Life Sciences, Vol. 46, pp. 635-645 Pergamon Press
Printed in the U.S.A.

COCAINE INHIBITION OF LIGAND BINDING AT DOPAMINE, NOREPINEPHRINE AND SEROTONIN TRANSPORTERS: A STRUCTURE-ACTIVITY STUDY

M.C. Ritz, E.J. Cone*, and M.J. Kuhar+

Molecular Pharmacology and Chemistry and
Drug Metabolism Laboratories*
NIDA Addiction Research Center, Box 5180
Baltimore, MD 21224

(Received in final form January 3, 1990)

Summary

Structure-activity relationships for cocaine and analog binding at the dopamine, norepinephrine and serotonin transporters were determined. Cocaine inhibition of ligand binding to each of these sites has a stereospecific requirement for the levorotatory isomer. Binding potencies of cocaine derivatives involving N-substitution, C2 and C3 substituent modifications, however, revealed differences in structure-activity relationships for cocaine binding at the transporters. Removal of the N-methyl groups produced little change in binding potency at the dopamine transporter site but produced increases in binding potency at norepinephrine and serotonin transporter sites. Changes in structure at the C2 substituent produced changes in binding potency at the dopamine transporter which were generally similar in direction, but not necessarily in magnitude at the norepinephrine and serotonin transporters. Modifications to the C3 substituent, especially substitution of a hydroxyl moiety, produce changes in affinity at norepinephrine and serotonin transporters which are much larger than those observed at dopamine transporters. In general, our results indicate that unique structural requirements exist for each transporter site, but that cocaine binding at norepinephrine and dopamine transporters can be described by more similar structure-activity relationships than those found for the serotonin transporter. Requirements for cocaine binding to the dopamine transporter, which we have previously shown to be associated with the reinforcing effects of cocaine, include levorotatory sterospecificity, the benzene ring at C3, at least some portions of the tropane ring, and the presence of the C2 methyl ester group in the β conformation.

A variety of psychoactive drugs are known to inhibit the active neurotransmitter uptake process and as well as ligand binding to transporter sites. Cocaine, in particular, has been shown to inhibit the transport of dopamine, norepinephrine and serotonin (1, 2, 3, 4, 5) and to interact with some receptors (6, 7, 8). Recently, Ritz et al. (9) presented evidence that the cocaine inhibition of ^{3}H-mazindol binding to the dopamine transporter may be related to the reinforcing properties of cocaine and related drugs.

+Address all correspondence to: Dr. Michael J. Kuhar, Neuroscience Branch, NIDA Addiction Research Center, P.O. Box 5180, Baltimore, MD 21224.

0024-3205/90 $3.00 +.00

There have been extensive studies of the dopamine uptake process, including structure activity studies (2, 10, 11, 12, 13, 14, 15). It has been possible to directly bind to the dopamine transporter using a variety of ligands including ^{3}H-mazindol (2), ^{3}H-GBR 12935 (12, 16, 17), ^{3}H-methylphenidate (18), ^{3}H-cocaine (19, 20, 21) and ^{3}H-nomifensine (22). In addition, several studies of cocaine metabolism and behavioral effects, including anesthesia, locomotor activity, self-administration and disruption of schedule-controlled behavior, have been performed in order to determine the relationship between the structure of cocaine and these measures (23, 24, 25, 26, 27, 28). There has also been a study of cocaine structure-activity relationships designed to assess the possible role of sodium channels in mediating the anesthetic and locomotor effects of cocaine (29). To date, there has been only one published report of cocaine structure-activity relationships related to monoamine uptake processes. In that study, the structure-activity relationships of cocaine and some derivatives at the ^{3}H-cocaine binding site related to the dopamine and serotonin transporters were examined (30).

In this paper, structure-activity relationships were determined for the binding of cocaine and a series of related compounds at striatal ^{3}H-mazindol binding sites on the dopamine transporter, previously shown to be associated with cocaine self-administration (9). In addition, we have studied structure-activity relationships for cocaine interactions with norepinephrine transporters labeled by ^{3}H-mazindol in frontal cortex as well as with serotonin transporters labeled by ^{3}H-paroxetine in mid-brain. We have utilized radiolabeled binding ligands other than cocaine, because they provide higher affinities, greater specificities and better specific-to-nonspecific binding ratios for the sites studied. We also determined the specificity or generality of these relationships by comparing the direction and magnitude of the effects of structural changes to (-)cocaine and its analogs on binding potencies at these three different transporter sites.

Materials and Methods

Tropacocaine was obtained from Sigma Chemical Co. (St. Louis, Mo.). m-Toluylecgonine methyl ester was synthesized (31). 4-Phenylpiperidine, 3-phenylpropylamine, propylamine, propylbenzene and phenethylamine were obtained from Aldrich. p-Aminobenzoylecgonine methyl ester and p-aminobenzoylecgonine were synthesized by Dr. F.I. Carroll. All other compounds were obtained from Research Triangle Institute (Research Triangle Park, N. Carolina).

The potencies of cocaine related compounds at monoamine transporter sites were assessed using *in vitro* receptor binding techniques. Tissues for all binding experiments were dissected from the brains of male Sprague-Dawley rats, age 60-120 days old. The rats were sacrificed by decapitation; their brains were removed and washed in cold saline; brain regions were then dissected and frozen and stored at -70 degrees C until used in the assay procedures. Tissues were homogenized in 20 volumes of the assay buffer, then centrifuged at 30,000 g for 10 minutes. The resulting pellet was washed, recentrifuged, and resuspended in buffer to yield the desired tissue concentration for addition to the assay.

^{3}H-mazindol was used to label dopamine uptake sites in rat striatal tissue, and norepinephrine uptake sites in rat frontal cortex (2). Nonspecific binding was defined by the addition of 10 uM nomifensine and 5 uM desmethylimipramine for these two sites, respectively. Approximately 1.5 and 4 mg original weight of homogenized tissue were incubated at 0 degrees C in buffer (50 mM Tris, 120 mM NaCl, 5 mM KCl; pH = 7.8) containing final ligand concentrations of 4 nM and

5 nM for dopamine and norepinephrine uptake sites, respectively. Final assay volumes were 0.5 ml in each case. ^{3}H-Paroxetine was used to label serotonin uptake sites in rat brainstem (32). Nonspecific binding was defined by the addition of 1 uM citalopram, and final assay volumes were 4 ml. Homogenized tissues (1.5 mg original weight per tube) were incubated at room temperature in buffer (50 mM Tris, 120 mM NaCl, 5 mM KCl; pH = 7.8) for 90 minutes with a final ligand concentration of 0.2 nM.

At the end of the incubation period, all assay mixtures were filtered through Whatman GF/C filters presoaked with 0.05% polyethylimine, and washed with buffer. Filters were placed into plastic vials and scintillation fluid was added. Vials were shaken for one hour and, finally, radioactivity in each was measured by liquid scintillation spectometry.

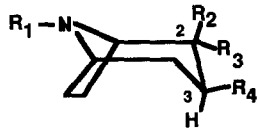
K_D values for ^{3}H-mazindol binding at dopamine and norepinephrine transporters and for ^{3}H-paroxetine binding at serotonin transporters were observed to be 18nM, 4nM and 20pM, respectively. Utilizing these values, K_i values were determined from analyses of competition curves using the nonlinear least-squares, curve fitting program, LIGAND (33). Mean values and standard errors were calculated for 3-5 assays for each test drug in each receptor system. As a means of assessing the effects of various structural changes on binding affinity, we determined the ratio of the K_i (uM) for the modified compound (B) to that of a starting compound (A) such that B/A provides an estimate of the magnitude of the changes in binding affinity.

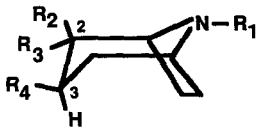
Results

The structure of natural cocaine consists of a central tropane ring with substituent groups at C2 and C3 (Figure 1). Both substituent groups are attached in the β-configuration, relative to the tropane nitrogen, and the molecule exists as the levorotatory (-)isomer. Analogs of cocaine representing five major structural modifications were tested in this study. The structural modifications of the cocaine molecule focused on changes in stereochemistry, nitrogen substituents, C2 substituents, C3 substituents, and in the tropane ring structure (Figure 1). Tables 1 and 2 summarize the effects of these various changes to the (-)cocaine molecule on binding potencies at monoamine transporter sites.

Table 1 indicates that the inhibitory potency of cocaine and its analogs at monoamine transporters varied substantially, exhibiting K_is from low nanomolar to greater than 10^{-3} M. WIN 35,428, WIN 35,065-2 and WIN 35,981, analogs of cocaine without an ester linkage between the C3 carbon atom of the tropane ring and the benzene ring, were the most potent inhibitors of ^{3}H-mazindol binding at this site. These compounds were also generally more potent than cocaine at norepinephrine and serotonin transporters. WIN 35,428, WIN 35,981 and norcocaine exhibited the greatest affinities at dopamine, norepinephrine and serotonin transporters, respectively. The metabolites of cocaine, ecognine derivatives, were generally only weak inhibitors of ligand binding or were inactive.

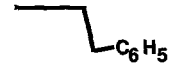
Table 1 also indicates that several compounds exhibit significantly different affinities for each of the three monoamine transporters. These compounds include WIN 35,428, WIN 35,981, phenethylamine and (-)benzoyl-norecgonine. Other compounds exhibit relative specificity for only one of the transporters. For example, (-)norcocaine and (+)pseudococaine are much more potent, and propylbenzene and pseudoecgonine methyl ester are less potent, at serotonin transporters than at either dopamine or norepinephrine transporters.



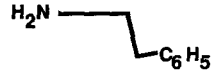


COMPOUND	R_1	R_2	R_3	R_4
COCAINE	CH_3	$COOCH_3$	H	$OCOC_6H_5$
NORCOCAINE	H	$COOCH_3$	H	$OCOC_6H_5$
COCAINE METHIODIDE	$(CH_3)_2$	$COOCH_3$	H	$OCOC_6H_5$
TROPACOCAINE	CH_3	H	H	$OCOC_6H_5$
PSEUDOCOCAINE	CH_3	H	$COOCH_3$	$OCOC_6H_5$
BENZOYLECGONINE	CH_3	COOH	H	$OCOC_6H_5$
META-TOLUYLECGONINE METHYL ESTER	CH_3	$COOCH_3$	H	$OCOC_6H_4CH_3$
P-AMINOBENZOYLECGONINE METHYL ESTER	CH_3	$COOCH_3$	H	$C_6H_4NH_2$
WIN 35,065	CH_3	$COOCH_3$	H	C_6H_5
WIN 35,981	H	$COOCH_3$	H	C_6H_5
WIN 35,428	CH_3	$COOCH_3$	H	C_6H_4F
ECGONINE METHYL ESTER	CH_3	$COOCH_3$	H	OH
P-AMINOBENZOYLECGONINE	CH_3	COOH	H	$C_6H_4NH_2$
PSEUDOECGONINE METHYL ESTER	CH_3	H	$COOCH_3$	OH
WIN 35,140	CH_3	H	$COOCH_3$	C_6H_5
ECGONINE	CH_3	COOH	H	OH
BENZOYLNORECGONINE	H	COOH	H	$OCOC_6H_5$

4-PHENYLPIPERIDINE

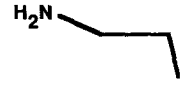


PROPYLBENZENE



PHENETHYLAMINE

3-PHENYLPROPYLAMINE



PROPYLAMINE

FIG. 1

The analogs of cocaine represent structural modifications from five major categories including stereochemistry, nitrogen substituents, C2 and C3 substituents and the tropane ring structure.

TABLE I

Inhibition of Binding at Dopamine, Norepinephrine and Serotonin Transporters

Structural Change		B/A (Potency Ratio)		
Compound A	Compound B	DA Transporter	NE Transporter	5HT Transporter
Stereochemistry:				
(-)cocaine	(+)cocaine	212	146	478
Nitrogen Substituent:				
(-)cocaine	(-)norcocaine	1.90	0.79	0.16
WIN 35,065-2	WIN 35,981	1.38	0.30	0.11
(-)benzoyl-ecgonine	(-)benzoyl-norecgonine	0.87	<0.04	0.02
(-)cocaine	(-)cocaine methiodide	31	28	60
C2 Substituent:				
(-)cocaine	(+)pseudococaine	181	38	31
WIN 35,065-2	WIN 35,140	1481	326	465
(+)cocaine	(-)pseudococaine	0.71	0.31	0.34
(-)cocaine	(-)benzoylecgonine	613	1218	1107
(-)cocaine	tropacocaine	23.0	4.30	5.30
p-aminoben-zoylecgonine methyl ester	p-aminobenzoyl-ecgonine	4.80	1.60	5.50
C3 Substituent:				
(-)cocaine	(-)ecgonine methyl ester	97.0	>625	>7142
(-)pseudo-cocaine	(-)pseudoecgonine methyl ester	0.90	0.30	<0.02
(-)cocaine	WIN 35,065-2	0.41	0.16	1.64
WIN 35,065-2	WIN 35,428	0.65	4.19	0.11
(-)cocaine	(-)meta-toluylecgonine methyl ester	62.5	22.5	6.60
Tropane Ring:				
WIN 35,981	4-phenylpiperidine	33.0	9.80	15.6
4-phenylpi-peridine	3-phenylpropylamine	3.50	3.0	6.40
3-phenyl-propylamine	phenethylamine	2.55	1.01	>23.0
3-phenyl-propylamine	propylbenzene	3.55	10.4	>23.0
3-phenyl-propylamine	propylamine	>42.0	>7.80	>23.0

The potencies of cocaine and related drugs at monoaminergic transporters, expressed as K_i values, were determined using receptor binding techniques as described in the METHODS. Standard errors for 3-5 assays were less than 10% of each K_i value. Hill coefficients describing competition curves at each uptake site were near 1.0 for all drugs exhibiting K_i values less than 1000.

Table 2 shows the effects of each of the five major categories of modifications to (-)cocaine. The results are as follows:

Stereochemistry Modifications

The results show that stereochemistry was very important for determining the potency of cocaine at the dopamine transporter. (-)Cocaine was more than 200 times more potent than (+)cocaine. At the norepinephrine and serotonin transporters, (+)cocaine was also considerably less potent.

N-Substituent Modifications

Changes related to the nitrogen atom had varying effects on binding potency at the dopamine transporter (Table 2). Replacement of the methyl group with a hydrogen atom in (-)cocaine, WIN 35,065-2 and benzoylecgonine to produce (-)norcocaine, WIN 35,981 and benzoylnorecgonine resulted in only minor changes in affinity. However, quaternization of the nitrogen by the addition of a second methyl group, as in cocaine methiodide, results in a permanent positive charge on that atom and a large loss in potency. (-)Cocaine methiodide was 31 fold less potent in inhibiting ^{3}H-mazindol binding at the dopamine transporter as a result of this change.

The effects of N-demethylation of (-)cocaine and analogs on binding to both norepinephrine and serotonin transporters provide an indication of the specificity of structure-activity relationships for cocaine binding to the dopamine transporter. N-demethylation of cocaine and its analogs appears to increase binding potencies more consistently at the norepinephrine and serotonin transporters, in contrast to the dopamine transporter, as observed by comparing the potencies of (-)norcocaine and (-)cocaine, WIN 35,981 and WIN 35,065-2, and (-)benzoylnorecgonine and (-)benzoylecgonine. For example, the magnitude of this increase is quite large when the affinity of (-)benzoylnorecgonine at either of these transporters is compared with that of the weak compound (-)benzoylecgonine (B/A $<$ 0.04). These changes in affinity due to N-demethylation are generally one to two orders of magnitude greater at norepinephrine and serotonin transporters than those observed for the dopamine transporter. In contrast, quaternization of the nitrogen by the addition of a second methyl group, to form cocaine methiodide, resulted in a loss in potency at norepinephrine and serotonin transporters which was similar to that observed at dopamine transporters.

C2 Substituent Modifications

Epimerization of (-)cocaine and WIN 35,065-2 at C2, producing (+)pseudococaine and WIN 35,140, resulted in 181 fold and 1481 fold decreases in potency to inhibit ^{3}H-mazindol binding, respectively. However, epimerization of the less potent (+)cocaine to (-)pseudococaine had little further effect on potency. Hydrolysis of the C2 ester group of (-)cocaine to form (-)benzoylecgonine, resulted in a large loss of potency (513 fold). Substitution of hydrogen for the carbomethoxy group at C2, as in tropacocaine, resulted in a smaller loss of potency. Tropacocaine was 23 times less potent than (-)cocaine. Similarly, substitution of hydrogen for the methyl group at C2 in p-aminobenzoylecgonine methyl ester results in a relatively small decrease in potency associated with p-aminobenzoylecgonine. Structural modifications involving the C2 substituents produced changes in binding potencies, which were generally similar in direction but not necessarily magnitude, at all three monoamine transporter binding sites.

C3 Substitutions

With respect to the dopamine transporter, substituting a hydroxyl group for the C3 benzoxy group of (-)cocaine resulted in the inactive compound (-)ecgonine methyl ester. However, this modification of the less potent derivative (-)pseudococaine to produce (-)pseudoecgonine methyl ester had little further effect on potency. In contrast, elimination of the ester linkage between the phenyl group and the tropane ring of cocaine, resulting in the direct attachment the phenyl group to the C3 carbon, produced only a minor change in binding affinity. WIN 35,065-2 was slightly more potent that (-)cocaine.

Addition of a methyl group to the aromatic ring of (-)cocaine in the meta position resulted in a relatively large decrease in binding potency.

TABLE II

Changes in the Binding Potency at Monoamine Transporters Resulting from Structural Modifications to (-)-Cocaine and Derivatives

Compound	Transporter DA	NE	5-HT
Cocaine and Analogs		**Ki (uM)**	
(-) WIN 35,428	0.17	1.09	0.03
(-) WIN 35,065-2	0.26	0.26	0.23
(-) WIN 35,981	0.36	0.01	0.03
(-) Cocaine	0.64	1.60	0.14
(-) Norcocaine	1.21	1.27	0.02
p-Aminobenzoylecgonine methyl ester	3.04	5.60	0.06
p-Aminobenzoylecgonine	4.51	8.90	0.33
4-Phenylpiperidine	12.0	2.55	3.60
Tropacocaine	15.0	6.90	0.74
(-) Cocaine Methiodide	20.0	45.5	8.35
(-) Meta-Toluylecgonine Methyl Ester	40.0	35.6	0.92
3-Phenylpropylamine	42.0	7.80	23.3
(-) Ecgonine Methyl Ester	62.0	> 1000	> 1000
(-) Pseudoecgonine Methyl Ester	87.0	22.0	> 1000
(-) Pseudococaine	97.0	74.0	23.2
Phenethylamine	107	7.90	>1000
(+) Pseudococaine	116	61.0	4.30
(+) Cocaine	136	235	67.0
Propylbenzene	149	81.0	>1000
(-) Benzoylnorecgonine	342	40.5	2.90
WIN 35,140	385	85.0	107
(-) Benzoylecgonine	392	3663	155
Propylamine	>1000	>1000	>1000
(-) Ecgonine	>1000	>1000	>1000
Neurotransmitters			
Dopamine	8.70	5.20	>1000
Norepinephrine	52.10	8.70	>1000
Serotonin	2.70	14.1	0.10

The ratio of the K_i (uM) for the modified compound (B) is divided by that of a starting compound (A) such that B/A provides an estimate of the magnitude of the changes in binding affinity resulting from specific structural modifications.

m-Toluylecgonine methyl ester was approximately 60 fold less potent than (-)cocaine. However, substitution of a highly electronegative fluorine at the para position of the benzene ring may result in a slight increase in potency as evidenced by comparing WIN 35,065-2 with WIN 35,428.

The different structure-activity relationships for cocaine binding at the different monoamine transporters is evidenced by substituting a hydroxyl group for the C3 benzoxy group of (-)cocaine to form (-)ecgonine methyl ester. This modification results in nearly 10 and 100 fold decreases in affinity at the norepinephrine and serotonin transporters, relative to dopamine transporters. This same modification to (-)pseudococaine, resulting in (-)pseudoecgonine methyl ester, produces a large increase in affinity at serotonin transporters, relative to dopamine and norepinephrine transporters. In addition, fluorination of WIN 35,065-2, resulting in WIN 35,428, increases potency at the serotonin transporter by 10-fold, while this structural modification leads to smaller changes in affinity at dopamine and norepinephrine transporters.

Tropane Ring Modifications

The necessity of an intact tropane ring structure with equivalent C2 and C3 substituent groups as those found in (-)WIN 35,065-2 was tested with two compounds, 4-phenylpiperidine and 3-phenylpropylamine. 4-phenylpiperidine can be considered a simplified version of (-)WIN 35,981, in which the carbomethoxy group at C2 has been substituted with hydrogen and the bicyclic tropane ring has been simplified to a piperidine ring system. These structural changes, combined in 4-phenylpiperidine, resulted in more than a 30 fold loss of affinity when compared with (-)WIN 35,981. Since the substitution of the C2 group of (-)cocaine with hydrogen in tropacocaine produced nearly as great a change (23 fold), it seems possible that the additional modification of the tropane ring in 4-phenylpiperidine is relatively less important for determining binding potency. Simplification of the cyclic piperidine ring to produce 3-phenylpropylamine, indicates that there is a further three to four fold decrease in affinity. Further modifications of 3-phenylpropylamine involving the elimination of one carbon atom, the amine group, or the benzene ring produce phenethylamine, propylbenzene or propylamine, respectively. Phenethylamine and propylbenzene, but not propylamine, retain slight activity at the dopamine transporter. Taken together, these results suggest that the tropane ring system as a whole and the nitrogen methyl group of cocaine may not be essential for its binding to the dopamine transporter. Nevertheless, a fully intact bicyclic tropane ring is more efficacious than more simplified structures.

Changes in the tropane ring system reveal generally similar changes in binding potency at the serotonin, norepinephrine and dopamine transporters. However, simplifications of 3-phenylpropylamine to phenethylamine, propylamine or propylbenzene leads to a change from moderate affinity to inactivity at the serotonin transporter in every case. In contrast, propylbenzene and phenethylamine retain at least moderate affinities at the dopamine or norepinephrine transporters.

Discussion

Important similarities are found among the structure-activity relationships for cocaine and analog binding at the dopamine, norepinephrine and serotonin transporters. Stereochemistry was an important factor in determining binding potencies of cocaine and its derivatives at all three transporters. (-)Cocaine was substantially more potent than was (+)cocaine. Quaternization of the nitrogen resulted in decreased binding potencies at all three transporter binding sites. Changes in the C2 substituents or in the tropane ring system

produced generally similar, though less consistent, changes in binding potency at the serotonin, norepinephrine and dopamine transporters. In general, cocaine binding at norepinephrine and dopamine transporters can be described by more similar structure-activity relationships than those found for the serotonin transporter. This result is consistent with the observed differences between the neurotransmitters themselves in binding potency at the three monoamine transporters. Serotonin is 10-100 fold less potent at dopamine and norepinephrine transporters than at its specific transport site. In contrast, dopamine and norepinephrine are quite inactive at the serotonin transporter. Only minor differences between dopamine and norepinephrine in affinity for the three transporters are observed.

The results of our studies also indicate important differences between structure-activity relationships for the three transporter sites. One of these was revealed by replacement of the N-methyl group of (-)cocaine, WIN 35,065-2 and (-)benzoylecognine with hydrogen. This relatively minor structural change resulted in increases in potency at both the norepinephrine and serotonin transporter, while producing little change in binding potency at the dopamine transporter. These potency differences probably reflect differences between the transporter structures, especially since the same ligand was used to label both the norepinephrine and dopamine transporters. Second, changes in structure at the C3 substituent produced both increases and decreases in binding potency at the dopamine transporter which were generally similar in direction at the norepinephrine and serotonin transporters, but of greater magnitude at these transporters. In particular, substitution of a hydroxyl moiety at C3 results in changes in affinities which are 10-100 fold greater at norepinephrine and serotonin transporters, relative to dopamine transporters. These potency differences may also reflect differences between the transporter structures. Alternatively, it might be hypothesized that cocaine may bind to protein structures which are common to all three transporters, but exist in different protein-lipid surroundings and conformations.

These results are consistent with those reported by Reith et al. (29) concerning the sodium-dependent ^{3}H-(-)cocaine binding site at striatal dopamine and sodium-independent ^{3}H-(-)cocaine binding at cortical serotonin transporters. The potency of (-)cocaine to inhibit striatal ^{3}H-mazindol binding (K_i = 0.64 uM) and cortical ^{3}H-paroxetine (K_i = 0.14 uM) binding in our study was similar to the K_D values determined for ^{3}H-cocaine in these regions. Reith et al. (29) examined more limited modifications to (-)cocaine involving epimerization at C2 and C3 groups and a change at the tropane nitrogen. In moderate contrast to our results, they suggest that there are generally similar structure-activity relationships for cocaine binding to dopamine and serotonin transporters. Our data suggest more specificity of cocaine structure-activity relationships at dopamine and serotonin transporters due to either the use of different ligands used to label the transporters or to the more extensive group of cocaine derivatives tested on our study. Their data are consistent with ours, however, in that it shows that replacement of the ester linkage between the tropane and phenyl rings of (-)cocaine with a direct attachment increases binding potency at these sites, and that the configuration of C2 groups is important for determining the affinity. Their data provides the information, additional to our results, that epimerization at the C3 group is moderately important for optimal binding potency at these sites.

It is not clear whether the phenyl ring and tropane nitrogen in cocaine can be related to these moieties in the dopamine molecule, and thus, whether there may be some similarity in the binding sites for these molecules at the dopamine transporter. Some inferences are possible, however. Schoemaker et al. (1985)

indicates that serotonin (IC_{50}=24.5 μM) may be slightly less potent than dopamine (IC_{50}=10.6 μM) in inhibiting ^{3}H-cocaine binding to striatal membranes. Our data indicates that serotonin (K_D=2.7 μM) may be slightly more potent in inhibiting ^{3}H-mazindol binding in striatum than dopamine (K_D=8.7 μM). Taken together, these results suggest that dopamine and serotonin do not exhibit large differences in their affinity for mazindol and cocaine binding sites on dopamine transporters. An increasing body of evidence suggests that these sites interact with but may not be identical to the dopamine recognition site in the channel, however. Thus, the two neurotransmitters may logically exhibit similar binding affinities for cocaine and mazindol binding sites, while exhibiting different affinities at the transport site itself and, consequently, different functional effects.

It appears that certain parts of the cocaine molecule are more important for inhibition of ^{3}H-mazindol binding to the dopamine transporter, the site which we have shown to be associated with the reinforcing effects of cocaine (9). Stereochemistry is important since the (-)isomer is substantially more potent than the (+)isomer. Also, the C2 substituents are important for determining potency since epimerization, hydrolyzing the methyl ester or removal of this substituent results in moderate to large losses in potency. An uncharged nitrogen on the tropane ring may be essential because quaternization results in a large loss of potency. Alternatively, a protonated and, thus, charged species may in fact be required for binding. In this case, the loss of potency observed might be due to steric factors. Finally, the phenyl ring at C3 is important because its removal results in a large loss of potency; interestingly, if the phenyl ring is positioned closer to the tropane ring by removal of the ester linkage, there is an increase in potency. Thus, lipophilicity may influence drug binding at this site. There appears to be steric limitations in the area of site attachment to the benzene ring to these transporter systems since the addition of a methyl group in the meta position resulted in a substantial loss of binding potency. In contrast, substition of a fluorine atom, a smaller group, onto WIN 35,065-2 to produce WIN 35,428 had much less effect and, in some cases, produced an increase in binding potency. It also appears that the entire bicyclic structure of the tropane ring is not required; simplification of bicyclic ring system to a linear chain containing a benzene ring and primary nitrogen group did not completely eliminate binding potency.

Our results are consistent with our previous work indicating that cocaine binding at a site related to the ^{3}H-mazindol sites on the dopamine transporter appears to be associated with its reinforcing properties (9). Cocaine metabolites, ecgonine derivatives, have been shown not to generalize to cocaine in previous operant behavioral studies (34). We have now shown that these compounds are also weak inhibitors of ^{3}H-mazindol binding at dopamine transporters. The structure-activity relationships for cocaine binding at this site suggest that ecgonine derivatives contain C2 or C3 substituents which reduce their binding potency relative to cocaine. Thus, the results of this study support our earlier hypothesis that cocaine inhibition of dopamine uptake is associated with its reinforcing effects.

Acknowledgements

The authors greatly appreciate excellent technical assistance from M. Milberger and M. Reihart, and expert clerical assistance from T. Pierce and C. Highkin.

References

1. J.T. COYLE and S.H. SNYDER, Science 166:899-901 (1969).
2. J.A. JAVITCH, R.O. BLAUSTEIN, and S.H. SNYDER, Mol. Pharmacol. 26:35-44 (1984).
3. S.B. ROSS and A.L. RENYI, Eur. J. Pharmacol. 7:270-277 (1969).
4. K.J. BLACKBURN, P.C. FRENCH, and R.J. MERRILLS, Life Sci. 6:1653 (1967).
5. A.S. HORN, C. CUELLO, and R.J. MILLER, J. Neurochem. 22:265-270 (1974).
6. J. SHARKEY, K.A. GLEN, S. WOLFE, and M.J. KUHAR, Eur. J. Pharmacol. 149:171-174, 1988.
7. J. SHARKEY, M.C. RITZ, J.A. SCHENDEN, R.C. HANSON, and M.J. KUHAR, J. Pharmacol. Exp. Ther. 246(3):1048-1052, 1988.
8. M.C. RITZ and M.J. KUHAR, J. Pharmacol. Exp. Ther. 248:1010-1017 (1989).
9. M.C. RITZ, R.J. LAMB, S.R. GOLDBERG, and M.J. KUHAR, Science. 237:1219-1223 (1987).
10. J.T. COYLE and S.H. SNYDER, J. of Pharmacol. Exp. Ther. 170:221-231 (1969).
11. S.H. SNYDER and J.T. COYLE, J. Pharmacol. Exp. Ther. 165:78-86 (1969).
12. P.H. ANDERSON. J. Neurochem. 48:1887-1896 (1987).
13. A.S. HORN, J.T. COYLE and S.H. SNYDER, Mol. Pharm. 7:66-80 (1971).
14. A.S. HORN and S.H. SNYDER, J. Pharmacol. Exp. Ther. 180:523-530 (1972).
15. A.S. HORN, Br. J. Pharmacol. 47:332-338 (1973).
16. A. JANOWSKY, P. BERGER, F. VOCCI., R. LABARCA, P. SKOLNICK, and S.M. PAUL, J. Neurochem. 46:1272-1276 (1986).
17. J. SHARKEY, M.C. RITZ and M.J. KUHAR, Society for Neuroscience Abstracts 13 (1987).
18. A. JANOWSKY, M.M. SCHWERI, P. BERGER, R. LONG, P. SKOLNICK, and S.M. PAUL, Eur. J. Pharmacol. 108:187-191 (1985).
19. L.T. KENNEDY and I. HANBAUER, J. Neurochem. 41:172-178 (1983).
20. M.E.A. REITH, H. SERSHEN, and A. LAJTHA, Life Sci. 27:1055-1062 (1980).
21. D.O. CALLIGARO and M.E. ELDEFRAWI, J. Pharmacol. Exp. Ther. 243:61-67 (1987)
22. M.L. DUBOCOVICH and N.R. ZAHNISER, Biochem. Pharmacol. 34:1137-1144 (1985).
23. M.E.A. REITH, B.E. MEISLER, and A. LAJTHA, Pharmacol. Biochem. Behav. 23:831-836 (1985).
24. A.L. MISRA, R.B. PONTANI, and S.J. MULE, Experientia 327:895-897 (1976).
25. R.L. CLARKE, S.J. DAUM, A.J. GAMBINO, M.D. ACETO, J. PEARL, M. LEVITT, W.R. CUMISKEY, and E.F. BOGADO, J. Med. Chem. 16:1260-1267 (1973).
26. D.A. SHRIVER, and J.P. LONG, Arch. Internat. Pharmacodyn. Ther. 189:198-208 (1971).
27. R.D. SPEALMAN, S.R. GOLDBERG, R.T. KELLEHER, D.M. GOLDBERG, and J.P. CHARLTON, J. Pharmacol. Exp. Ther. 202:500-509 (1977).
28. R.D. SPEALMAN and R.T. KELLEHER, J. Pharmacol. Exp. Ther. 216:532-536 (1981)
29. M.E. REITH, S.S. KIM, and A. LAJTHA, J. Biol. Chem. 35:1123-1129 (1986).
30. M.E.A. REITH, B.E. MEISLER, H. SERSHEN, and A. LAJTHA, Biochem. Pharm. 35:1123-1129 (1986).
31. P. JACOB, III, B.A. ELIAS-BAKER, R.T. JONES, and N.L. BENOWITZ, J. Chromatography 417:277-286 (1987).
32. E. HABERT, D. GRAHAM, L. TAHRAOUI, Y. CLAUSTRE, and S.Z. LANGER, Eur. J. Pharmacol. 118:107-114 (1985).
33. P.J. MUNSON and D. RODBARD, Anal. Biochem. 107:220-239 (1980).
34. H. SCHOEMAKER, C. PIMOULE, S. ARBILLA, B. SCATTON, F. JAVOY-AGID and S.Z. LANGER, Naunyn-Schmiedeberg Arch. Pharmacol. 329:227-235 (1985).
35. M.L. MCKENNA, B.T. HO, and L.F. ENGLERT, Pharmacol. Biochem. Behav. 10:273-276 (1979).