obvious side effects. All animals were found to reliably bar press for rewarding brain stimulation.

Following 1–3 days of training, six stimulation frequencies (158–16 Hz in 0.2 descending steps) were collected in 90-s trials for the next 3 weeks (stabilization) at the optimum current. Each frequency trial begun with a free stimulation burst and was signaled by house light illumination, which was extinguished for 5 s between trials. It took 15 min to collect the rate–frequency curve. Rats were tested daily under baseline conditions until LOR and MAX became stable. Stability was defined as less than 0.1 log Hz variation in LOR and less than a 20% daily variation in MAX with no up or down trend in either statistic over 5 test days. All stimulation and operant controls were delivered by a Stimtek stimulator/microcontroller connected to an IBM XT in network with four Stimteks and one PC. When the initial training was completed, a VI-3 s schedule was imposed with a 0.1-s time-out during receipt of the stimulation burst delivery, while time-out was in effect responses were not counted. This manipulation serves to eliminate false data from spurious stimulation-induced responding. After VI lever pressing had been established, at the same set of frequencies as indicated before, stimulation current and

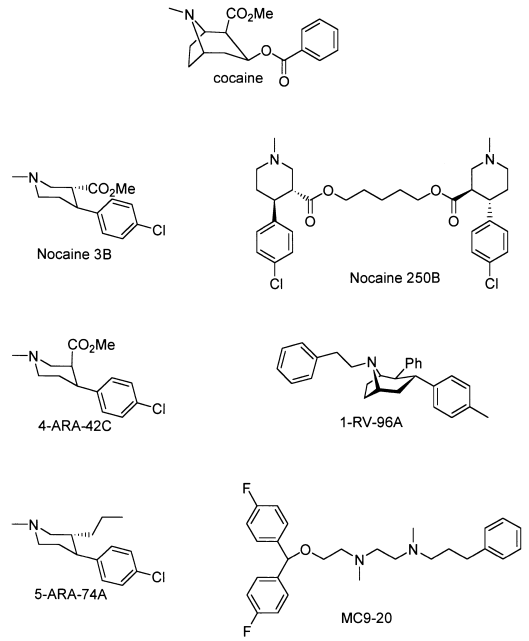

Fig. 1. Chemical structure of cocaine and novel monoamine transporter ligands.

Table 1
In vitro pharmacology (average K_i values (in nM) and S.E.M. values) of the novel monoamine transporter ligands

		Uptake			Selectivity		
	Binding [^{3}H]mazindol	DAT	SERT	NET	SE/DA	NE/DA	NE/SE
Cocaine	375 ± 68	423 ± 150	154.7 ± 0.4	108.0 ± 3.5	0.37	0.26	0.70
Nocaine-250B	63.8 ± 2.8	56.0 ± 4.7	24.9 ± 5.4	182 ± 8.0	0.44	3.3	7.3
Nocaine-3B	248 ± 8	228 ± 30	5880 ± 440	89.7 ± 5.2	26	0.39	0.015
4-ARA-42C	28.0 ± 2.0	69.0 ± 8.1	391 ± 27	87.6 ± 2.9	5.7	1.3	0.22
5-ARA-74A	12.27 ± 0.41	19.6 ± 2.4	228 ± 22	6.53 ± 0.71	12	0.33	0.029
1-RV-96A	0.95 ± 0.2	1.63 ± 0.17	182 ± 11	13.8 ± 1.9	112	8.5	0.08
MC9-20	12.6 ± 0.5	23.3 ± 0.1	562 ± 108	35.2 ± 7.6	24	1.5	0.063

Abbreviations: DAT, dopamine (DA) transporter; SERT, serotonin (SE) transporter; NET, norepinephrine (NE) transporter.

burst duration were adjusted to produce vigorous responding with no signs of aversiveness (turning away from the lever, defecation, and vocalization). Following intraperitoneal administration, cocaine produces statistically significant LOR shifts in the order of 0.15 to 0.40 log units (30–60%), and a shift of 0.1 log units is usually considered to be meaningful (Stellar and Rice, 1989). Thus, when combined with cocaine, a promising candidate should be capable of reducing cocaine-induced reward enhancement from 0.1 to 0.3 log units (20–50%).

1.3. Drug preparation and treatment

Once behavioral stability was achieved cocaine hydrochloride (Sigma-RBI) was dissolved in 0.9% bacteriostatic saline, and each dose of a novel compound was dissolved in 25% propylene glycol and 75% distilled water to circumvent potential solubility problems. All solutions were prepared fresh daily. (Neither saline nor the combination of propylene glycol and distilled water alone produced any significant shifts in the LOR or MAX, $n = 4$). All injections were administered by an intraperitoneal route in a volume of 1 ml/kg of body weight. A dose–response curve was established by administering three to five doses of each compound, with each dose given once in a random order, 5 min prior to testing. The reason for choosing such a short time delay between injection and behavioral testing was to be able to observe nearly immediate drug effects, if present. A washout period of 24 h was imposed between the testing of each dose, during which baseline responding (an evaluation of stability) was reassessed. In some instances when LORs did not return to baseline, an additional 24-h "washout" period was given. Each animal was closely monitored for adverse side effects, such as increased salivation, urination, and hyperactivity. The day following the evaluation of the last dose of a compound, cocaine-induced reward enhancement was assessed 10 min prior to testing by a single injection of 10 mg/kg cocaine hydrochloride, which produced significant leftward shifts in the rate–frequency curve. It should be noted that doses as little as 2 mg/kg have been shown to also potentiate BSR frequency thresholds (Bauco and Wise, 1997). Finally, rats were injected with one of two doses (5 or 10 mg/kg, ip) of the novel compound that had the least effect on baseline LOR and were thus deemed hedonically neutral; 5 min later, 10 mg/kg cocaine hydrochloride was administered. Testing begun 10 min following cocaine injection. The same procedure was repeated 48 h later for the second selected dose of the novel compound. Testing 15 min after the injection of a novel compound mimicked procedures, which were previously shown to be effective in pilot studies (Pimentel et al., 1998; Pimentel et al., 1999).

1.4. Data analysis

The LOR and MAX measures were obtained from each rate–frequency curve by a broken line curve fitting (Camp-

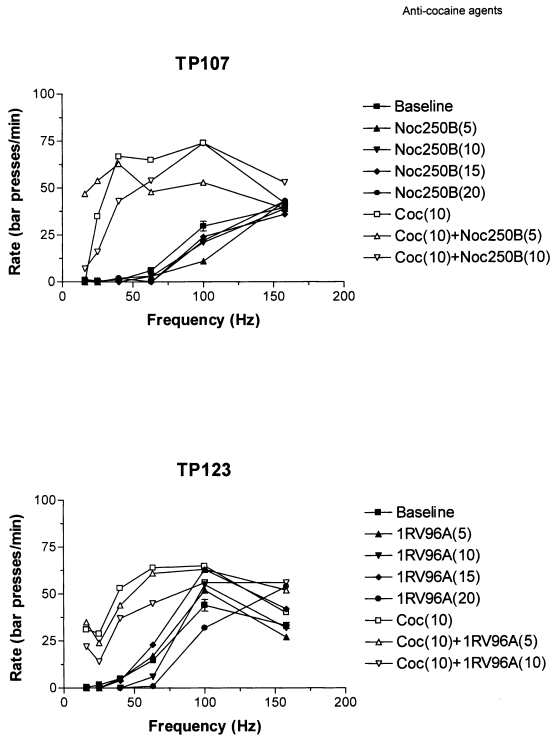

Fig. 2. Representative rate–frequency data of two subjects, TP107 and TP123, that were evaluated under several conditions: baseline (no drug), cocaine (Coc) alone, Novel compound alone: nocaine-250B (Noc-250B) or 1-RV-96A and cocaine plus a novel agent combination. The numbers in parentheses indicate doses of respective compounds in milligrams per kilogram (mg/kg).

bell et al., 1985) program and later reported as "difference from baseline" data, which were derived by subtracting the value obtained for the average baseline value (no drug condition) from the value under the drug condition. The values derived in this manner were analyzed by analysis of variance (ANOVA), with dose and subject as a repeated measure. Significant differences were further examined by performing Tukey's protected t comparisons between relevant experimental groups.

2. Results

Fig. 1 illustrates the chemical structure of the novel compounds and for comparison that of cocaine and Table 1 lists the K_i values for the binding at a mazindol site on the DAT, uptake at each of the three transporters, as well as the selectivity of the novel agents.

Fig. 2 shows representative rate–frequency curves for two animals. In both cases, two of the six novel monoamine transporter ligands did not significantly shift the rate–frequency curves as compared to baseline (except for the highest dose, 20 mg/kg, ip, of 1-RV-96A), indicating that by themselves these agents are neutrally hedonic. Cocaine lead to substantial leftward shifts (decrease in LOR or enhancement of reward), which was significantly counteracted (shift back to baseline) by the coadministration of 10 mg/kg, ip, of either of the novel candidates.

Fig. 3 illustrates derived data, LOR (difference from baseline) as a function of dose for each of the com-

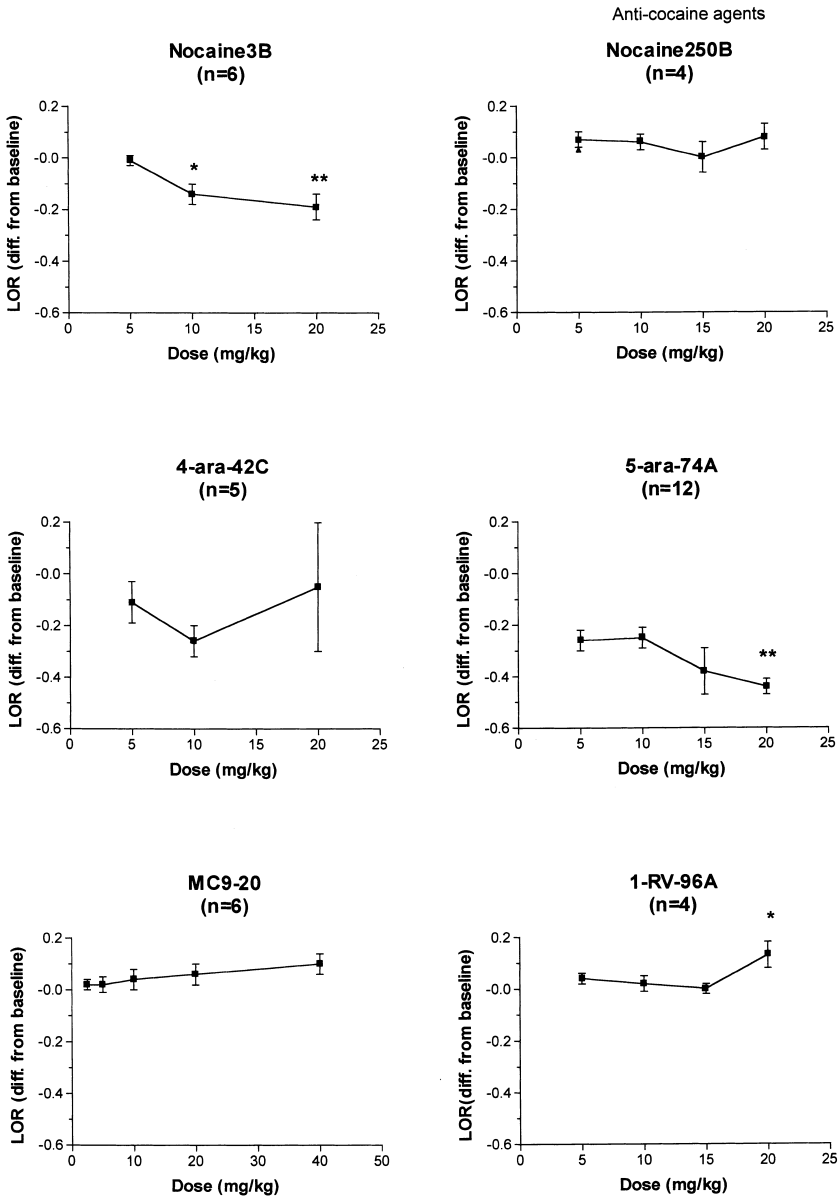

Fig. 3. LOR (difference from baseline) as a function of dose for all six compounds: $*P<.05$, $**P<.01$ different from all.

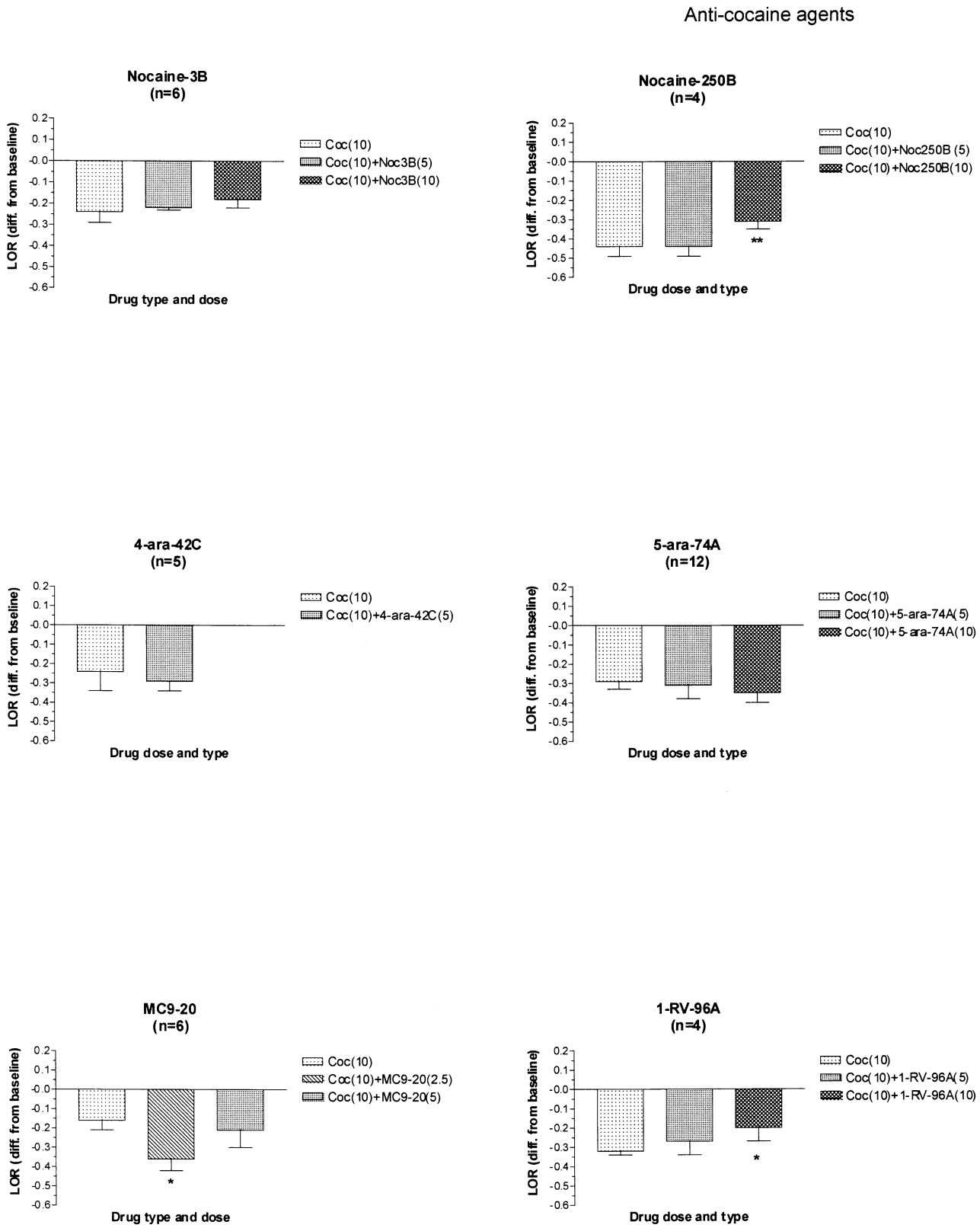

Fig. 4. Effects of cocaine alone (10 mg/kg, ip) in comparison to a combination of cocaine plus selected doses of one of the six novel ligands: $*P<.05$, $**P<.01$ different from all. Numbers in parentheses indicate a given dose in milligrams per kilogram (mg/kg).

pounds. No significant effect of dose on reward threshold (LOR) was found for 4-ara-42C, nocaine-250B (Fig. 2), and MC9-20. However, there was a significant effect of dose for nocaine-3B [$F(2,14)=7.68$, $P<.01$] and 5-ara-

74A [$F(3,33) = 11.53$, $P < .01$], indicating that these compounds significantly enhanced reward (lowered LOR). Post hoc analyses revealed that for nocaine-3B the LOR at 10 and 20 mg/kg ($P < .01$) was significantly more reduced than LOR at 5 mg/kg, for 5-ara-74A, LOR at 20 mg/kg was significantly smaller from 5 and 10 mg/kg ($P < .01$). In contrast, 1-RV-96A (Fig. 2) significantly reduced reward [$F(3,9) = 4.04$, $P < .05$] at the highest dose tested, 20 mg/kg. The LOR was significantly greater at that dose in comparison to 10 and 15 mg/kg.

There were occasional side effects associated with the administration of 5-ara-74A that were not observed for other compounds. These included increased urination within minutes after injection and secretions around the eyes and nose, suggesting a possible cholinomimetic action. In addition, some animals injected with 5-ara-74A required an additional test day, resulting in a 48-h rather than a 24-h time delay between the administration of each dose, in order for the LOR to return to its baseline value.

Cocaine reduced LOR (enhanced reward) from 0.15 to 0.40 log units, as reported previously (Bauco and Wise, 1997; Maldonado-Irizzary et al., 1994; Wise, 1996). When cocaine (10 mg/kg, ip) was combined with 10 mg/kg, but not 5 mg/kg, of either nocaine-250B or 1-RV-96A (Figs. 2 and 4), a significant reduction in cocaine-induced enhancement of reward was observed for 1-RV-96A [$F(2,6) = 5.84$, $P < .05$] and nocaine-250B [$F(2,6) = 15.13$, $P < .01$], respectively (0.13 log units, or 41%, for both compounds). In addition, the LOR produced by the combination of cocaine and 10 mg/kg of either compound was significantly reduced in comparison to the combination of cocaine and 5 mg/kg of either drug ($P < .05$ for 1-RV-96A and $P < .01$ for nocaine-250B). Conversely, a combination of cocaine (10 mg/kg, ip) and MC9-20 (2.5 mg/kg, ip) lead to enhancement of cocaine-induced reward-enhancement [$F(2,8) = 6.34$, $P < .05$] by 0.2 log units or 53%. In contrast, 5 or 10 mg/kg of either nocaine-3B, 4-ara-42C, or 5-ara-74A were ineffective at altering cocaine-induced reward enhancement.

No differences in MAX were noted (either at the individual doses of each compound or when selected doses were combined with cocaine), as exemplified in Fig. 2.

3. Discussion

The present investigation demonstrated that two of six novel monoamine reuptake inhibitors (nocaine-250B and 1-RV-96A) fulfilled the basic behavioral criteria for an effective pharmacotherapeutic candidate, i.e., they significantly reduced (41%) cocaine-induced reward enhancement while possessing minimal hedonic effects when administered alone (with the exception of the highest dose tested of 1-RV-96A, which was slightly negatively hedonic). Thus, with these agents achieving patience compliance could be possible, a characteristic that among the potential therapeutic agents proposed thus far has been lacking (e.g., Carroll et al., 1999; Mendelson and Mello, 1996; Smith et al., 1999). A 41% reduction in cocaine's reward-enhancing effects is substantial since cocaine alone causes leftward shifts in the rate–frequency curve (reduction in LOR) in the order of 30–60%. In addition, thus far, only DA antagonists are able to completely nullify the reward-enhancing effect of psychostimulants (i.e., bring the leftward shifted curve back to baseline) (e.g., Wise, 1996). However, these agents would not constitute good pharmacotherapeutic candidates against cocaine's euphorigenic effects because they tend to produce anhedonia and lead to extrapyramidal side effects (e.g., Stellar and Rice, 1989).

The effects of the screened analogues on LOR changes are difficult to explain based on the activity of the drugs at a single transporter (Table 1). While the available data do not contain sufficient data points to make a strong conclusion about the monoamine selectivity vs. LOR, some general trends can be proposed. In examining the selectivities, there does seem to be a rough correlation between the NET/DAT selectivity and the ability of the compounds to induce a reduction in the LOR (increase reward). For example, the NET/DAT selectivities for hedonically positive compounds, cocaine, nocaine-3B, and 5-ARA-74A lie in the range of 0.26–0.39, while the hedonically neutral compounds, nocaine-250B, 4-ARA-42C, and MC9-20 are in the range of 3.3–1.5. Consistent with this analysis, 1-RV-96A has a NET/DAT selectivity of 8.5 and appears to be anhedonic, but only at the highest dose. These findings appear to support the earlier reports of the effects of other NET-selective agents, mainly tricyclic antidepressants, such as desipramine. For example, chronic (Valentino et al., 1991), but not acute (McCarter and Kokkinidis, 1988; Moreau et al., 1992) treatment with desipramine appears to reduce threshold for BSR (increase reward). It is possible that although those substances that inhibit the reuptake at NET, such as nocaine-3B and 5-ara-74A may potentiate reward when given alone, they do not significantly influence cocaine's reward-enhancing properties. This might be so, perhaps because DAT and NET are already inhibited by cocaine. The behavioral results for 4-ara-42C and 5-ara-74A fit with the in vitro binding data, which showed that 5-ara-74A was more potent than 4-ara-42C at both binding to the mazindol site on the DAT and also inhibiting the reuptake of DA.

Nocaine-250B strongly inhibits the reuptake of serotonin (SE; $K_i = 25$ nM), while nocaine-3B ($K_i = 5880$ nM) is much less potent. Thus, it is possible that some of the reduction in cocaine-induced reward enhancement might be due to the activity of this compound at the SERT, rather than the DAT transporter. Interestingly, inhibition of SERT has been shown to reduce self-administration of psychostimulants, such as D-amphetamine (Leccese and Lyness, 1984; Lyness et al., 1980). In addition, chronic, but not acute pretreatment with fluoxetine (an SE reuptake inhibitor and an antidepressant) appears to block the reward-enhancing effect of chronic amphetamine (Lin et al., 1999a). However, there are some discrepant reports on the effects of chronic treatment with fluoxetine on BSR

thresholds, which indicate either no effect (Lin et al., 1999a) or decrease in reward (Lee and Kornetsky, 1998).

Based on the in vitro binding data, if SERT was uniquely critical in cocaine-induced reward enhancement, then we should expect different results for 1-RV-96A than for nocaine-250B. The former exhibits a significantly greater binding affinity at the DA site on the DAT than the SE site on the SERT, however, its behavioral effects are comparable to that of nocaine-250B, especially when considering the effects on cocaine-induced reward enhancement. Interestingly, the present results may not be surprising, in light of the finding that the potency of the WIN-series compounds, which are highly lipophilic, to induce hyperlocomotion in vivo is unrelated to the in vitro potency of these agents at the DAT (Izenwasser et al., 1994). It is also possible that unlike in vitro, the in vivo distribution of these compounds in the two structures differs, as different pharmacokinetic properties may exist between these compounds. The behavioral results obtained in the BSR screen may however, be explained by the observation that tropanes, such as 1-RV-96A, are more potent than cocaine at the DAT site (Smith et al., 1999). Thus, the reduction in cocaine-induced reward enhancement may be due to 1-RV-96A's ability to effectively displace cocaine from its site on the DAT, while only somewhat reducing DA reuptake.

Another compound, MC9-20, a modified GBR-like molecule, had no significant hedonic effects on its own, yet when combined with cocaine increased cocaine-induced enhancement of reward. Interestingly, cocaine-induced sensitization (a potential index of abuse vulnerability) does not generalize to GBR 12909 (Elmer et al., 1996; Tella et al., 1996). Also, when rats are given a chance to substitute cocaine for GBR 12909, they do not readily do so (Tella et al., 1996). In addition, GBR 12909 and its slowly dissociating, low-efficacy analogues can reduce or delay intravenous self-administration for cocaine in both rats and primates without affecting food intake (Glowa et al., 1996; Lewis et al., 1999). Previous BSR study showed that both GBR 12909 and cocaine enhance reward, yet have different effects on other behavioral measures, such as general activity (Maldonado-Irizzary et al., 1994). The possible explanation for the hedonic behavioral effects is that GBR 12909 and its analogues are lipophilic, and hence are generally less potent in vivo than would be expected by their in vitro binding affinities at the DAT (Bonnet and Costentin, 1986; Kelley and Lang, 1989). Recently, it has also been shown that another GBR-like compound, 4-CI-BZT binds with a strong affinity to the DAT in vitro, yet in vivo it lacks cocaine-like behavioral effects in a drug discrimination paradigm (e.g., Kline et al., 1997).

It is unknown at the present time the degree and rate at which these compounds penetrate the blood–brain barrier in relation to cocaine, their relative distribution within the brain and their onset of action. An extensive behavioral screen will require testing these compounds over a longer time period (extending the duration of a session), as well as screening their effectiveness against varying doses of cocaine. Since an effective pharmacological intervention would be administered to an already addicted individual, assessing the biological and behavioral effects of these novel promising agents following chronic exposure to cocaine is also critical. Indeed, it has been shown that depending on the experimental protocol used and brain regions studied, chronic cocaine administration can lead to either a decrease (e.g., Lee and Kornetsky, 1998; Wilson et al., 1996) or an increase (e.g., Robinson and Berridge, 1993) in the density of the DAT mRNA in the mesocorticolimbic DA system. Such changes could affect the efficacy with which the candidate agents might reduce cocaine-induced reward enhancement.

Further, in the event that these compounds are so effective that in the presence of elevated blood levels of cocaine the pharmacological blockage could still be achieved, another potential problem would surface: a state of a ''pharmacological abstinence''. The reason why this may be problematic is that inducing abstinence or withdrawal does little to control craving for the drug (e.g., Smith et al., 1999; Rothman, 1990). Hence, future search for an effective pharmacotherapeutic agent would also need to address this issue. However, the mechanisms of craving are not well defined (e.g., Altman et al., 1996) and there is little consensus about which experimental paradigm is best suited to measure craving, although the intravenous self-administration/reinstatement model seems to be reliable in this regard (e.g., Erb et al., 1998; Self et al., 1998). Thus, whether candidate pharmacotherapeutic agents, in addition to normalizing the hedonic state and thus enhancing patient compliance, could also reduce craving, as measured by the intravenous self-administration/reinstatement paradigm, remains to be determined.

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References

Alterman AI, O'Brien CP, McLellan AT. Effectiveness and costs of inpatient versus day hospital cocaine. Rehabilitation. J Nerv Ment Dis 1994;182:157–63.

Altman J, Everitt BJ, Galutier S, Markou A, Nutt D, Oretti R, Phillips GD, Robbins TW. The biological, social and clinical bases of drug addiction, commentary and debate. Psychopharmacology (Berlin) 1996;125: 285–345.

Backus LI, Stellar JR, Jacob J, Hesse GH, Shashoua VE. Novel GABA agonists depress the reward effect of lateral hypothalamic stimulation in rats. Pharmacol, Biochem Behav 1988;30:657–63.

Bauco P, Wise RA. Synergic effects of cocaine with lateral hypothalamic brain stimulation reward: lack of tolerance or sensitization. J Pharmacol Exp Ther 1997;283:1160–7.

Boja JW, Markham L, Patel A, Uhl G, Kuhar MJ. Expression of a single dopamine transporter cDNA can confer two cocaine binding sites. NeuroReport 1992;3:247–8.

Bonnet J-J, Costentin J. GBR 12783, a potent and selective inhibitor of dopamine uptake: biochemical studies in vivo and ex vivo. Eur J Pharmacol 1986;121:199–209.

Campbell KA, Evans G, Gallistel CR. A microcomputer-based method for physiologically interpretable measurement of the rewarding efficacy of brain stimulation. Physiol Behav 1985;35:395–403.

Carroll FI, Lewin AH, Boja JW, Kuhar MJ. Cocaine receptor: biochemical characterization and structure–activity relationship of cocaine analogues at the dopamine transporter. J Med Chem 1992;35:969–81.

Carroll FI, Howell LL, Kuhar MJ. Pharmacotherapies for treatment of cocaine abuse: preclinical aspects. J Med Chem 1999;42:2721–36.

Choi S-W, Elmaleh D, Hanson RN, Fischman AJ. Design, synthesis, and biological evaluation of novel non-piperazine analogues of 1-[2-(diphenylmethoxy)ethyl]- and 1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl)piperazine as dopamine transporter inhibitors. J Med Chem 1999;42:3647–56.

Dewey SL, Morgan AE, Ashby CR, Horan B, Kushner SA, Loga J, Volkow ND, Fowler JS, Gardner EL, Brodie JD. A novel strategy for the treatment of cocaine addiction. Synapse 1998;30:119–29.

Dutta AK, Coffey LL, Reith MEA. Potent and selective ligands for the dopamine transporter (DAT): structure–activity relationship studies of novel 4-[2-(diphenylmethoxy)ethyl]-1](3-phenylpropyl)piperidine analogues. J Med Chem 1998;41:699–705.

Elmer GI, Brockington DA, Gorelick FI, Carol KC, Rice KC, Matecka D, Goldberg SR, Rothman RB. Cocaine cross-sensitization to dopamine uptake inhibitors: unique effects of GBR-12909. Pharmacol, Biochem Behav 1996;53:911–8.

Erb S, Shaham Y, Stewart J. The role of corticotropin-releasing factor and corticosterone in stress- and cocaine-induced relapse to cocaine seeking in rats. J Neurosci 1998;18:5529–36.

Gallistel CR. Determining the quantitative characteristics of a reward pathway. In: Church RM, Commons ML, Stellar JR, Wagner AR, editors. Biological determinants of reinforcement. Hillsdale, NJ: Lawrence Erlbaum Associates, 1987. pp. 1–30.

Gawin FH. Cocaine addiction: psychology and neurophysiology. Science 1991;251:1580–6.

Glowa JR, Fantegrossi WE, Lewsi DB, Matecka D, Rice KC, Rothman RB. Sustained decrease in cocaine-maintained responding in rhesus monkeys with 1-[-2-[bis(4-fluorophenyl)methoxy]ethyl]-4-(-3-hydroxy-3-phenylpropyl)piperazinyl decanoate, a long-acting ester derivative of GBR 12909. J Med Chem 1996;39:4689–91.

Glowa JR, LeSage M, Stafford D, Vocci F. Novel pharmacotherapies for cocaine abuse — 1995 to present. Expert Opin Ther Pat 1997;7:1459–76.

Izenwasser S, Terry P, Heller B, Witkin JM, Katz JL. Differential relationship among dopamine transporter affinities and stimulant properties of various uptake inhibitors. Eur J Pharmacol 1994;263:277–83.

Johnson KM, Bergmann J, Kozikowski AP. Cocaine and amphetamine differentially protect [^{3}H]mazindol binding sites from alkylation by *N*-ethylmaleimide. Eur J Pharmacol, Mol Pharmacol Ser 1992;227:411–5.

Kelley AE, Lang CG. Effects of GBR 12909, a selective dopamine uptake inhibitor, on motor activity and operant behavior in the rat. Eur J Pharmacol 1989;167:385–95.

Kitayama S, Shimada S, Xy H, Markham L, Donovan DM, Uhl GR. Dopamine transporter site-directed mutations differentially alter substrate transport and cocaine binding. Proc Natl Acad Sci 1992;89:7782–5.

Klein M. Research issues related to development of medications for treatment of cocaine addiction. Ann NY Acad Sci 1998;844:75–91.

Kline RH, Izenwasser S, Katz JL, Joseph DB, Bowen WD, Newman AH. 3′-Chloro-3a-(diphenylmethoxy)tropane, but not 4′-chloro-3a-(diphenylmethoxy)tropane produces a cocaine-like behavioral profile. J Med Chem 1997;40:851–7.

Kozikowski AP, Araldi GL, Boja J, Meil WM, Johnson KM, Flippen-Anderson JL, George C, Saiah E. Chemistry and pharmacology of the piperidine-based analogues of cocaine. Identification of potent DAT inhibitors lacking the tropane skeleton. J Med Chem 1998;41:1962–9.

Leccese AP, Lyness WH. The effects of putative 5-hydroxytryptamine receptor active agents on D-amphetamine self-administration in controls and rats with 5,7-dihydroxytryptamine medial forebrain bundle lesions. Brain Res 1984;303:153–62.

Lee K, Kornetsky C. Acute and chronic fluoxetine treatment decreases the sensitivity of rats to rewarding brain stimulation. Pharmacol, Biochem Behav 1998;60:539–44.

Lewis DB, Matecka D, Zhang Y, Hsin L-W, Dersch CM, Stafford D, Glowa RB, Rothman RB, Rice KC. Oxygenated analogues of 1-[2-(diphenylmethoxy)ethyl]- and 1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl)piperazine (GBR 12935 and GBR 12909) as potential extended-action cocaine-abuse therapeutic agents. J Med Chem 1999;42:5029–42.

Lin D, Koob GF, Markou A. Differential effects of withdrawal from chronic amphetamine or fluoxetine administration on brain stimulation reward in the rat-interactions between the two drugs. Psychopharmacology (Berlin) 1999a;145:283–94.

Lin Z, Wang W, Kopajtic T, Revay S, Uhl GR. Dopamine transporter: transmembrane phenylalanine mutations can selectively influence dopamine update and cocaine analog recognition. Mol Pharmacol 1999b;56:434–47.

Lyness WH, Friedle NM, Moore KE. Increased self-administration of D-amphetamine after destruction of 5-hydroxytryptaminergic neurons. Pharmacol, Biochem Behav 1980;12:937–41.

Maldonado-Irizzary CS, Stellar JR, Kelley AE. Effects of cocaine and GBR-12909 on brain stimulation reward. Pharmacol, Biochem Behav 1994;48:915–20.

McCarter BD, Kokkinidis L. The effects of long-term administration of antidepressant drugs on intracranial self-stimulation responding in rats. Pharmacol, Biochem Behav 1988;31:243–7.

Mendelson JH, Mello NK. Management of cocaine abuse and dependence. N Engl J Med 1996;334:965–72.

Miliaressis E, Rompre P-P, Laviolette LP, Philippe L, Coulombe D. The curve–shift paradigm in self-stimulation. Physiol Behav 1986;37:85–91.

Moreau JL, Jenck F, Martin JR, Mortas P, Haefely WE. Antidepressant treatment prevents chronic unpredictable mild stress-induced anhedonia as assessed by ventral tegmentum self-stimulation behavior in rats. Eur Neuropsychopharmacol 1992;2:43–9.

SAMHSA. *Preliminary Results From the 1997 National Household Survey on Drug Abuse.* DHHS Pub. No. (SMA) 98-3200. Rockville, MD: Substance Abuse and Mental Health Services Administration, 1998. Also available electronically at www.samhsa.gov or at www.health.org, pp. 9–14.

Newman AH, Allen AC, Izenwasser S, Katz JL. Novel 3a-(diphenylmethoxy)tropane analogs: potent dopamine uptake inhibitors without cocaine-like behavioral profiles. J Med Chem 1994;37:2258–61.

Pimentel P, Crowell A, Stellar JR, Hanson R, Choi SW, Elmaleh D, Babich J, Kozikowski AP. Testing novel anti-cocaine drugs MC9-20 and nocaine-B with a rate–frequency brain stimulation reward enhancement paradigm. Soc Neurosci Abstr 1998;24:957.

Pimentel P, Trzcińska M, Crowell A, Stellar JR, Zhang J-R, Kozikowski AP, Smith M, Babich JW. Effects of novel piperidines on cocaine-induced enhancement of reward — Brain Stimulation Reward Study. Soc Neurosci Abstr 1999;25:1873.

Prakash KRC, Tamiz AP, Araldi GL, Zhang M, Johnson KM, Kozikowski AP. *N*-Phenylalkyl-substituted tropane analogs of boat conformation with high selectivity for the dopamine versus serotonin transporter. Bioorg Med Chem Lett 1999;9:3325–8.

Ritz MC, Lamb RJ, Goldberg RJ, Kuhar SR. Cocaine receptors on dopamine transporter are related to self-administration of cocaine. Science 1987;237:1219–23.

Robinson TE, Berridge KC. The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res Rev 1993;18:247–91.

Rothman RB. High affinity dopamine reuptake inhibitors as potential co-

caine antagonists: a strategy for drug development. Life Sci 1990;46: PL17–21.

Self DW, Genova LM, Hope BT, Barnhart WJ, Spencer JJ, Nestler EJ. Involvement of cAMP-dependent protein kinase in the nucleus accumbens in cocaine self-administration and relapse of cocaine-seeking behavior. J Neurosci 1998;18:1848–59.

Simoni D, Stoelwinder J, Kozikowski AP, Johnson KM, Bergmann JS, Ball RG. Methoxylation of cocaine reduces binding affinity and produces compounds of differential binding and dopamine uptake inhibitory activity: discovery of a weak cocaine "antagonist". J Med Chem 1993;36: 3975–7.

Smith MP, Hoepping A, Johnson KM, Trzcińska M, Kozikowski AP. Dopaminergic agents for the treatment of cocaine abuse. Drug Discovery Today 1999;4:322–32.

Stellar JR, Rice MB. Pharmacological basis of intracranial self-stimulation reward. In: Liebman JM, Cooper SJ, editors. The pharmacological basis of reward. Oxford: Clarendon Press, 1989. pp. 14–65.

Stellar JR, Kelley A, Corbett D. Effects of peripheral and central dopamine blockage on lateral hypothalamic self-stimulation: evidence for both reward and motor deficits. Pharmacol, Biochem Behav 1983;18:433–42.

Stellar JR, Waraczynski M, Bruno JP. Neonatal dopamine depletions spare lateral hypothalamic stimulation reward in adult rats. Pharmacol, Biochem Behav 1988;30:365–70.

Tamiz AP, Zhang J, Zhang M, Wang CZ, Johnson KM, Kozikowski AP. Application of the bivalent ligand approach to the design of novel dimeric serotonin reuptake inhibitors. J Am Chem Soc (submitted for publication).

Tella SR, Landenheim B, Andrews AM, Goldberg SR, Cadet JL. Differential reinforcing effects of cocaine and GBR-12909: biochemical evidence for divergent neuroadaptive changes in the mesolimbic dopaminergic system. J Neurosci 1996;16:7416–27.

Valentino DA, Riccitelli AJ, Dufresne RL. Chronic DMI reduces thresholds for brain stimulation reward in the rat. Pharmacol, Biochem Behav 1991;39:1–4.

Vaughan RA, Kuhar MJ. Dopamine transporter ligand binding domains. Structural and functional properties revealed by limited proteolysis. J Biol Chem 1996;271:21672–80.

Volkow ND, Wang GJ, Fischman MW, Foltin RW, Fowler JS, Abumrad NN, Vitkun S, Logan J, Gatley SJ, Pappas N, Hitzemann R, Shea CE. Relationship between subjective effects of cocaine and dopamine transporter occupancy. Nature 1997;386:827–30.

Wayment H, Meiergerd FM, Schenk JO. Relationship between catechol substrate binding and amphetamine, cocaine and mazindol binding sites in a kinetic model of the striatal transport of dopamine in vitro. J Neurochem 1998;70:1941–9.

Wilson J, Kish M. The vesicular monoamine transporter, in contrast to the dopamine transporter, is not altered by chronic cocaine self-administration in the rat. J Neurosci 1996;16:3507–10.

Wise RA. Addictive drugs and brain stimulation reward. Annu Rev Neurosci 1996;19:319–40.

Woolverton WL, Johnson KM. Neurobiology of cocaine abuse. Trends Pharmacol Sci 1992;13:193–200.