Scatchard analysis suggested that the high-affinity site (0.34 nM K_D) shown under these conditions is the D_2 component of [^{3}H]haloperidol binding. The identity of the 3.3 nM K_D site is difficult to determine. Since R43448, serotonin and phentolamine did not inhibit that component of [^{3}H]haloperidol binding, it was unlikely that [^{3}H]haloperidol was binding to alpha-adrenoceptors or serotonergic receptors, even though haloperidol has been reported to compete with high affinity for alpha-1 adrenergic sites [19]. It is also unlikely that, under our assay conditions, we would be measuring a state of the D_2 receptor that has high affinity for dopamine agonists, as previously reported by Creese and coworkers [20].

The IC_{50} values for inhibition data (Fig. 1) for some neuroleptics (fluphenazine, clozapine, piflutixol, alpha-flupenthixol), but not for haloperidol, are similar to those for [^{3}H]flupenthixol binding (which corresponds to D_1 or dopamine stimulated adenylate cyclase sites [21–23]). However, the data are insufficient, as yet, to determine whether binding was also occurring at the D_1 site; clearly, a reevaluation of some [^{3}H]haloperidol binding studies is necessary, where only one set of sites has been assumed in the past.

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Cocaine: comparative effect on dopamine uptake in extrapyramidal and limbic systems

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Dopamine (DA) performs many roles in the central nervous system (CNS) [1–8]. First of all, it is the major neurotransmitter of the nigrostriatal (extrapyramidal) system. A notable advance in modern neurology has been the discovery that DA deficiency in this system leads to Parkinsonism and that replacement therapy via levo-3,4-dihydroxyphenylalanine (*l*-DOPA) corrects the disorder [3, 4].

The role of DA in the limbic system may be equally important though it is less well understood. It has been proposed that this DA system modulates emotional behavior and mood [1, 2, 4–8]. The DA cell bodies for this system are found in the ventral tegmental area of the mesencephalon. Axons of these neurons are projected to the olfactory bulbs, to the nucleus accumbens and associated nuclei (mesolimbic system) and to the prefrontal, cingulate and entorrhinal cortex (mesocortical system) [1]. It has been postulated that DA dysfunction may be responsible for schizophrenia and other emotional disorders [5]. This has been based largely on the fact that many neuroleptics are DA receptor antagonists [3, 5]. A defect in limbic DA neurotransmission would best fit this disorder. Moreover, it has been proposed that affective deterioration in such organic diseases as progressive supranuclear palsy and Parkinsonism may result from degeneration of limbic DA neurons [4, 6–8].

It is evident from animal studies that the extrapyramidal and limbic DA systems respond preferentially to various stimuli and drugs. It has been reported that the mesocortical and mesolimbic DA tracts respond to self-stimulation, long-acting neuroleptics, and foot-shock induced stress,

while in the striatum DA activity remains unperturbed by these stimuli [2]. We have reported similar findings in fighting animals and for still other conditions [7, 8]. DA also modulates release of certain pituitary hormones (tuberoinfundibular system) and performs still other CNS functions [1].

In the present communication, we report important quantitative differences in DA uptake inhibition by cocaine in synaptosomes obtained from the striatum (A9 extrapyramidal system) versus those obtained from the prefrontal cortex and olfactory bulbs (A10 DA limbic system). We used cocaine because it is a potent competitive inhibitor of DA uptake [9], an effect we have confirmed for the first time in the central nervous system following *in vivo* administration of cocaine [10]. Thus, cocaine may provide us with information concerning the affinity of DA uptake sites or carrier molecules in these respective DA systems.

Adult, male ICR mice were obtained from Flow Laboratories (Dublin, VA). They were individually housed under controlled conditions of temperature, humidity and lighting. They received standard lab. chow and water *ad lib.*

On the day of experimentation, they were killed by cervical dislocation and the brains were rapidly removed over ice. The striata, prefrontal cortices and olfactory bulbs were dissected, weighed and respectively homogenized in 0.32 M sucrose in a Teflon pestle, glass mortar, motor driven system (six gentle up-and-down strokes). The nuclei and debris were removed by centrifuging at 1000 *g*/10 min. The synaptosome-rich homogenate (3 mg of original tissue) was placed in a medium consisting of Krebs–Henseleit bicarbonate to which had been added 2 mg/ml glucose, 0.05 mg/ml Na_2EDTA, 0.2 mg/ml L-ascorbic acid, 10^{-5} M pargyline and 0.2 μM [^{3}H]DA (sp. act. 20.1 Ci/mmole, New England Nuclear Corp., Boston, MA). The samples were incubated for 5 min under 5% CO_2/95% O_2. Desmethylimipramine (DMI), 500 μM (USV Pharmaceuticals, Tuckahoe, NY). was added to prevent uptake of DA into norepinephrine (NE) terminals. Cocaine–HCl was added to various samples in concentrations ranging from 0.1 μM to 10 mM. For comparison, cocaine-free samples were incubated simultaneously to determine total uptake and also at 4° to determine passive diffusion (see Fig. 1). The reaction was stopped with ice-cold physiologic saline, and the tissue was pelleted in a refrigerated centrifuge and then extracted in a Triton-X100, POPOP/PPO* toluene mixture. Radioactivity was determined in a Beckman LS5600 liquid scintillation counter. Preliminary experiments were carried out to determine uptake velocities during a time interval, with a tissue concentration and at a substrate concentration for which uptake was linear.

Cocaine produced a striking dose-dependent inhibition of DA uptake in the striatum across the total range of drug concentrations measured. In contrast, in both the prefrontal cortex and the olfactory bulbs, uptake reached a plateau at 10 μM cocaine and below (Fig. 1). Determination of IC_{50} for cocaine uptake inhibition vs − log of cocaine concentration showed the following values for the three regions: 3.75 μM for striatum, 2.75 μM for olfactory bulbs, and 0.95 μM for prefrontal cortex.

The striking difference between the effect of cocaine on DA uptake in the striatum and the effects on the olfactory bulbs and prefrontal cortex could have been due to several factors acting alone or in concert. First, the prefrontal cortex and olfactory bulbs are relatively poor in DA terminals as compared with the striatum. Exogenous DA uptake may have occurred in neurotransmitter terminals not affected by cocaine or DMI. This possibility is supported by the similar IC_{50} inhibition values. Second, there may have been non-specific uptake of DA by still other

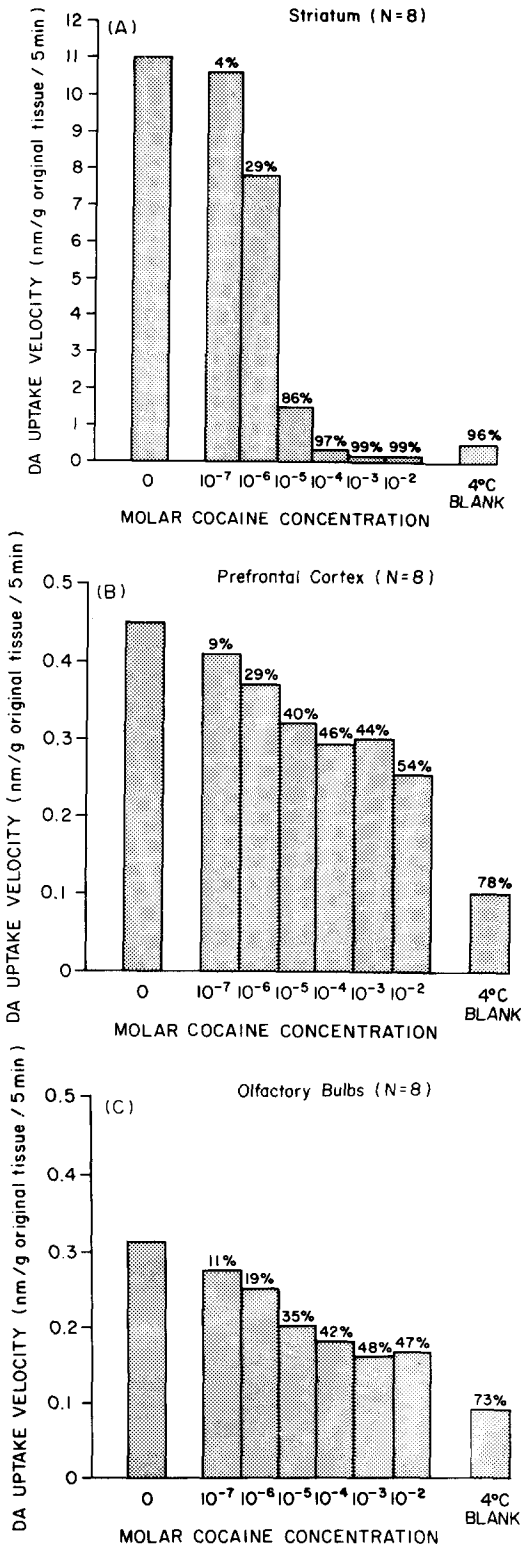

Fig. 1. Effect of cocaine on dopamine uptake in (A) striatum, (B) prefrontal cortex and (C) olfactory bulbs. DA uptake is expressed as velocity (nmoles DA/g of original tissue over a 5-min period) vs the molar concentration of cocaine. The percent inhibition produced by cocaine and 4°, as compared with the drug-free 37° value (first bar), is given at the top of the bars. The standard errors averaged less than 10% (4.4 to 16.6%).

* POPOP = 1,4-bis-[2-(4-methyl-5-phenylyoxazolyl)]-benzene; and PPO = 2,5-diphenylopazole.

structures that are more abundant in the prefrontal cortex and olfactory bulbs than in the striatum (compare the passive uptake values). Third, the properties of DA uptake receptor sites may be different in the limbic system than in the extrapyramidal system. This is consistent with the differential effects of certain neuroleptics on limbic and extrapyramidal DA activity as reported by others [2]. It is also consistent with the fact that cocaine inhibited DA uptake less effectively in the limbic system than did chilling (Fig. 1, panels B and C). If this latter mechanism contributed, even in part, to the present findings, then there may be important differences in DA neurotransmission in the various DA systems (i.e. a configurational difference in uptake sites in the respective carrier molecules). A search for such differences may lead us to better understand and treat "functional" and organic disorders related to DA activity.

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Hypoglycin toxicity: studies of ammonia metabolism

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Hypoglycin A, α-amino (methylenecyclopropyl) propionic acid, is an amino acid found in unripe ackee fruit and in the seeds of several varieties of maple trees [1, 2]. Ingestion in man causes Jamaican vomiting sickness, a disorder characterized by hypoglycemia [3], encephalopathy, small vacuole fatty degeneration of the viscera [4], and a distinctive organic aciduria dominated by acidic metabolites of hypoglycin and substrates of FAD-dependent dehydrogenases, e.g. glutarate, ethylmalonate and β-hydroxyisovalerate [5]. The precise biochemical mechanisms responsible for the *in vivo* blocks in gluconeogenesis and fatty acid metabolism are not clear. Hypoglycin A is metabolized by transamination and oxidative decarboxylation to (methylenecyclopropyl) acetyl-CoA (MCPA-CoA) [6]. The toxicity is due to simultaneous inhibition of several acyl-CoA dehydrogenases by MCPA-CoA [7, 8]. In addition, mitochondrial sequestration of carnitine and/or coenzyme A by MCPA and other fatty acids may contribute to the toxicity [9].

Pent-4-enoate is structurally related to MCPA, and administration to the rat causes hypoglycemia, hyperammonemia, encephalopathy and fatty degeneration of the liver [10], presumably due in part to inhibition of fatty acid oxidation by a metabolite, pent-2,4-dienoyl-CoA [11].

Jamaican vomiting sickness is also similar in many respects to Reye's syndrome, a human disorder of obscure etiology, in which hyperammonemia is common.

Similarities between Jamaican vomiting sickness, intoxication due to pent-4-enoate, and Reye's syndrome have prompted speculation of possible hyperammonemia in hypoglycin intoxication [12, 13]; however, direct measurement of blood ammonia in hypoglycin intoxication has not been reported.

Hypoglycin A was prepared from Jamaican ackee by the method of Kean [14]. It eluted from UR-30 anion exchange resin on a Beckman model 120 amino acid analyzer as a single peak which co-chromatographed with leucine and had a molecular weight of 141 by mass spectrometry. (Methylenecyclopropyl) acetic acid (MCPA) was prepared from the hypoglycin [6]; the trimethylsilyl (TMS)-ester chromatographed as a single peak (M.U. 11.72) by GC on OV-22 and had the appropriate molecular weight (184) and spectrum by combined gas chromatography-mass spectrometry.

Sprague–Dawley rats (approx. 150 g) were maintained on commercial rat chow. They were fasted but allowed free access to water beginning 15 hr prior to i.p. injection of