Pharmacology Biochemistry and Behavior, Vol. 57, No. 3, pp. 505–512, 1997

0091-3057/97 $17.00 + .00

ELSEVIER

PII S0091-3057(96)00441-8

Relations Between Heterogeneity of Dopamine Transporter Binding and Function and the Behavioral Pharmacology of Cocaine

JONATHAN L. KATZ, AMY H. NEWMAN AND SARI IZENWASSER

Psychobiology Section, NIDA Addiction Research Center, National Institutes of Health, Baltimore, MD 21224

KATZ, J. L., A. H. NEWMAN AND S. IZENWASSER. *Relations between heterogeneity of dopamine transporter binding and function and the behavioral pharmacology of cocaine.* PHARMACOL BIOCHEM BEHAV **57**(3) 505–512, 1997.— Both in vitro binding studies and studies of dopamine uptake have indicated that there is a heterogeneity of action of cocaine and cocaine analogs. Both high- and low-affinity binding sites have been identified. Some drugs that bind to the dopamine transporter show both high- and low-affinity components whereas others do not. Behavioral studies have indicated that the high-affinity component appears to be the one most directly involved in the actions of cocaine related to abuse. These conclusions are based on correlations of affinities and psychomotor stimulant effects. In addition, tolerance to the psychomotor stimulant effects of cocaine occurs with a concomitant change in only the high-affinity component for dopamine uptake. Certain dopamine uptake inhibitors may have only actions mediated by the low-affinity component. These drugs bind to the dopamine transporter and inhibit dopamine uptake; however, they do not have behavioral effects like those of cocaine. This finding is a critical point of inquiry for the dopamine hypothesis because, based on the neurochemical data, these drugs should have behavioral actions like those of cocaine. In contrast, some of these drugs antagonize the behavioral effects of cocaine, suggesting that the low-affinity site somehow modulates the actions mediated by the high-affinity site. Recently, some benztropine analogs have been discovered that bind to the dopamine transporter and inhibit dopamine uptake monophasically but have behavioral effects that are dissimilar to those of cocaine. These compounds may prove useful in determining the behavioral significance of heterogeneity of actions at the dopamine transporter. Further, these studies may provide leads to novel therapeutics for the treatment of cocaine abuse. © 1997 Elsevier Science Inc.

Cocaine Dopamine Drug effects Heterogeneity of binding Two-state model Uptake transport

AS DESCRIBED elsewhere in this special issue, the actions of dopamine as a neurotransmitter are terminated by transport of released transmitter back into the presynaptic cell. Ligand binding techniques have shown that cocaine has affinity for the dopamine transporter protein, and functional assays have shown that cocaine increases concentrations of labeled dopamine in presynaptic terminals. Further, the behavioral effects of cocaine appear to be mediated by this activity at the dopamine transporter, although cocaine also inhibits the uptake of norepinephrine and serotonin. Kuhar, Ritz, and colleagues (36) examined the relationship between the affinity of cocaine and several related drugs for the various monoamine uptake sites and reinforcing effects. The potency of these drugs to maintain behavior in self-administration procedure was compared to the affinity of the drugs in displacement of labeled mazindol from transporters of dopamine (striatum) or norepinephrine (frontal cortex) and in displacement of paroxetine from serotonin transporters (brain stem). A direct relationship was found between the potency to inhibit the binding to the dopamine transporter and the potency with which these drugs maintain self-administration behavior. In addition, the relationship was stronger for the affinity of these drugs at the dopamine transporters than it was for the others. From this result, Kuhar and his colleagues argued that binding to the dopamine transporter was the important action for the reinforcing effects in abuse of cocaine. This correlation has been reported for other behavioral effects of cocaine, such as the psychomotor stimulant effects on locomotor activity [(7); but see also (25,37,44)] and the stimulation of operant behavior (42).

A number of recent studies have shown that there is a heterogeneity in the actions of some drugs at the dopamine transporter. For example, Madras and her colleagues (30) have found that the binding of cocaine is better described by a two-site model than by a one-site model. In addition, the binding of the cocaine analog WIN 35,428 has also been reported to

Requests for reprints should be addressed to Jonathan L. Katz, Intramural Research Program, Psychobiology Section, NIH NIDA, PO Box 5180, Baltimore, MD 21224.

be better fit by a two-site model than by single-site models (23,31). Further, this heterogeneity of binding appears to be due to more than one binding site on the dopamine transporter, as opposed to binding to other distinct receptors, because the binding of WIN 35,428 to the cloned dopamine transporter is better described as two sites than as one site (4). It has also been shown that mazindol and GBR 12935 may bind to a site on the dopamine transporter that is different from the site for cocaine binding (3), further suggesting the presence of more than one binding site.

In addition, functional assays of drug actions at the dopamine transporter have also indicated some heterogeneity in the inhibition of dopamine transport produced by cocaine. Most often, inhibition of dopamine uptake is assessed by measuring accumulation of radiolabeled dopamine in synaptosomal preparations. Under these conditions, the inhibition of uptake by cocaine and other drugs is monophasic [e.g., (12)]. However, when uptake is assessed in a chopped tissue preparation in which the tissue is left in a relatively more intact state compared with synaptosomes, the inhibition of dopamine uptake produced by cocaine and some of its analogs can be resolved into two components (24). Figure 1 shows uptake inhibition produced by cocaine in striatal synaptosomes (open symbols) and in the chopped tissue preparation (filled symbols). The right panel shows these studies on tissue from nucleus accumbens. These data show two distinct components to the curve for the chopped tissue preparation, which result in a significant nonlinear fit to the concentration–response curve. In contrast, in the synaptosomes, there is only one linear component. In striatal tissue (left panel), the difference between the two preparations is expressed as a curve that is broader for the chopped tissue than for the synaptosomes. Thus, heterogeneity of action at the dopamine transporter also can be obtained in a functional assay. The relation between the binding heterogeneity and the two components of uptake inhibition observed in the functional assay is an important area of research that needs to be fully explored.

The significance of the heterogeneity of interactions of drugs with dopamine transport for drug abuse and the behavioral pharmacology of cocaine has not been explored. The purpose of this paper is to examine those implications to the extent possible with the currently available research findings. This is an emerging story; however, at this stage, results are sufficient to at least suggest that heterogeneity of actions at the dopamine transporter appears to have a behavioral significance that may have implications for how cocaine and its congeners produce their behavioral effects and may explain why some dopamine uptake inhibitors are subject to abuse while others are not. Finally, this information may also provide leads for the development of useful treatments for cocaine abuse.

HIGH-AFFINITY COMPONENT

As indicated above, correlations between the affinity of ligands for the dopamine transporter and potency have been reported for several behavioral effects, most notably reinforcing effects (36). Studies by Heikkila and his colleagues (16–19) were among the first to examine this relationship. Both stimulation of locomotor activity and rotation in unilaterally 6-OHDA-treated animals were found to be related to the dopamine uptake-inhibiting effects of cocaine and phenyltropane congeners, analogs of mazindol, and a series of dialkylpiperazines (GBR 12909 and analogs). These previous results were further supported by Cline et al. (7), who found that the potency to produce stimulation of locomotor activity in mice by a limited number of cocaine analogs was directly related to the in vivo binding affinities. Similar results were reported for the relation between in vitro binding affinity and psychomo-

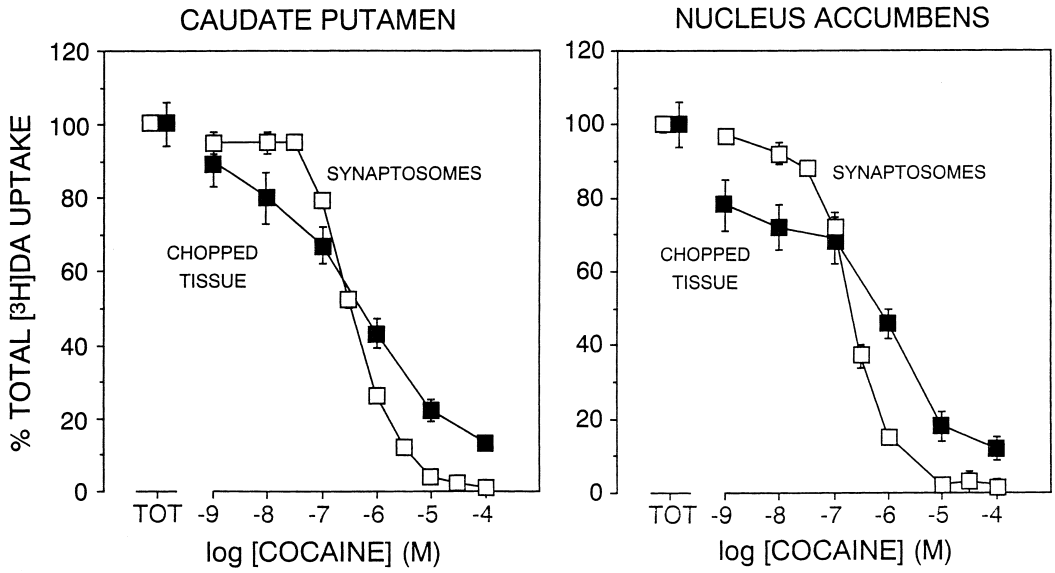

FIG. 1. Effects of increasing concentrations of cocaine on [^{3}H]dopamine uptake in chopped tissue slices (filled symbols) and synaptosomes (open symbols) prepared from rat striatum (left) and nucleus accumbens (right). Uptake is expressed as percent of total uptake (in the absence of drug). Values represent mean ± SEM from triplicate samples in each of *n* independent experiments. Sample sizes: striatum: slices ($n = 9$), synaptosomes ($n = 3$); nucleus accumbens: slices ($n = 7$), synaptosomes ($n = 3$). Mean total [^{3}H]dopamine uptake values for these experiments, expressed as pmol/mg protein: striatum: slices (1.5 ± 0.4), synaptosomes (19.4 ± 0.9); nucleus accumbens: slices (0.7 ± 0.1), synaptosomes (2.0 ± 0.1). [Adapted from Izenwasser et al. (24).]

tor stimulant effects as assessed by stimulation of operant behavior in squirrel monkeys (42).

Vaugeois et al. (44) further examined the relationship between activity of a number of dopamine uptake inhibitors at the dopamine transporter and psychomotor stimulant effects. In that study, the ED_{50} doses for displacement of [^{3}H]GBR 12783 from striatal membranes in vivo were assessed, at the time of maximal occupancy, for their stimulant effects on locomotor activity. Whereas some of the dopamine uptake inhibitors produced large increases in locomotor activity, some were without effects. The authors suggested that the drugs should have produced comparable degrees of activation at these doses if their actions are due exclusively to the degree of transporter occupancy produced.

Several studies by Rothman and colleagues support the notion that there are differences in activity among dopamine uptake inhibitors at comparable occupancies. In those studies, either locomotor activation or level of extracellular dopamine was assessed. Initial observations were that the increase in striatal extracellular levels of dopamine produced by cocaine were of a smaller magnitude in subjects previously treated with GBR 12909 (38,39). Those findings were replicated in subsequent studies of a wider range of doses examining dopamine levels in the nucleus accumbens (2). In other studies, Rothman and colleagues (37) suggested that comparable locomotor stimulant and stereotypy-inducing effects of cocaine and GBR 12909 were obtained at doses that produced different levels of transporter occupancy. Despite these results suggesting differences in intrinsic activity of cocaine and GBR 12909 in vivo, in vitro studies failed to confirm a pharmacology of GBR 12909 that resembled that of a partial agonist (14). Further, the in vivo findings of Vaugeois et al. (44), albeit using only analogs of GBR 12909, were different from those of Rothman and colleagues. In the Vaugeois et al. study, GBR 12783 and GBR 13069 produced greater locomotor stimulation than did cocaine at the ED_{50} doses for in vivo displacement of [^{3}H]GBR 12783.

Izenwasser et al. (25) also examined actions at the dopamine transporter and locomotor stimulant effects. In that study, the in vitro binding affinities of a series of uptake inhibitors were related to the ED_{50} value for stimulation of locomotor activity in mice. As discussed above, binding to the dopamine transporter can be characterized by a two-site model, therefore this study determined affinities of each displacer drug for both the [^{3}H]WIN 35,428 high- and low-affinity binding sites, if the two-site model fit better than a one-site model. In general, the correlation among affinities and behavioral ED_{50} values was higher when the values for the high-affinity site were used, compared with an overall affinity (using a one-site model) or the values for the low-affinity site. In addition, there was a marked difference between the relationship of affinity and behavioral ED_{50} value for analogs of cocaine and the dopamine uptake inhibitors that were not congeners of cocaine. Indeed, the relationship between binding affinity and behavioral ED_{50} values for the structurally distinctive drugs was weak. These results suggest that the interaction between dopamine uptake inhibitors and the transporter may be fundamentally different for some of the drugs, and suggest, as in the study by Vaugeois et al. (44), that the translation of action at the transporter to behavior may not depend solely on transporter occupancy.

The heterogeneity of interactions of drugs with the dopamine transporter has also been supported by results of studies of chronic cocaine treatment. In one study (21), rats were exposed to cocaine via osmotic minipumps over a period of 7 days at a dose of 50 mg/kg/day. Figure 2 shows the results on the inhibition of dopamine uptake in both striatum (left panel) and nucleus accumbens (right panel). The rats exposed

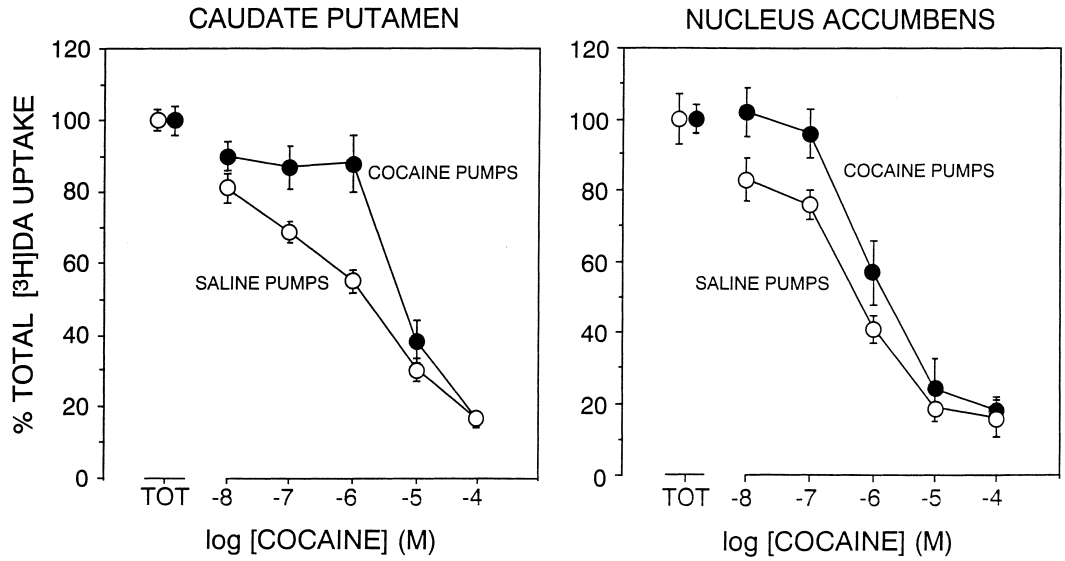

FIG. 2. Effects of increasing concentrations of cocaine on [^{3}H]dopamine uptake in chopped striatum (left) or nucleus accumbens (right) from animals who had been treated with either continuous cocaine infusions (filled symbols) or saline (open symbols). Uptake is expressed as percent of total [^{3}H]dopamine for each treatment group. Values represent pooled mean ± SEM from triplicate samples in each of n independent experiments. Sample sizes: striatum: saline (n = 9), cocaine (n = 5); nucleus accumbens: saline (n = 7), cocaine (n = 5). Control values for mean total [^{3}H]dopamine uptake, expressed as pmol/mg protein: striatum: saline (1.47 ± 0.37), cocaine (1.41 ± 0.39); nucleus accumbens: saline (0.73 ± 0.11), cocaine (0.69 ± 0.21). [Adapted from Izenwasser and Cox (21).]

to saline (open points) showed a biphasic uptake inhibition curve, as detailed above. Rather than shifting the cocaine dose–effect curve to the right in a parallel manner, exposure to cocaine (filled points) changed the shape of the curve. The rats exposed to cocaine showed an essentially monophasic curve that appeared to lack the high-affinity component of the biphasic curve obtained in the saline-treated subjects. Thus, it appears from these results that the high-affinity component of the dopamine uptake inhibition produced by cocaine could be regulated independently of the low-affinity component.

The continuous exposure produced by the osmotic minipumps also has been reported to have behavioral effects. For example, Reith et al. (35) found decreases in the stimulation of locomotor activity in mice exposed to cocaine via osmotic minipumps. This tolerance to the stimulant effects of cocaine may correspond to the selective elimination of the high-affinity component of cocaine's inhibition of dopamine uptake observed by Izenwasser and Cox (21). Indeed, studies of locomotor activation and uptake inhibition in rats exposed to cocaine via osmotic minipumps showed the selective elimination of the high-affinity component after cocaine exposures that produced partial tolerance to the locomotor activating effects of cocaine (20).

Correlations between the affinity of ligands for the dopamine transporter and potency also have been suggested for the discriminative stimulus effects of dopamine uptake inhibitors (8), although extensive studies have not been reported. One recent study (22) of the behavioral effects of meperidine may relate to the behavioral implications of heterogeneity of action at the dopamine transporter. Meperidine is an atypical opioid agonist with some stimulant effects, and it has structural characteristics resembling those of the phenyltropane analogs of cocaine. Meperidine has established activity as an inhibitor of serotonin uptake (5), and in the present study inhibited [^{3}H]dopamine uptake in chopped rat caudate putamen. However, meperidine differed from cocaine in that its effects could be characterized as having predominantly a single component that corresponded closely with the high-affinity component of cocaine's actions. Morphine was not active in inhibiting [^{3}H]dopamine uptake, indicating that the effect of meperidine was not indirectly initiated via a classic μ-opioid agonist action. Further, meperidine but not morphine displaced [^{3}H]WIN 35,428 binding. These data suggest that some activity of meperidine might be due to direct activity at the dopamine transporter mediated by the high-affinity component of the inhibition of dopamine transport.

In behavioral studies, squirrel monkeys were trained to discriminate injections of 0.3 mg/kg cocaine from saline. Previous studies had established the pharmacological specificity of this behavioral performance in rats [e.g., (1,9,45)] and primates (27,28,41). As in the previous studies, cocaine produced a dose-related substitution, with maximal substitution occurring at the training dose. Neither morphine (open triangles) nor meperidine (open circles) substituted for cocaine (Fig. 3). However, in monkeys pretreated with 0.1 mg/kg naltrexone, meperidine produced cocaine-like discriminative stimulus effects completely substituting in all monkeys (filled circles), whereas morphine in combination with naltrexone still did not substitute for cocaine (filled triangles). The results with naltrexone indicate that an opioid action that disrupted behavior was interfering with the expression of cocaine-like discriminative stimulus effects in the meperidine-treated subjects. When this opioid effect was antagonized by naltrexone, as evidenced by a shift to the right in the effects of meperidine on response rates (Fig. 3; lower panel), behavior was restored and the cocaine-like activity was revealed. This study suggests that the dopamine uptake inhibition produced by meperidine, rather than its opioid actions, was responsible for the cocaine-like discriminative stimulus effects and, further, suggests that the high-affinity component of cocaine's effects may be critical for the production of the subjective effects of cocaine on which the discriminative stimulus effects are based.

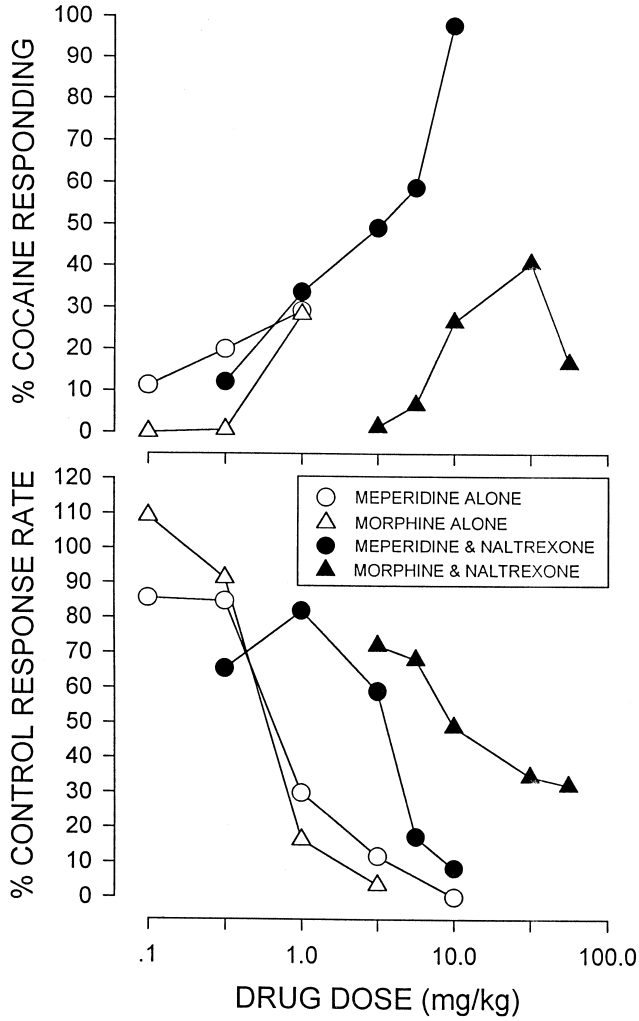

FIG. 3. Substitution of meperidine and morphine, alone and in combination with 0.1 mg/kg naltrexone, for cocaine in squirrel monkeys trained to discriminate cocaine (0.3 mg/kg) from saline. Abscissae: drug dose in mg/kg. Ordinates for upper panel: percentage of responses on the cocaine-appropriate lever. Ordinates for lower panel: response rate as a percentage of the rate obtained after saline injections. Open points: effects of meperidine (circles) or morphine (triangles) when administered alone; filled points: effects of the two drugs when administered with naltrexone (0.1 mg/kg). The average percentages of cocaine-appropriate responses during training sessions in which saline or cocaine was administered were 1.89 ± 3.21 or 98.78 ± 2.12, respectively. The average response rate (in responses per second) during training sessions in which saline or cocaine was administered were 3.33 ± 0.81 or 1.89 ± 0.60, respectively. Each point represents the effect in five or six squirrel monkeys. Note that neither drug substituted for cocaine when administered alone, whereas meperidine substituted for cocaine in subjects also treated with naltrexone. [Adapted from Izenwasser et al. (22).]

LOW-AFFINITY COMPONENT

The lack of drugs selective for each of the components has impeded any attempts to characterize their behavioral significance. Currently, there are no compounds that can differentially label high- and low-affinity binding sites, and there are no compounds that have been shown conclusively to inhibit dopamine uptake only through a low-affinity component. However, several compounds characterized as sigma ligands inhibit dopamine uptake in a monophasic manner, have relatively low potency in doing so, and have relatively low affinity for the dopamine transporter in studies of displacement of [^{3}H]WIN 35,428 (23).

Several results suggest a concordance among structures of drugs that bind to sigma receptors and those that bind to the dopamine transporter. Cocaine has been shown to bind to sigma receptors at high concentrations (40). The photoaffinity label [^{125}I]iodoazido-cocaine recognizes both high- and low-affinity cocaine binding sites (26). However, when the compound is photoactivated, it derivatizes a polypeptide that has the pharmacology of a sigma receptor and a 26-kDa molecular mass, which differs from that of the dopamine transporter (15). Finally, GBR 12909, a compound selective among monoamine transporters for the dopamine transporter, also has relatively high affinity for sigma receptors (10). All of these results together suggest an overlap of structural requirements for sigma- and cocaine-binding sites.

Interestingly, although some sigma ligands inhibit dopamine uptake (presumably through actions at the dopamine transporter), in behavioral studies sigma ligands generally are not locomotor stimulants. For example, in one study (32) the sigma ligands rimcazole, haloperidol, (+)-3-PPP, and BMY 14802 only depressed locomotor activity. This depression of activity occurred despite activity at the dopamine transporter, as indicated in the study by Izenwasser et al. (23). Thus, these drugs represent a group of compounds that bind to the dopamine transporter and inhibit dopamine uptake, yet do not have behavioral effects like those of cocaine and are not abused. The lack of cocaine-like behavioral activity runs counter to what would be expected based on the dopamine hypothesis of the reinforcing actions of cocaine (29). It is possible that the cocaine-like behavioral effects of these compounds that would be imparted by their actions at the dopamine transporter are mitigated by other activities, such as sigma-mediated actions. In fact, most of the sigma ligands studied by Izenwasser et al. had higher affinity at sigma receptors than they did at the dopamine transporter. However, rimcazole, which has been suggested to be a highly selective sigma ligand (13), had higher affinity at the dopamine transporter than at sigma receptors. Thus, it is also possible that sigma activity is not responsible for the lack of stimulant effects.

One interesting aspect of several sigma receptor ligands is their activity in combination with cocaine. Menkel et al. (32) showed that rimcazole and BMY 14802 were capable of antagonizing the locomotor stimulant effects of cocaine in mice. This antagonist effect occurred at doses that were without effects when given alone; thus, the antagonism was not merely a due to the locomotor depressant actions of these drugs precluding stimulation by cocaine. Similar effects of another selective sigma ligand, DuP 734, were reported in an abstract (11). In addition, that abstract reported antagonism of the discriminative stimulus effects of cocaine by DuP 734. Because some sigma ligands have affinity for the dopamine transporter (23), it is possible that the interactions of sigma receptor ligands with cocaine are due to actions at a common site rather than their activity at sigma receptors.

If in fact selected sigma ligands, such as rimcazole, are interacting with a low-affinity component on the dopamine transporter, then efforts to explore structure–activity relations with one of these compounds as a template would be an important approach to further characterizing these sites. One approach is to prepare an irreversible ligand that covalently binds to the dopamine transporter. An irreversible ligand derived from a compound that interacts with a low-affinity component of the dopamine transporter would eliminate those sites from interaction with other dopamine uptake inhibitors, such as cocaine. Efforts directed at the development of such an affinity label are in progress, and it is hoped that these compounds may provide essential tools with which to study the pharmacological significance of the low-affinity sites on the dopamine transporter.

Several studies have suggested that analogs of benztropine may provide some answers to questions about heterogeneity of actions at the dopamine transporter. Benztropine is a dopamine uptake inhibitor (12) that also has antimuscarinic activity and is used in the treatment of Parkinson's disease. A previous study (43) found that the 4′-chloro analog of benztropine inhibited dopamine uptake more potently and selectively than did the parent drug, benztropine. The benztropine structure is of interest because it shares some of the structural features of cocaine and GBR 12909, a selective dopamine uptake inhibitor (Fig. 4). Benztropine and its analogs have the diphenyl-ether system of GBR 12909 and the *N*-methyl-tropane function of cocaine. Newman and colleagues (33,34) developed phenyl ring-substituted analogs of benztropine with much greater affinity for the dopamine transporter than the parent compound. Specifically, halogenation with F or Cl at the 3′-, 4′-, or 4′,4′′-positions resulted in highly selective and potent dopamine uptake inhibition. In addition, the binding

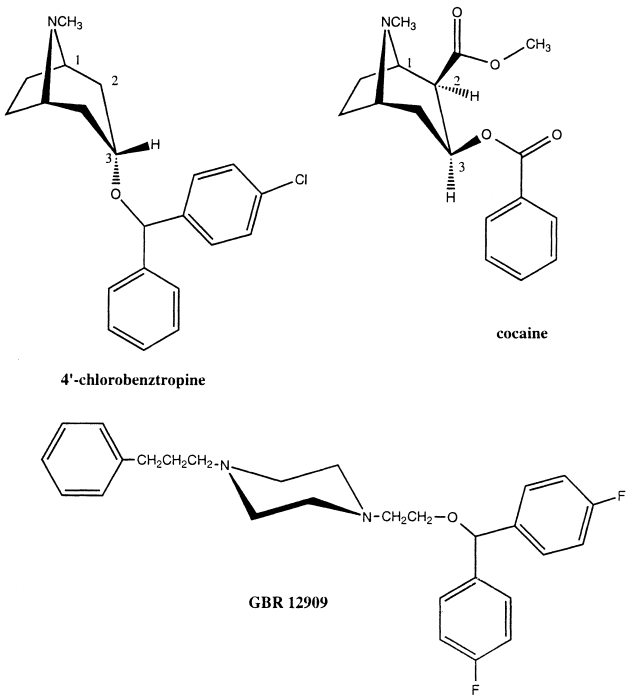

FIG. 4. Molecular structures of 4′chlorobenztropine, cocaine, and GBR 12909.

data for these drugs was generally best fit by a one-site model. Further, these drugs inhibit dopamine transport in vitro, and the effect was characterized as having one component.

Several aspects of the structure–activity relationships for these drugs suggest that they are interacting with the dopamine transporter in a manner that is distinct from the way in which cocaine analogs interact with the transporter. First, these compounds do not have a substitution at the 2-position of the tropane ring and retain high affinity for the transporter. Within the series of cocaine analogs, a substituent at that position is considered important to retain activity (6). Further, the axial (α) stereochemistry at position 3 of the oxygen in the diphenyl-ether moiety is preferred within the benztropine series (33), whereas the opposite conformation of the ester linkage is preferred within the series of cocaine analogs (6). Finally, large differences in potency result from different *para*-substitutions on the phenyl rings within the benztropine series (33,34), whereas within the series of cocaine analogs, different *para*-substituents do not have as great an influence on binding affinity (6). Thus, there are distinct differences in the structure–activity relationships for benztropine and cocaine, suggesting important differences between these drugs and how they are binding to the dopamine transporter.

The behavioral effects of several of these compounds have been examined. Figure 5 shows the results obtained with a representative compound, 4′-chlorobenztropine. Cocaine, as has been shown many times previously, produced a profound stimulation of locomotor activity in mice. In contrast, 4′-chlorobenztropine produced a more modest elevation of activity that was significant only at a single dose. In addition, 4′-chlorobenztropine did not produce cocaine-like discriminative stimulus effects. Figure 6 shows the dose-related substitution of cocaine in rats trained to discriminate injection of 10 mg/kg cocaine from saline. As with the stimulation of locomotor activity, 4′-chlorobenztropine was distinctive from cocaine; across the range of behaviorally active doses there was no significant difference from saline. As can be seen in the lower panel, 4′-chlorobenztropine was studied at doses that ranged from inactive to those that completely suppressed all behavior. Therefore, the lack of activity is not likely due to the study of insufficient dose levels. Thus, benztropine analogs represent a group of dopamine uptake inhibitors that demonstrate neurochemical similarities to cocaine and other related dopamine

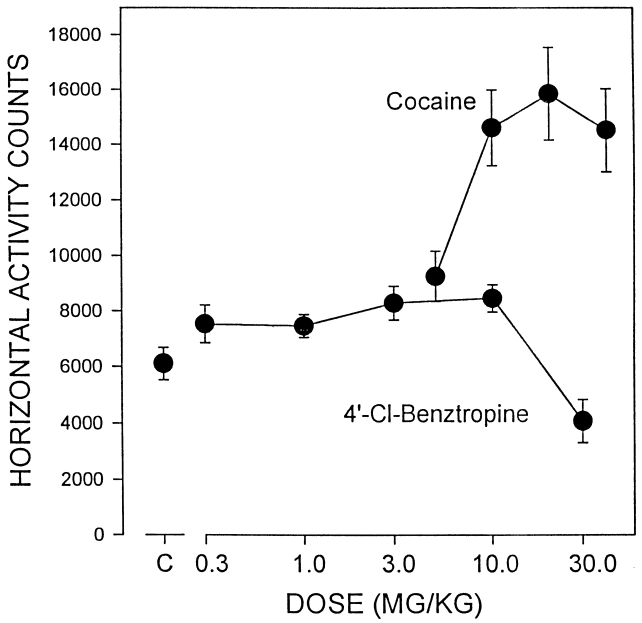

FIG. 5. Dose-dependent effects of cocaine and 4′-chlorobenztropine on locomotor activity in mice. Ordinate: horizontal activity counts after drug administration. Abscissa: dose of drug in mg/kg (log scale). Filled point above C represents the effects of saline vehicle control. Each point represents the average effect determined in eight mice. The data are from the first 30-min period after drug administration, in which the greatest stimulant effects were obtained. [Adapted from Newman et al. (33).]

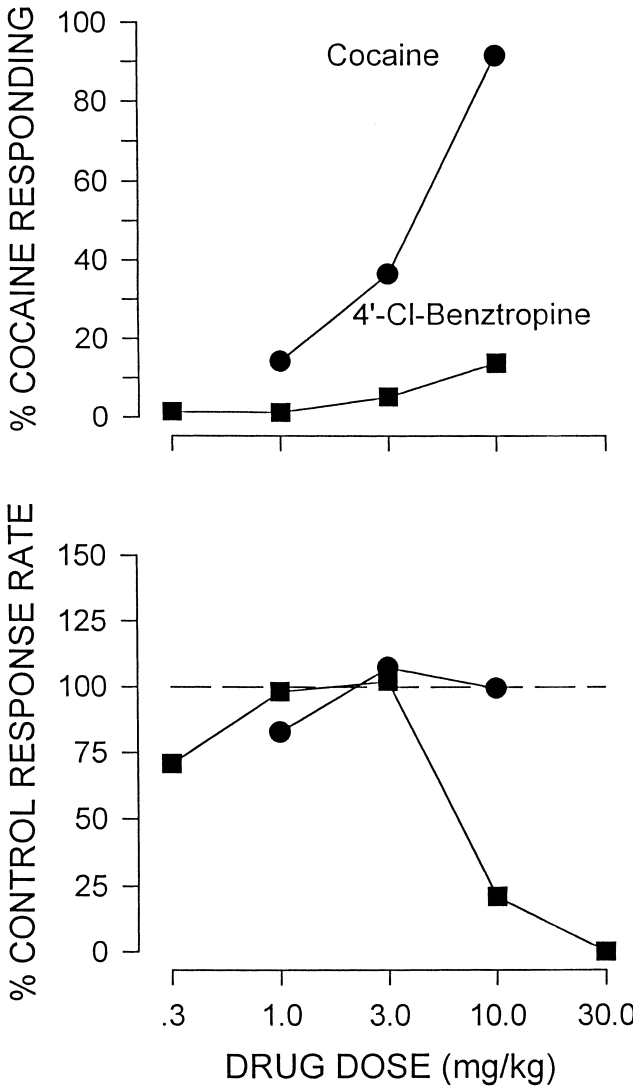

FIG. 6. Effects of cocaine and 4′-chlorobenztropine in rats trained to discriminate injections of cocaine from saline. Ordinates: percentage of responses on the cocaine-appropriate lever. Abscissae: drug dose in mg/kg (log scale). Top panel: percentage of responses emitted on the lever on which rats were trained to respond after injections of cocaine. Bottom panel: rates at which responses were emitted as a percentage of response rate after saline administration. Each point represents the effect in four or six rats. [Adapted from Newman et al. (33).]

uptake inhibitors but are behaviorally distinct. The possibility of interaction at the low-affinity component, which may not mediate psychomotor stimulant actions, must be considered. These compounds differ from cocaine in that they appear to interact at the dopamine transporter monophasically. Because these compounds clearly bind to the dopamine transporter differently from cocaine, as illustrated by their vastly different structure–activity relations, another binding domain is suggested. Further exploration of the differences in structure–activity relations among benztropine analogs and cocaine analogs is an area for further study that may prove to have implications for a better understanding of the behavioral significance of heterogeneity of actions at the dopamine transporter.

SUMMARY AND CONCLUSIONS

Previous in vitro studies have established that there are distinct components to the binding of cocaine to brain tissue as well as to its inhibition of dopamine uptake. However, the significance of this heterogeneity for behavior or cocaine abuse is not clear. In the present paper, we have examined whether this heterogeneity can be related to distinct behavioral outcomes. Clearly, some dopamine uptake inhibitors are not subject to abuse like that of cocaine, and some of these drugs have a spectrum of behavioral activity that is distinct from that of cocaine. For example, a number of dopamine uptake inhibitors, such as some sigma-receptor ligands and analogs of benztropine, despite having activity at the dopamine transporter, do not stimulate locomotor activity. These compounds also lack cocaine-like discriminative stimulus effects. In contrast, the majority of dopamine uptake inhibitors have behavioral activity like that of cocaine. Meperidine appears to inhibit dopamine uptake in vitro in a manner that corresponds to the high-affinity component of the inhibition of dopamine uptake. In addition, when the opioid actions of meperidine are antagonized, a cocaine-like discriminative stimulus effect is revealed. These results suggest that the behavioral effects of cocaine that confer its abuse liability are due to activity mediated by the high-affinity component. Further elucidation of the roles of these components in the pharmacological actions of cocaine will require advances in their isolation and independent study. Finally, it is possible that the heterogeneity of action at the dopamine transporter may have important implications for the development of useful therapeutics for cocaine abusers. Because some sigma ligands that have activity at the dopamine transporter can antagonize the effects of cocaine, it is tempting to speculate that the low-affinity component appears not to be related to the abuse liability of cocaine and may initiate actions that are incompatible with the induction of pharmacological events related to cocaine abuse. Development of a better understanding of how actions mediated by these two components are translated into pharmacological activity will prove useful in furthering our understanding of cocaine pharmacology and the medical treatment of cocaine abuse.

ACKNOWLEDGEMENTS

We thank Ms. Patty Ballerstadt for her technical help in preparation of this manuscript. Animals used in this study were maintained in facilities accredited by the American Association for the Accreditation of Laboratory Animal Care, and the experiments were conducted in accordance with the Guide for Care and Use of Laboratory Animals provided by the National Institutes of Health and adopted by the National Institute on Drug Abuse.

REFERENCES

1. Barrett, R. L.; Appel, J. B.: Effects of stimulation and blockade of dopamine receptor subtypes on the discriminative stimulus properties of cocaine. Psychopharmacology 99:13–16; 1989.
2. Baumann, M. H.; Char, G. U.; De Costa, B. R.; Rice, K. C.; Rothman, R. B.: GBR12909 attenuates cocaine-induced activation of mesolimbic dopamine neurons in the rat. J. Pharmacol. Exp. Ther. 271:1216–1222; 1994.
3. Berger, P.; Elsworth, J. D.; Reith, M. E. A.; Tanen, D.; Roth, R. H.: Complex interaction of cocaine with the dopamine uptake carrier. Eur. J. Pharmacol. 176:251–252; 1990.
4. Boja, J. W.; Markham, L.; Patel, A.; Uhl, G.; Kuhar, M. J.: Expression of a single dopamine transporter cDNA can confer two cocaine binding sites. NeuroReport 3:247–248; 1992.
5. Carlsson, A.; Lindqvist, M.: Central and peripheral monoaminergic membrane-pump blockade by some addictive analgesics and antihistamines. J. Pharm. Pharmacol. 21:460–464; 1969.
6. Carroll, F. I.; Lewin, A. H.; Boja, J. W.; Kuhar, M. J.: Cocaine receptor: Biochemical characterization and structure–activity relationships of cocaine analogues at the dopamine transporter. J. Med. Chem. 35:969–981; 1992.
7. Cline, E. J.; Scheffel, U.; Boja, J. W.; Carroll, F. I.; Katz, J. L.; Kuhar, M. J.: Behavioral effects of novel cocaine analogs: A comparison with in vivo receptor binding. J. Pharmacol. Exp. Ther. 260:1174–1179; 1992.
8. Cline, E. J.; Terry, P.; Carroll, F. I.; Kuhar, M. J.; Katz, J. L.: Stimulus generalization from cocaine to analogs with high in vitro affinity for dopamine uptake sites. Behav. Pharmacol. 3:113–116; 1992.
9. Colpaert, F. C.; Niemegeers, C. J. E.; Janssen, P. A. J.: Discriminative stimulus properties of cocaine: Neuropharmacological characteristics as derived from stimulus generalization experiments. Pharmacol. Biochem. Behav. 10:535–546; 1979.
10. Contreras, P. C.; Bremer, M. E.; Rao, T. S.: GBR-12909 and fluspirilene potently inhibited binding of [^{3}H] (+) 3-PPP to sigma receptors in rat brain. Life Sci. 47:PL-133–PL-137; 1990.
11. Cook, L.; McElroy, J. F.; Rohrbach, K. W.: DuP 734 differentially antagonizes the locomotor stimulant effects of cocaine, δ-amphetamine and apomorphine in mice and rats. Soc. Neurosci. Abstr. 18:548; 1992.
12. Coyle, J. T.; Snyder, S. H.: Antiparkinsonian drugs: Inhibition of dopamine uptake in the corpus striatum as a possible mechanism of action. Science 166:899–901; 1969.
13. Ferris, R. M.; Tang, F. L. M.; Chang, K.-J.; Russell, A.: Evidence that the potential antipsychotic agent rimcazole (BW 234U) is a specific, competitive antagonist of sigma sites in brain. Life Sci. 38:2329–2337; 1986.
14. Gifford, A. N.; Bergmann, J. S.; Johnson, K. M.: GBR 12909 fails to antagonize cocaine-induced elevation of dopamine in striatal slices. Drug Alcohol Depend. 32:65–71; 1993.
15. Grigoriadis, D. E.; Wilson, A. A.; Lew, R.; Sharkey, J. S.; Kuhar, M. J.: Dopamine transport sites selectively labeled by a novel photoaffinity probe: ^{125}I-DEEP. J. Neurosci. 9:2664–2270; 1989.
16. Heikkila, R. E.; Babington, R. G.; Houlihan, W. J.: Pharmacological studies with several analogs of mazindol: Correlation between effects on dopamine uptake and various in vivo responses. Eur. J. Pharmacol. 71:277–286; 1981.
17. Heikkila, R. E.; Cabbat, F. S.; Duvoisin, R. C.: Motor activity and rotational behavior after analogs of cocaine: Correlation with dopamine uptake blockade. Commun. Psychopharmacol. 3:285–290; 1979.

18. Heikkila, R. E.; Cabbat, F. S.; Manzino, L.; Duvoisin, R. C.: Rotational behavior induced by cocaine analogs in rats with unilateral 6-hydroxydopamine lesions of the substantia nigra: Dependence upon dopamine uptake inhibition. J. Pharmacol. Exp. Ther. 211:189–194; 1979.
19. Heikkila, R. E.; Manzino, L.: Behavioral properties of GBR 12909, GBR 13069 and GBR 13098: Specific inhibitors of dopamine uptake. Eur. J. Pharmacol. 103:241–248; 1984.
20. Izenwasser, S.: Interactions between chronic cocaine and morphine on locomotor activity and dopamine receptor function. Soc. Neurosci. Abstr. 21:717; 1995.
21. Izenwasser, S.; Cox, B. M.: Inhibition of dopamine uptake by cocaine and nicotine: Tolerance to chronic treatments. Brain Res. 573:119–125; 1992.
22. Izenwasser, S.; Newman, A. H.; Cox, B. M.; Katz, J. L.: The cocaine-like behavioral effects of meperidine are mediated by activity at the dopamine transporter. Eur. J. Pharmacol. 297:9–17; 1996.
23. Izenwasser, S.; Newman, A. H.; Katz, J. L.: Cocaine and several sigma receptor ligands inhibit dopamine uptake in rat caudate-putamen. Eur. J. Pharmacol. 243:201–205; 1993.
24. Izenwasser, S.; Rosenberger, J. G.; Cox, B. M.: Inhibition of [^{3}H]dopamine and [^{3}H]serotonin uptake by cocaine: Comparison between chopped tissue slices and synaptosomes. Life Sci. 50: 541–547; 1992.
25. Izenwasser, S.; Terry, P.; Heller, B.; Witkin, J. M.; Katz, J. L.: Differential relationships among dopamine transporter affinities and stimulant potencies of various uptake inhibitors. Eur. J. Pharmacol. 263:277–283; 1994.
26. Kahoun, J. R.; Ruoho, A. E.: (^{125}I)Iodoazidococaine, a photoaffinity label for the haloperidol-sensitive sigma receptor. Proc. Natl. Acad. Sci. USA 89:1393–1397; 1992.
27. Katz, J. L.; Sharpe, L.; Jaffe, J. H.; Shores, E. I.; Witkin, J. M.: Discriminative stimulus effects of inhaled cocaine in squirrel monkeys. Psychopharmacology 105:317–321; 1991.
28. Kleven, M. S.; Anthony, E. W.; Woolverton, W. L.: Pharmacological characterization of the discriminative stimulus effects of cocaine in rhesus monkeys. J. Pharmacol. Exp. Ther. 254:312–317; 1990.
29. Kuhar, M. J.; Ritz, M. C.; Boja, J. W.: The dopamine hypothesis of the reinforcing properties of cocaine. Trends Neurosci. 14:299–302; 1991.
30. Madras, B. K.; Fahey, M. A.; Bergman, J.; Canfield, D. R.; Spealman, R. D.: Effects of cocaine and related drugs in nonhuman primates. I. [^{3}H]cocaine binding sites in caudate-putamen. J. Pharmacol. Exp. Ther. 251:131–141; 1989.
31. Madras, B. K.; Spealman, R. D.; Fahey, M. A.; Neumeyer, J. L.; Saha, J. K.; Milius, R. A.: Cocaine receptors labeled by [^{3}H]2β-carbomethoxy-3β-(4-fluorophenyl)tropane. Mol. Pharmacol. 36: 518–524; 1989.
32. Menkel, M.; Terry, P.; Pontecorvo, M.; Katz, J. L.: Selective σ ligands block stimulant effects of cocaine. Eur. J. Pharmacol. 201:251–252; 1991.
33. Newman, A. H.; Allen, A. C.; Izenwasser, S.; Katz, J. L.: Novel 3α- (diphenylmethoxy)tropane analogs: Potent dopamine uptake inhibitors without cocaine-like behavioral profiles. J. Med. Chem. 37:2258–2261; 1994.
34. Newman, A. H.; Kline, R. H.; Allen, A. C.; Izenwasser, S.; George, C.; Katz, J. L.: Novel 4′- and 4′,4′-substituted-3α-(diphenylmethoxy)tropane analogs are potent and selective dopamine uptake inhibitors. J. Med. Chem. 38:3933–3940; 1995.
35. Reith, M. E. A.; Benuck, M.; Lajtha, A.: Cocaine disposition in the brain after continuous or intermittent treatment and locomotor stimulation in mice. J. Pharmacol. Exp. Ther. 243:281–287; 1987.
36. Ritz, M. C.; Lamb, R. J.; Goldberg, S. R.; Kuhar, M. J.: Cocaine receptors on dopamine transporters are related to self-administration of cocaine. Science 237:1219–1223; 1987.
37. Rothman, R. B.; Greieg, N.; Kim, A.; De Costa, B. R.; Rice, K. C.; Carroll, F. I.; Pert, A.: Cocaine and GBR12909 produce equivalent motoric responses at different occupancy of the dopamine transporter. Pharmacol. Biochem. Behav. 43:1135–1142; 1992.
38. Rothman, R. B.; Mele, A.; Reid, A. A.; Akunne, H. C.; Greig, N.; Thurkauf, A.; De Costa, B. R.; Rice, K. C.; Pert, A.: GBR 12909 antagonizes the ability of cocaine to elevate extracellular levels of dopamine. Pharmacol. Biochem. Behav. 40:387–397; 1991.
39. Rothman, R. B.; Mele, A.; Reid, A. A.; Akunne, H.; Greig, N.; Thurkauf, A.; Rice, K. C.; Pert, A.: Tight binding dopamine reuptake inhibitors as cocaine antagonists. A strategy for drug development. FEBS Lett. 257:341–344; 1989.
40. Sharkey, J.; Glen, K. A.; Wolfe, S.; Kuhar, M. J.: Cocaine binding at σreceptors. Eur. J. Pharmacol. 149:171–174; 1988.
41. Spealman, R. D.; Bergman, J.; Madras, B. K.; Melia, K. F.: Discriminative stimulus effects of cocaine in squirrel monkeys: Involvement of dopamine receptor subtypes. J. Pharmacol. Exp. Ther. 258:945–953; 1991.
42. Spealman, R. D.; Madras, B. K.; Bergman, J.:Effects of cocaine and related drugs in nonhuman primates. II. Stimulant effects on schedule-controlled behavior.J. Pharmacol. Exp. Ther. 251:142–149; 1989.
43. van der Zee, P.; Koger, H. S.; Gootjes, J.; Hespe, W.: Aryl 1,4-dialk(en)ylpiperazines as selective and very potent inhibitors of dopamine uptake. Eur. J. Med. Chem. Chim. Ther. 15:363–370; 1980.
44. Vaugeois, J.-M.; Bonnet, J.-J.; Duterte-Boucher, D.; Costentin, J.: In vivo occupancy of the striatal dopamine uptake complex by various inhibitors does not predict their effects on locomotion. Eur. J. Pharmacol. 230:195–201; 1993.
45. Witkin, J. M.; Nichols, D. E.; Terry, P.; Katz, J. L.: Behavioral effects of selective dopaminergic compounds in rats discriminating cocaine injections. J. Pharmacol. Exp. Ther. 257:706–713; 1991.