Pergamon

Biochemical Pharmacology, Vol. 50, No. 7, pp. 1007–1014, 1995.

0006-2952/95 $9.50 + 0.00

0006-2952(95)00230-8

A COCAINE-SENSITIVE ACTIVE DOPAMINE TRANSPORT IN HUMAN LYMPHOCYTES*

BAHJAT A. FARAJ,†‡ ZBIGNIEW L. OLKOWSKI§ and RICHARD T. JACKSON‖

Departments of †Radiology (Division of Nuclear Medicine), §Radiation Oncology and ‖Surgery, Emory University School of Medicine, Atlanta, GA 30322, U.S.A.

(*Received* 17 *October* 1994; *accepted* 22 *March* 1995)

Abstract—Human lymphocytes possess a cocaine-sensitive high-affinity transport system for [^{3}H]dopamine. [^{3}H]Dopamine uptake was saturated with increasing dopamine concentrations and followed Michaelis–Menten kinetics. The uptake was temperature, sodium, and chloride dependent and was affected by the co-addition of ouabain, phloridzin, potassium cyanide, gramicidin, and other metabolic inhibitors. The uptake of dopamine was blocked significantly in a concentration-dependent manner by cocaine and its congeners. Furthermore, preliminary evidence is presented linking the possible relationship between decreased lymphocyte [^{3}H]dopamine uptake and chronic cocaine abuse in humans.

Key words: lymphocyte; dopamine; monoamine uptake; cocaine; addiction; structure–activity relationship

A wide variety of studies have demonstrated that interactions occur between the immune and neuronal systems [1–3]. Several neurotransmitters influence immune reactivity [4]. Serotonin has been shown to affect the function of natural killer (NK) cells, macrophages, T cells and pre-B lymphocytes [5–9]. For such interactions to occur, immune cells may require specific receptors to mediate an intracellular event that leads to enhancement or suppression of immune processes. More recently, serotonin receptors have been identified and partially characterized on Jurkat cells [10]. Pharmacologic analysis revealed that these receptors resemble the 5-HT1a receptors found in the brain. Sympathetic innervation of lymphoid tissue has been described [11]. Mann *et al.* [12] demonstrated β-adrenergic receptors on peripheral lymphocytes that can be used as an accessible and convenient marker of β-adrenergic responsiveness. Furthermore, LeFur *et al.* [13] reported the presence of specific binding sites for the D2 dopamine receptor antagonist spiroperidol on rat and human peripheral lymphocytes. A significant increase in [^{3}H]spiroperidol binding was described in lymphocytes of schizophrenic patients [14–16]. In contrast, a decrease in the binding capacity for the dopamine receptor has been detected in lymphocytes of patients with idiopathic Parkinson's disease [17], a neurological disorder characterized by pronounced degeneration of dopaminergic neurons in basal ganglia.

The observation that human lymphocytes possess dopamine binding sites that share functional characteristics with those of D2 dopamine receptors in brain suggested to us that perhaps an uptake mechanism for dopamine may exist in human lymphocytes. In support of this thesis, we reported that freshly isolated lymphocytes from human blood are capable of active dopamine transport [18]. We also showed that cocaine, which is known to block the uptake of dopamine, serotonin and norepinephrine in central and peripheral sympathetic neurons [19–21], caused a marked inhibition of dopamine uptake in human lymphocytes. These findings raised two questions. How specific is the inhibition by cocaine of dopamine uptake into human lymphocytes? Do the lymphocytes isolated from the blood of chronic cocaine abusers transport dopamine differently from the lymphocytes of non-cocaine users? The aim of the present investigation, therefore, was to determine the structure–activity relationship of cocaine and its congeners in inhibiting the uptake of [^{3}H]dopamine by human lymphocytes. Moreover, preliminary evidence is presented linking the possible relationship between decreased lymphocyte [^{3}H]dopamine uptake and chronic cocaine abuse among cocaine addicts.

MATERIALS AND METHODS

Materials

The chemicals used were purchased from the following sources: *l*-cocaine hydrochloride, ecgonine methyl ester hydrochloride, (WIN 35,428¶, RTI-55, α-CIT, GBR 12909, Lu 19-005, bupropion dihydrochloride and nomifensine from Research Biochemical, Inc. (Natick, MA); dopamine hydrochloride, ouabain octahydrate, iodoacetic acid (sodium salt), phloridzin, gramicidin, and Hanks' balanced salts containing calcium chloride (0.185 g/L) from the Sigma Chemical Co. (St. Louis, MO); and 3,4[ring-2,5,6-^{3}H]dopamine hydrochloride

* Presented in part at the 55th Annual Scientific Meeting of the College on Problems of Drug Dependence, Inc., June 12–17, 1993, Toronto, Canada.

‡ Corresponding author: Bahjat A. Faraj, Ph.D., Department of Radiology, Emory University School of Medicine, 1215 Woodruff Memorial Building, Atlanta, GA 30322. Tel. (404) 727-5901; FAX (404) 727-3488.

¶ Abbreviations: WIN 35,428, (2β-carbomethoxy-3β-(4-fluorophenyl)tropane 1,5-naphthalene disulfonate; WIN 35,065-2, 2β-carbomethoxy-3β-phenyltropane hydrochloride; WIN 35,065-3, an enantiomer of WIN 35,065-2; RTI-55, 2β-carbomethoxy-3β-(4-iodophenyl)tropane tartrate; α-CIT, 2α-carbomethoxy-3β-(4-iodophenyl)tropane 1,5-naphthalene disulfonate; GBR 12909, 1-[2-bis(4-fluorophenylmethoxy)ethyl]-4-(3-phenylpropyl)-piperazine dihydrochloride; Lu 19-005, (±)*trans*-3-(3,4-dichlorophenyl)-*N*-methyl-1-indanamine hydrochloride; RPMI 1640, tissue culture medium supplemented with 10% calf serum and 1% glutamine; and LSM, lymphocyte separating medium.

(sp. act. 31 Ci/mmol) from the Dupont Co. Biotechnology Systems (Wilmington, DE). A RPMI 1640 was obtained from Gibco Laboratories (Life Technologies, Inc., Grand Island, NY); and LSM from Organon Teknika (Durham, NC). The cocaine analogs WIN 35,065-2, and WIN 35,065-3 were obtained from the National Institute on Drug Abuse, Addiction Research Center (Baltimore, MD). All other chemicals were of analytical grade. Mazindol was a gift from the Sandoz Research Institute (Hanover, NJ).

Subjects

The cocaine addicts evaluated in this study consisted of six female and four male adults between the ages of 20 and 45 years (31 ± 14). Of these addicts, six were black and four were white. They were admitted for inpatient treatment at the Addictive Disorders Unit of Emory University Hospital. The cocaine addicts selected for this investigation met the DSM-III-R criteria for cocaine dependence [22]. The patients had positive urine drug screen for cocaine upon initial presentation to the hospital. Cocaine addicts who were co-dependent on alcohol and other substances of abuse were excluded from the study. Patients who were HIV positive or had active liver disease, cancer, or primary psychiatric illness were excluded from the study. The control group consisted of six female and four male adults that were age-, race- and sex-matched with the patients evaluated in this study. They were members of the faculty and staff of Emory University School of Medicine. These individuals had no history of alcoholism or cocaine addiction, had no physical or psychiatric illness, were on no medications, and had no history of any chronic medical problem. Informed consent, approved by the Human Investigations Committee of Emory University, was obtained from all subjects prior to enrollment in the study. Blood samples were taken in the morning after an overnight fast.

Sample collection

Blood samples for the determination of [^{3}H]dopamine uptake were drawn from a peripheral vein into evacuated tubes containing heparin as an anticoagulant.

Preparation of lymphocytes

Human lymphocytes were isolated on a Ficoll Hypaque gradient according to a modified procedure of Faraj *et al.* [18]. Ninety milliliters of heparinized blood was layered over 30 mL of LSM, and this was centrifuged at 400 *g* for 30 min at 18°. The upper plasma layer was aspirated and discarded. The mononuclear cell layer containing lymphocytes, platelets, and monocytes was withdrawn with a plastic transfer pipette into a tissue culture flask (Corning) and diluted with RPMI 1640 and incubated for 30 min at 37°. Nonadherent cells (mainly lymphocytes) were dislodged from adherent monocytes by gentle agitation and poured into a polypropylene graduated conical tube and centrifuged at 200 *g* for 30 min at 18°. The supernatant containing platelets was removed and discarded. The lymphocyte pellet was washed three times with Hanks' buffer and centrifuged as described above; the final pellet was resuspended in Hanks' buffer and counted with a hemocytometer. Viability of isolated suspended lymphocytes assessed by trypan blue exclusion was found to be greater than 90%. Contamination with platelets accounted for less than 1% of the total lymphocyte preparation.

[^{3}H]Dopamine uptake assay

The [^{3}H]dopamine uptake assay was performed by incubating 1×10^7 cells of suspended lymphocytes in Hanks' buffer (1 mL) (pH 7.2) with a 3–500 nM concentration of [^{3}H]dopamine for 10 min at 37°. The incubation buffer contained L-ascorbate (10^{-5} M) and pargyline (10^{-5} M) to retard oxidative metabolism of dopamine. For competition experiments, each assay tube contained buffer or buffer plus test drug (1–100,000 nM), [^{3}H]dopamine (20 nM) and 1 mL of lymphocyte suspension (1×10^7 cells). The incubation was terminated by removing the samples to an ice water bath for 2–4 min, and then the mixture was centrifuged at 400 *g* for 15 min at 10° to collect particulate matter. The supernatant was discarded, and the lymphocyte layer was washed twice with 1 mL of Hanks' buffer, as previously described. The lymphocyte pellet was then homogenized in 1 mL of buffer by sonication (3 times at 15 sec at 40 W). An aliquot of the lymphocyte homogenate (0.5 mL) was added to a scintillation vial containing 10 mL of liquid scintillation fluid. The radioactivity in the sample was counted to an accuracy of ±2%. Non-specific accumulation of [^{3}H]dopamine was determined by measuring the uptake of [^{3}H]dopamine in the presence of 100 μM non-radioactive dopamine.

Statistical analysis

Correlation analyses were done using Pearson products moment estimates of linear association. Comparison of lymphocyte [^{3}H]dopamine uptake between cocaine addicts and controls was done using the Kruskal–Wallis test. Correlation between IC_{50} values obtained for the compounds in inhibiting the uptake of [^{3}H]dopamine by human lymphocytes with IC_{50} values reported for the same compounds in inhibiting the uptake of [^{3}H]dopamine by human lymphocytes with IC_{50} values reported for the same compounds in inhibiting the uptake of [^{3}H]dopamine into brain tissue preparation was determined by linear regression analysis. Statistical significance was set at the 0.05 level.

RESULTS

General characteristics of lymphocyte [^{3}H]dopamine uptake

The uptake of [^{3}H]dopamine by human lymphocytes was concentration dependent, with evidence of saturable kinetics (Fig. 1). Specific uptake of [^{3}H]dopamine, obtained by subtracting the non-specific uptake in the presence of 100 μM dopamine from total uptake, was 5–6 times greater than non-specific uptake. Kinetic analysis of the uptake data as computed by regression analysis from Lineweaver–Burk plots yielded a K_m of 102 ± 20 nM and a V_{max} of 10.0 ± 3.11 pmol/10^7 cells. When NaCl was present in the uptake buffer, uptake of [^{3}H]dopamine at a concentration of 20 nM was very rapid and increased with time. The uptake was linear for at least 10 min; this incubation time was used in further experiments. However, when NaCl in the uptake buffer was replaced by choline chloride, uptake of [^{3}H]dopamine was reduced markedly at all time points (Fig. 2). The sodium-dependent dopamine uptake into lymphocytes

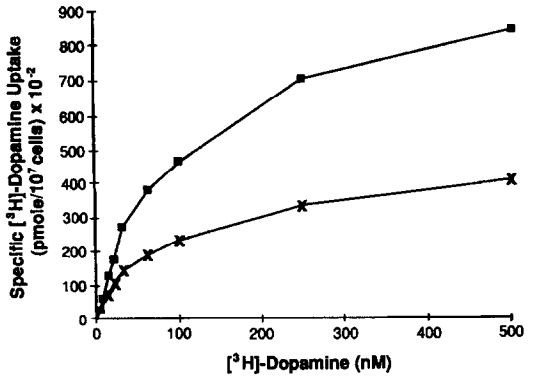


Fig. 1. Uptake velocity observed in the presence of various concentrations of [^{3}H]dopamine (3–500 nM) in lymphocytes isolated from blood of controls (■—■) and cocaine addicts (×—×). Each point represents the average of six experiments. The range was <10% of each value.

exhibited an absolute requirement for Cl^-. Substitution of Cl^- with gluconate drastically reduced the uptake of [^{3}H]dopamine. The uptake of [^{3}H]dopamine by human lymphocytes was also temperature dependent. Maximal uptake occurred at 37°. At 0–4°, specific uptake was represented by about 5% of the uptake that occurred at 37° (Fig. 2).

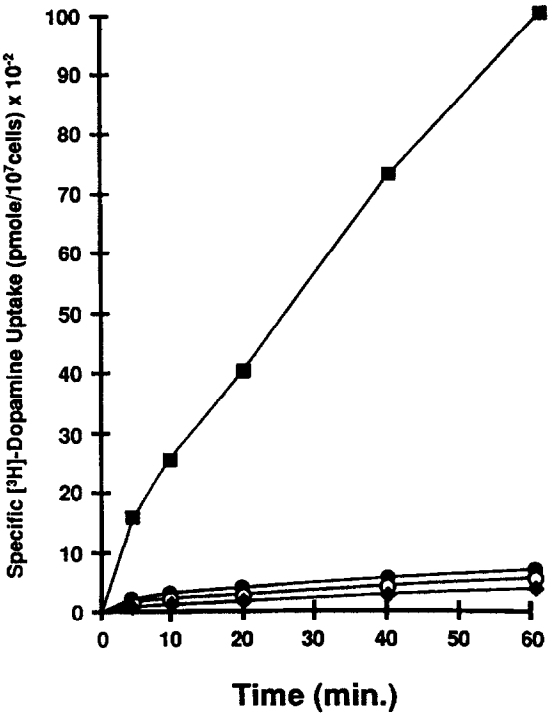


Fig. 2. Time course of [^{3}H]dopamine uptake by human lymphocytes. Uptake of [^{3}H]dopamine by lymphocytes was determined in a medium containing NaCl (■—■), choline chloride (●—●), and sodium gluconate (○—○). Uptake of [^{3}H]dopamine by lymphocytes in a medium containing NaCl at 4° (♦—♦) was also measured. The concentration of [^{3}H]dopamine in the uptake medium was 20 nM. Each point represents the average of five experiments. The range was <10% of each value.

Structure–activity relationship for the ability of cocaine and its congeners to inhibit [^{3}H]dopamine uptake by human lymphocytes

[^{3}H]Dopamine uptake by human lymphocytes was inhibited by cocaine and its congeners with a wide range of potencies. The result of this study showed that stereochemistry was very important for determining the potency of cocaine. WIN 35,065-3 is a pure enantiomer of WIN 35,065-2; the two compounds are *d*- and *l*-isomers, respectively, of the phenyltropane analog of cocaine. The *l*-isomer (WIN 35,065-2) was markedly more effective in inhibiting the uptake of [^{3}H]dopamine by human lymphocytes ($IC_{50} = 70 \pm 20$ nM) than its *d*-isomer (WIN 35,065-3; IC_{50} = 15,000 ± 2,000 nM) (Table 1, Fig. 3). Epimerization of RTI-55 at C-2 produced α-CIT, which was 800-fold less potent than RTI-55 in inhibiting the uptake of [^{3}H]dopamine into human lymphocytes (Fig. 3, Table 1). Modification to the C-3 substituent produced significant changes in inhibitory potency. Substitution of an hydroxyl group for the C-3-benzoxy group of *l*-cocaine resulted in the inactive compound *l*-ecgonine methyl ester. *l*-Ecgonine methyl ester (IC_{50} > 100,000 nM) was about 1000-fold less potent than *l*-cocaine (IC_{50} = 100 ± 30 nM) in inhibiting the uptake of [^{3}H]dopamine by human lymphocytes (Table 1). In contrast, elimination of the ester linkage between the phenyl group and the tropane ring of cocaine, resulting in direct attachment of the phenyl group to the C-3 carbon, produced cocaine analogs that were more potent than cocaine in inhibiting the uptake of [^{3}H]dopamine by human lymphocytes (RTI-55 > WIN 35,428 > WIN 35,065-2 > *l*-cocaine) (Fig. 3, Table 1).

Effects of other monoamine uptake inhibitors on lymphocyte [^{3}H]dopamine uptake

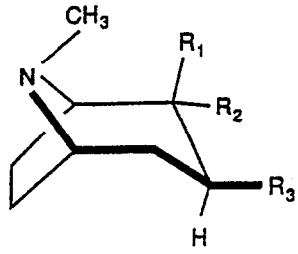
Mazindol, Lu 19-005, GBR 12909, bupropion and nomifensine all inhibited the uptake of [^{3}H]dopamine in a concentration-dependent manner, with Lu 19-005 and mazindol being the most potent compounds (Table 2).

Studies in humans

Patient profile. A majority of the subjects evaluated in the study smoked crack cocaine. The average amount of cocaine used was 5 g/week. The reported presence of alcoholism and drug addiction among immediate family members of cocaine addicts studied here was about 50%.

Characteristics of [^{3}H]dopamine uptake into lymphocytes of cocaine addicts and controls. Dopamine uptake was determined in lymphocytes isolated from the blood of cocaine addicts and controls. In this preliminary investigation, a significant ($P < 0.002$) decrease in the uptake of [^{3}H]dopamine (assayed at 100 nM [^{3}H]dopamine concentration) was observed in lymphocytes of cocaine addicts (N = 10; velocity = 2.0 ± 0.75 pmol/10^7 cells) as compared with controls (N = 10; velocity = 4.8 ± 1.12 pmol/10^7 cells) (Figs. 1 and 4). Kinetic analysis revealed a substantially lower ($P < 0.01$) maximal velocity (V_{max}) for the uptake of [^{3}H]dopamine in lymphocytes of cocaine addicts (V_{max} = 4.0 ± 2.22 pmol/10^7 cells) as compared with that of controls ($V_{max} = 10 \pm 3.1$ pmol/10^7 cells). The changes in the values for the apparent affinity constant (K_m) of the uptake system for

Table 1. Inhibition of lymphocyte [^{3}H]dopamine uptake by cocaine congeners*



Compound	R_1	R_2	R_3	IC_{50}† (nM)
(−)Cocaine	$COOCH_3$	H	$OCOC_6H_5$	100 ± 30
WIN 35,065-2	$COOCH_3$	H	C_6H_5	70 ± 20
WIN 35,065-3	$COOCH_3$	H	C_6H_5	15,000 ± 2,000
WIN 35,428	$COOCH_3$	H	C_6H_4F	25 ± 5.1
RTI-55	$COOCH_3$	H	C_6H_4I	1.9 ± 0.3
α-CIT	H	$COOCH_3$	C_6H_4I	850 ± 51
Ecgonine methyl ester	$COOCH_3$	H	OH	>100,000

* [^{3}H]Dopamine (20 nM) and lymphocyte preparation (1×10^7 cells/mL) were incubated with various concentrations of competing drugs (1–100,000 nM), as described in Materials and Methods.

† The IC_{50} values represent the means ± SD of four or five independent experiments, each performed in duplicate; IC_{50} is defined as the concentration of the drug required to produce 50% inhibiton of the specific uptake of [^{3}H]dopamine by human lymphocytes.

[^{3}H]dopamine in lymphocytes of cocaine addicts versus controls were not significant (cocaine addicts, $K_m = 63 \pm 29$ nM; controls, $K_m = 102 \pm 20$ nM).

This preliminary study showed that among cocaine addicts no significant difference in lymphocyte dopamine uptake was observed between African-Americans and Caucasians, as well as between male and female subjects. Also, no difference in dopamine uptake was observed in lymphocytes of white versus black and male versus female individuals within the control group.

Correlation analysis. A significantly inverse association was noted between the V_{max} of [^{3}H]dopamine uptake and the reported amount of cocaine used ($y = -0.403x + 6.421$; $r = 0.60$, $P < 0.05$) (Fig. 5). A significant correlation was noted between IC_{50} values of cocaine analogs and non-tropane compounds that inhibited the uptake of [^{3}H]dopamine by lymphocytes with that of the same compounds in inhibiting the uptake of [^{3}H]dopamine by brain (Fig. 6; $y = 0.76x + 0.36$; $r = 0.77$; $P < 0.005$).

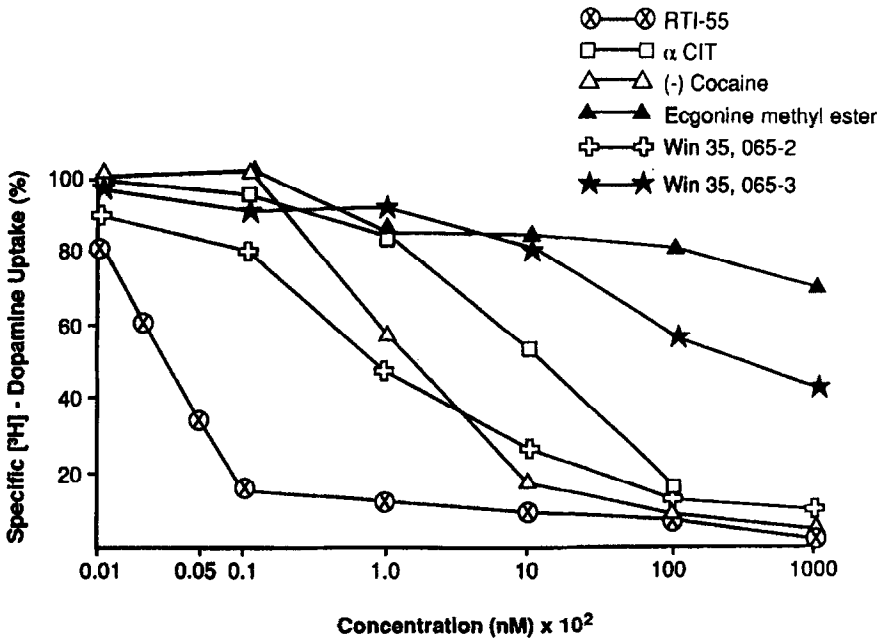


Fig. 3. Inhibition curves depicting pharmacological sensitivity of [^{3}H]dopamine uptake (20 nM) by human lymphocytes in the presence of various concentrations of cocaine congeners [(−)cocaine, RTI-55, α-CIT, WIN 35,065-2, WIN 35,065-3, and ecgonine methyl ester]. Each point represents the average of five experiments. The range was <10% of each value.

Table 2. Comparison of the inhibition of [^{3}H]dopamine uptake in lymphocytes by cocaine analogs and other monoamine uptake inhibitors with the reported inhibition of these compounds of the uptake of [^{3}H]dopamine in brain preparations

Compound	IC_{50} (nmol/L) Lymphocyte [^{3}H]dopamine uptake	Brain [^{3}H]dopamine uptake
Phenyltropane		
(–)Cocaine	100	600 [23]
WIN 35,428	25	100 [24]
WIN 35,065-2	70	400 [25,26]
WIN 35,065-3*	15,000	10,000 [25,26]
RTI-55	2.00	1.6 [27]
α-CIT	850	87 [27]
Ecgonine methyl ester	>100,000	>10,000 [24]
Non-phenyltropane		
GBR 12909	1,000	1 [28]
Lu 19-005	3.5	12 [29]
Mazindol	30	29 [30]
Nomifensine	700	240 [31]
Bupropion	10,000	1,300 [32]

* Compound WIN 35,065-3 is an enantiomer of WIN 35,065-2.

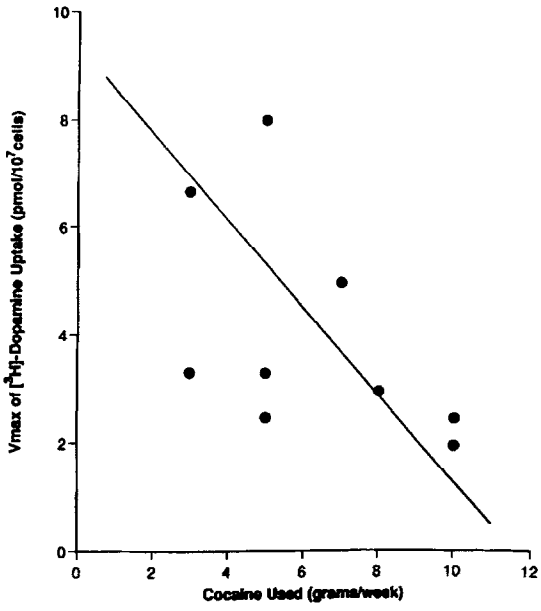


Fig. 5. Inverse relationship between the V_{max} of [^{3}H]dopamine uptake into lymphocytes of cocaine addicts with the amount of cocaine used among these subjects ($y = -0.403x + 6.421$; $r = 0.60$, $P < 0.05$).

DISCUSSION

Data presented in this study demonstrate that lymphocytes from human blood possess a high-affinity active uptake process for dopamine. This uptake system shares certain characteristics that are in common with the active transport of monoamine neurotransmitters in neurons of the peripheral and central nervous systems [19–21]. These characteristics include: (a) dependence on a sodium and potassium gradient across the cell membrane, (b) the ability to transport substrate "uphill" against a concentration gradient, (c) saturability, i.e. initial uptake rates follow Michaelis–Menten kinetics, (d) temperature sensitivity, and (e) specificity for the inhibition of the uptake by certain inhibitors of monoamine transport.

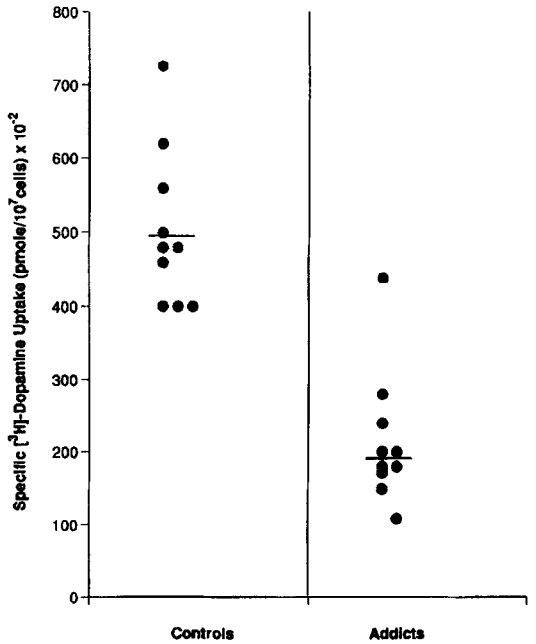


Fig. 4. Scattergram of [^{3}H]dopamine uptake by lymphocytes of controls and cocaine addicts.

Cocaine, which has been shown to block the neuronal uptake of dopamine, serotonin and norepinephrine [19, 21], caused a marked inhibition of dopamine uptake in human lymphocytes. Furthermore, structure–activity relationships were determined for cocaine and its congeners to inhibit the uptake of [^{3}H]dopamine by human lymphocytes. The structure of cocaine consists of a central tropane ring with substituent groups at C-2 and C-3 (Table 1). Both substituent groups are attached in the β-configuration, relative to the tropane nitrogen, and the molecule exists as the levorotatory (*l*) isomer. Analogs of cocaine representing these three major structural modifications were tested in this study.

Stereochemistry was very important for determining the potency of cocaine in inhibiting the uptake of dopamine by human lymphocytes. WIN 35,065-3 is a pure enantiomer of WIN 35,065-2; the two compounds are *d*- and *l*-isomers, respectively, of the phenyltropane analog of cocaine. WIN 35,065-3 was about 200-fold less potent than WIN 35,065-2 in inhibiting the uptake of dopamine by human lymphocytes. This was in agreement with Clarke *et al.* [25] and Kuhar *et al.* [26], who demonstrated similar stereospecificity for the interaction of these compounds with the dopamine transporter in neuronal tissues. The marked stereospecificity noted here was comparable to that of *l*-cocaine, which was exceedingly more potent than *d*-cocaine in inhibiting the uptake of dopamine by neuronal tissue [24]. Stereochemistry at C-2 has the second largest effect on activity. Epimerization of 2β-carbomethoxy-3β-(4-iodophenyl)tropane (designated as β-CIT under the code RTI-55), producing

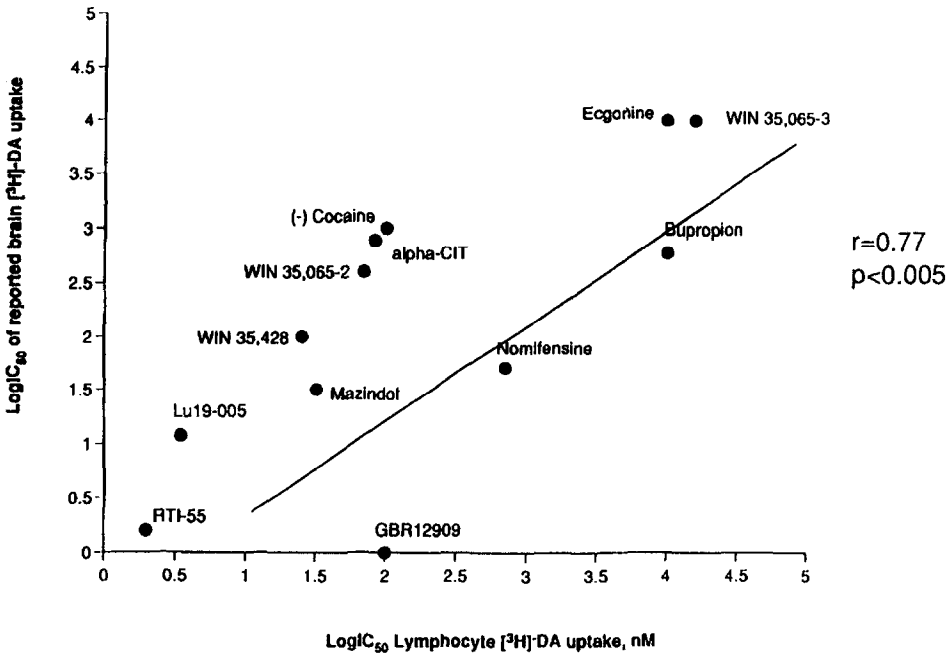


Fig. 6. Correlation between the IC_{50} values of various phenyltropane and non-phenyltropane compounds in inhibiting the uptake of [^{3}H]dopamine (DA) into lymphocytes with the IC_{50} values of the same compounds in inhibiting the uptake of [^{3}H]dopamine into brain ($P < 0.005$, $r = 0.77$).

α-CIT, resulted in an 800-fold decrease in inhibiting the uptake of [^{3}H]dopamine by lymphocytes. These results were consistent with the findings of Neumeyer *et al.* [27], who demonstrated that β-CIT (RTI-55) had a much higher affinity for the dopamine transporter in baboon and rat brains than its C-2 epimer α-CIT.

Moreover, the uptake of dopamine into lymphocytes was affected markedly by cocaine analogs with various substituents at the C-3 position. Phenyltropane derivatives of cocaine, which lack an ester link between the phenyl group and the tropane ring and have para-substitutions on the phenyl ring, are 10–100 times more potent than cocaine in inhibiting the uptake of dopamine by human lymphocytes. Our findings are in agreement with those of Boja *et al.* [33], Cline *et al.* [34] and Madras *et al.* [35], who demonstrated that these compounds were more potent than cocaine in binding to the cocaine receptor and in inhibiting the uptake of [^{3}H]dopamine by brain synaptosomal preparations.

A key structural feature of the phenyltropane molecule required for the inhibition of the uptake of dopamine is the existence of a phenyl ring moiety at C-3. Ecgonine methyl ester, a hydrolytic metabolite of cocaine, was a very weak inhibitor of dopamine uptake in human lymphocytes. This is in agreement with previously reported studies [24], which demonstrated that ecgonine methyl ester was a poor inhibitor of dopamine uptake in brain synaptosomal preparation.

Moreover, we compared the IC_{50} values obtained for the cocaine analogs used in inhibiting the uptake of [^{3}H]dopamine by human lymphocytes with the IC_{50} values reported for the same compounds in inhibiting the uptake of [^{3}H]dopamine by brain synaptosomal preparations. A significant correlation was noted between the IC_{50} of these compounds in inhibiting the uptake of [^{3}H]dopamine into lymphocytes with that of the uptake of [^{3}H]dopamine into brain. With the exception of GBR 12909, a similar association was noted between the IC_{50} of the non-tropane compounds (Lu 19-005, mazindol, nomifensine and bupropion) in inhibiting the uptake of [^{3}H]dopamine by human lymphocytes with that of the uptake of [^{3}H]dopamine by animal brain tissue preparations (Fig. 6). It is not certain as to why GBR 12909, a very potent and selective inhibitor of dopamine transporter [28], was a thousand-fold less potent in inhibiting the uptake of [^{3}H]dopamine into lymphocytes as compared with the inhibition of the uptake of [^{3}H]dopamine into brain. The results with GBR 12909 suggest that the uptake of dopamine by lymphocytes occurs via a monoamine transporter system that may not be identical to the one described in brain [36]. Recently, Ramamoorthy *et al.* [37] provided evidence for the existence of a high-affinity uptake system for dopamine in human placental syncytiotrophoblast. The uptake of dopamine was inhibited very effectively by cocaine but much less effectively by bupropion and GBR 12909. Molecular and pharmacologic studies of this uptake process indicated that it is the norepinephrine transporter, not the dopamine transporter, that is responsible for the uptake.

Apart from the fulfillment of a specific structural activity requirement, the inhibition of [^{3}H]dopamine by cocaine occurred at pharmacologically significant concentrations. Blood levels of cocaine in humans that are associated with the subjective feelings of a "high" are in the nanomolar range [38]. This concentration range is in close proximity to the IC_{50} of cocaine in inhibiting the uptake of dopamine by human lymphocytes. This presented us with an opportunity to use the lymphocyte preparation as a readily accessible tissue to examine the influence of cocaine use on the uptake of [^{3}H]dopamine in humans. Consequently, we carried out a preliminary study to determine whether or not the uptake of

[^{3}H]dopamine by lymphocytes of chronic cocaine abusers is different from that of non-users.

The results of this preliminary study demonstrated that cocaine addicts, upon initial presentation to the hospital for in-patient treatment, had a significant decrease in the uptake of [^{3}H]dopamine by their lymphocytes compared with controls. Kinetic analysis revealed that changes in uptake among cocaine addicts were due primarily to a change in maximum velocity (V_{max}) with only a moderate change in the affinity constant (K_m) of the uptake system toward dopamine. In spite of individual variability, we noted a significantly inverse correlation between the V_{max} of [^{3}H]dopamine uptake and the reported amount of cocaine used. The results noted here of decreased dopamine uptake by lymphocytes of cocaine addicts closely resemble the diminished uptake of serotonin in platelets of cocaine patients [39].

In view of the disproportionate representation of blacks among cocaine addicts, it is imperative that racial variability be taken into consideration before conclusions on differences in lymphocyte dopamine uptake can be drawn between addicts and non-addicts. Appropriate statistical analysis was performed and the results of the study revealed that the difference in dopamine uptake between the two groups was not race-related.

The effects of cocaine abuse on dopamine uptake into peripheral blood lymphocytes noted in this study may be mediated by direct and indirect mechanisms. The direct mechanism may be representative of the blockage of neuronal dopamine reuptake by cocaine. Inhibition of neuronal uptake of released dopamine by cocaine may expose more of the dopamine to metabolism in non-neuronal tissues. In support of this thesis, Faraj *et al.* [40] observed that cocaine abuse caused a marked increase in the concentration of the extracellular metabolite of dopamine, dopamine sulfate, in the plasma of chronic cocaine abusers. The indirect mechanism may be indicative of putative alterations in dopamine uptake, probably as a result of long-term neuronal adaptation to chronic cocaine exposure [41].

Inhibition of the active transport of dopamine in lymphocytes by cocaine may have significant implications. Lymphocytes represent a critical effector arm of the immune system and are responsible for both the cell-mediated responses (T-lymphocytes) and antibody production, or the humoral response (B-lymphocytes). Like the opiates, cocaine is known to alter certain immune functions [42, 43]. Effects of cocaine on immune function represent an important concern in light of the findings that drug abusers have a much greater risk of exposure to HIV and the subsequent development of AIDS [44]. While sharing of HIV-contaminated drug paraphernalia is most likely the primary culprit for the elevated rate of HIV infectivity, there is now some evidence to suggest that cocaine and other drugs of abuse themselves may also be important contributors. Peterson *et al.* [45] demonstrated that cocaine potentiated the replication of HIV-1 in human lymphocytes. It remains to be established whether the inhibition of dopamine transport by cocaine in human lymphocytes partly contributes to the effect of cocaine on immune function. Van Dyke *et al.* [46] suggested that the significant increase in natural killer cell activity following exposure of human subjects to cocaine *in vivo* may be related to the stimulatory effect of cocaine on catecholamine release or to an inhibition of catecholamine reuptake.

In summary, human lymphocytes possess a high-affinity transport system for dopamine. The transport system exhibits characteristics that are similar to those of the transport of monoamines in neuronal tissues. The data presented here showed that the uptake of dopamine is markedly sensitive to cocaine inhibition. Important similarities were found among the structure–activity relationship for cocaine and its analogs in inhibiting the uptake of dopamine in human lymphocytes to that reported in neuronal tissues. The uptake of dopamine in lymphocytes isolated from the blood of cocaine addicts was reduced significantly as compared with controls, and an inverse relationship was noted between the V_{max} for the uptake and the severity of cocaine use among these subjects. This may be an important finding in view of the recent report that the pleasurable effects of cocaine and the reinforcement that makes it habit-forming have been linked specifically to its inhibition of dopamine uptake [47]. This raises the possibility, therefore, that the measurement of plasma dopamine uptake in peripheral blood lymphocytes in conjunction with the measurement of plasma dopamine sulfate [40] may potentially present us with a specific biochemical marker for assessing the severity of cocaine addiction in humans.

Acknowledgements—This project was supported, in part, by BRSGS07RR05364 awarded by the Biomedical Research Support Grant Program, Division of Research Resources, National Institute of Health and by funds from Otolaryngology Research Department of Surgery, Emory University School of Medicine.

REFERENCES

1. Farrar WL, Hill JM, Harel-Bellan A and Vinocour M, The immune logical brain. *Immunol Rev* **100:** 362–378, 1987.
2. Ader R, Felten D and Cohen N, Interactions between the brain and the immune system. *Annu Rev Pharmacol Toxicol* **30:** 561–602, 1990.
3. Neveu PJ and LeMoal M, Physiological basis for neuroimmunomodulation. *Fundam Clin Pharmacol* **4:** 281–286, 1990.
4. Plau M, Lymphocyte hormone receptors. *Annu Rev Immunol* **5:** 621–669, 1987.
5. Hellstrand K and Hermodsson S, Role of serotonin in the regulation of human natural killer cell cytotoxicity. *J Immunol* **139:** 869–875, 1987.
6. Sternberg EM, Trial J and Parker CW, Effect of serotonin on murine macrophages: Suppression of Ia expression by serotonin and its reversal by 5-HT$_2$ serotonergic receptor antagonists. *J Immunol* **137:** 276–282, 1986.
7. Choquet D and Korn H, Dual effects of serotonin on a voltage-gated conductance in lymphocytes. *Proc Natl Acad Sci USA* **85:** 4557–4561, 1988.
8. Nordlind K and Sundstrom E, Different modulating effects of the monoamines, adrenaline, noradrenaline and serotonin on the DNA synthesis response of human peripheral blood T lymphocytes activated by mercuric chloride and nickel sulfate. *Int Arch Allergy Appl Immunol* **87:** 317–320, 1988.
9. Kut JL, Young MRI, Crayton JW, Wright MA and Young ME, Regulation of murine T-lymphocyte function and exogenous serotonin. *Immunopharmacol Immunotoxicol* **14:** 783–796, 1992.
10. Aune TM, McGrath KM, Sarr T, Bombara MP and Kelley KA, Expression of 5HT1a receptors on activated human T cells. Regulation of cyclic AMP levels and T cell proliferation by 5-hydroxytryptamine. *J Immunol* **151:** 1175–1183, 1993.
11. Felten DL, Felten SY, Carlson JA, Olschowka JA and

Livnat S, Noradrenergic and peptidergic innervation of lymphoid tissue. *J Immunol* **135:** 755s–765s, 1985.
12. Mann JJ, Brown RP, Halper JP, Sweeney JA, Kocsis JH, Stokes PE and Bilezikian JP, Reduced sensitivity of lymphocyte beta-adrenergic receptors in patients with endogenous depression and psychomotor agitation. *N Engl J Med* **313:** 715–720, 1985.
13. Le Fur G, Phan T and Uzan A, Identification of stereospecific [^{3}H]spiroperidol binding sites in mammalian lymphocytes. *Life Sci* **26:** 1139–1148, 1980.
14. Halbach M and Henning U, Abnormal glucocorticoid dependent increase of spiperone binding sites on lymphocytes from schizophrenics *in vitro*. *Pharmacopsychiatry* **22:** 168–173, 1989.
15. Grodzicki J, Pardo M, Schved G, Schlosberg A, Fuchs S and Kanety H, Differences in [^{3}H]-spiperone binding to peripheral blood lymphocytes from neuroleptic responsive and nonresponsive schizophrenic patients. *Biol Psychiatry* **27:** 1327–1330, 1990.
16. Bondy B and Ackenheil M, ^{3}H-Spiperone binding sites in lymphocytes as possible vulnerability marker in schizophrenia. *J Psychiatr Res* **21:** 521–529, 1987.
17. Le Fur G, Meininger V, Phan T, Gérard A, Baulac M and Uzan A, Decrease in lymphocyte [^{3}H]spiroperidol binding sites in Parkinsonism. *Life Sci* **27:** 1587–1591, 1980.
18. Faraj BA, Olkowski ZL and Jackson RT, Binding of [^{3}H]-dopamine to human lymphocytes: Possible relationship to neurotransmitter uptake sites. *Pharmacology* **42:** 135–141, 1991.
19. Iversen LL, Uptake processes for biogenic amines. In: *Biochemistry of Biogenic Amines* (Eds. Iversen LL, Iversen SD and Snyder SH), Vol. 3, pp. 381–442. Plenum Press, New York, 1975.
20. Horn AS, Dopamine uptake: A review of progress in the last decade. *Prog Neurobiol* **34:** 387–400, 1990.
21. Hendley D, Neurotransmitter uptake. In: *Handbook of Neurochemistry* (Ed. Lajtha A), Vol. 6, pp. 441–429. Plenum Press, New York, 1984.
22. American Psychiatric Association, *DSM-III-R: Statistical Manual of Mental Disorders*, 3rd Edn, revised. APA, Washington, DC, 1987.
23. Masserano JM, Venable D and Wyatt RJ, Effects of chronic cocaine administration on [^{3}H]dopamine uptake in the nucleus accumbens, striatum and frontal cortex of rats. *J Pharmacol Exp Ther* **270:** 133–141, 1994.
24. Ritz MC, Cone EJ and Kuhar MJ, Cocaine inhibition of ligand binding at dopamine, norepinephrine, and serotonin transporters: A structure–activity study. *Life Sci* **46:** 635–645, 1990.
25. Clarke RL, Daum SJ, Gambino AJ, Aceto MD, Pearl J, Levitt M, Cumiskey WR and Bogado EF, Compounds affecting the central nervous system. 4. 3β-Phenyltropane-2-carboxylic esters and analogs. *J Med Chem* **16:** 1260–1267, 1973.
26. Kuhar MJ, Ritz MD, Grigoriadis D and Lew R, A cocaine receptor associated with dopamine transport and drug self-administration. In: *Cocaine: Pharmacology, Physiology and Clinical Strategies* (Eds. Lakoski JM, Galloway MP and White FJ), pp. 191–202. CRC Press, Boca Raton, 1992.
27. Neumeyer JL, Wang S, Milius RA, Baldwin RM, Zea-Ponce Y, Hoffer PB, Sybirska E, Al-Tikriti M, Charney DS, Malison RT, Laruelle M and Innis RB, [^{123}I]-2β-Carbomethoxy-3-β-(4-iodophenyl)tropane: High-affinity SPECT radiotracer of monoamine reuptake sites in brain. *J Med Chem* **34:** 3144–3146, 1991.
28. Andersen PH, The dopamine uptake inhibitor GBR 12909: Selectivity and molecular mechanism of activity. *Eur J Pharmacol* **166:** 493–504, 1989.
29. Hyttel J and Larsen J, Neurochemical profile of Lu 19-005, a potent inhibitor of uptake of dopamine, norepinephrine and serotonin. *J Neurochem* **44:** 1615–1622, 1985.
30. Javitch JA, Blaustein RO and Snyder SH, [^{3}H]Mazinol binding associated with neuronal dopamine and norepinephrine uptake sites. *Mol Pharmacol* **26:** 35–44, 1984.
31. Schoemaker H, Pimoule C, Arbilla S, Scatton B, Javoy-Agid F and Langer SZ, Sodium dependent [^{3}H]cocaine binding associated with dopamine uptake sites in the rat striatum and human putamen decrease after dopaminergic denervation and in Parkinsons disease. *Naunyn Schmiedebergs Arch Pharmacol* **329:** 227–235, 1985.
32. Madras BK, Fahey MA, Bergman J, Canfield DR and Spealman RD, 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.
33. Boja JW, Carroll FI, Rahman MA, Phillip A, Lewin AH and Kuhar MJ, New potent cocaine analogs: Ligand binding and transport studies in rat striatum. *Eur J Pharmacol* **184:** 329–332, 1990.
34. Cline EJ, Scheffel U, Boja JW, Carroll FI, Katz JL and Kuhar MJ, Behavioral effects of novel cocaine analogs: A comparison with *in vivo* receptor binding potency. *J Pharmacol Exp Ther* **260:** 1174–1179, 1992.
35. Madras BK, Spealman RD, Fahey MA, Neumeyer JL, Saha JK and Milius RA, Cocaine receptors labeled by [^{3}H]2β-carbomethoxy-3β-(4-fluorophenyl) tropane. *Mol Pharmacol* **36:** 518–524, 1989.
36. Uhl GR, Neurotransmitter transporters (plus): A promising new gene family. *Trends Neurosci* **15:** 265–268, 1992.
37. Ramamoorthy S, Prasad PD, Kulanthaivel P, Leibach FH, Blakely RD and Ganapathy V, Expression of a cocaine-sensitive norepinephrine transporter in the human placental syncytiotrophoblast. *Biochemistry* **32:** 1346–1353, 1993.
38. Javaid JI, Fischman MW, Schuster CR, Dekirmenjan H and Dvais JM, Cocaine plasma concentration: Relation to physiological and subjective effects in humans. *Science* **202:** 227–228, 1978.
39. Dackis CA, Marcy A, Dackis P, Martin D, Pottash ALC and Gold MS, Platelet serotonin transporter in cocaine patients. In: *Problems of drug dependence* (Ed. Harris LS), NIDA Research Monograph 95, pp. 164–169. NIDA, Rockville, MD, 1989.
40. Faraj BA, Camp VM, Davis DC, Kutner M, Cotsonis GA and Holloway T, Elevated concentrations of dopamine sulfate in plasma of cocaine abusers. *Biochem Pharmacol* **46:** 1453–1457, 1993.
41. Zahniser NR, Peris J, Dwoskin LP, Curella P, Yasuda RP, O'Keefe L and Sally JB, Sensitization to cocaine in the nigrostriatal dopamine system. In: *Problems of Drug Dependence* (Ed. Harris LS), NIDA Research Monograph 95, pp. 55–77. NIDA, Rockville, MD, 1989.
42. Ou DW, Shen ML and Luo YD, Effects of cocaine on the immune system of Balb/C mice. *Clin Immunol Immunopathol* **52:** 305–312, 1989.
43. Bagasra O and Forman L, Functional analysis of lymphocyte subpopulations in experimental cocaine abuse. I. Dose-dependent activation of lymphocyte subsets. *Clin Exp Immunol* **77:** 289–293, 1989.
44. Ginzburg HM, Weiss SH, MacDonald MG and Hubbard RL, HTLV-III exposure among drug users. *Cancer Res* **45** (Suppl): 4605S–4608S, 1985.
45. Peterson PK, Gekker G, Chao CC, Schut R, Molitor TW and Balfour HH Jr, Cocaine potentiates HIV-1 replication in human peripheral blood mononuclear cell cultures. *J Immunol* **146:** 81–84, 1991.
46. Van Dyke C, Stesin A, Jones R, Chuntharapai A and Seaman W, Cocaine increases natural killer cell activity. *J Clin Invest* **77:** 1387–1390, 1986.
47. Kuhar MJ, Ritz MC and Boja JW, The dopamine hypothesis of the reinforcing properties of cocaine. *Trends Neurosci* **14:** 299–302, 1991.