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# A Portion of [$^3$H]Cocaine Binding in Brain Is Associated with Serotonergic Neurons

M. E. A. REITH, H. SERSHEN, D. L. ALLEN, AND A. LAJTHA

*Center for Neurochemistry, Rockland Research Institute, Ward's Island, New York, New York 10035*

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SUMMARY

Three lines of evidence are brought forward in support of an association in the brain cortex of some, but not all, of the cocaine binding sites with serotonergic nerve terminals. The first is based upon the significant correlation observed between the inhibition of cocaine binding by various drugs and the inhibition of neuronal uptake of serotonin in the mouse cerebral cortex. The second is based upon the demonstration of cocaine binding in human blood platelets, a model system for central serotonergic neurons. The third comes from experiments in which rats were treated with *p*-chloroamphetamine and 5,7-dihydroxytryptamine (serotonin neurotoxins), 6-hydroxydopamine (catecholamine neurotoxin), or *p*-chlorophenylalanine (inhibitor of tryptophan hydroxylase). Only the serotonin neurotoxins decreased the binding of [$^3$H]cocaine in the rat cerebral cortex, but to a lower extent than the binding of [$^3$]imipramine, which is known to be associated with serotonergic terminals. In contrast to the cocaine binding in the mouse cerebral cortex, the binding in the rat cerebral cortex included a considerable portion of low-affinity binding that was relatively unaffected by lesions of serotonergic neurons.

## INTRODUCTION

Cocaine is used in clinical medicine as a local and regional anesthetic (1). In addition, it is a powerful central stimulant causing euphoria when inhaled through the nose, injected intravenously, or smoked as paste (1). Cocaine's local anesthetic potency in blocking the electrical activity of nerve fibers is presumably attributable to interactions with specific ion channels (2); its central activity may be related to interference with uptake and synthesis of catecholamines and indoleamines (3, 4).

Recent studies in our laboratory (5–8) and by Toth Kennedy and Hanbauer (9) have demonstrated the existence in brain of membrane-associated sites that saturably bind [$^3$H]cocaine. Analogues of cocaine are available, some with potent central stimulatory activity and others with only local anesthetic activity. It is interesting that only the analogues with central activity appreciably compete with [$^3$H]cocaine for the binding sites in brain; both the central effect and the binding show stereospecificity (5). The affinity ($K_d$ = 0.4–0.8 $\mu$M) of the central cocaine binding reported by us (5, 8) and by Toth Kennedy and Hanbauer (9) is in the same range as brain concentrations of cocaine (11–23 $\mu$M) that are achieved by i.v. or s.c. injections, and as plasma concentrations (0.5–15 $\mu$M) that result from systemic, intranasal, or pulmonary administration of cocaine (for citations see refs. 1 and 6).

It is well known that cocaine inhibits *in vitro* the neuronal uptake of norepinephrine, dopamine, and serotonin (3, 10, 11) with $IC_{50}$ values comparable to those in inhibiting [$^3$H]cocaine binding. This raises the question of whether the cocaine binding sites in brain are related to the neurotransmitter reuptake systems in nerve terminals. The present results indicate an association of part of the cocaine binding in the brain cortex with serotonergic nerve terminals based on (a) correlation studies between inhibition of neurotransmitter uptake into brain slices and of binding of [$^3$H]cocaine to brain membranes; (b) the demonstration of binding of [$^3$H]cocaine to platelets, a model system for neuronal uptake of serotonin (12, 13); and (c) the effects of neurotoxins aimed at serotonergic or catecholaminergic neurons on the central binding of [$^3$H]cocaine.

## MATERIALS AND METHODS

*Tissues.* In the studies on mouse brain we used adult male BALB/cBy mice (20–25 g; Jackson Laboratories, Bar Harbor, Maine). The experiments involving neurotoxins were carried out on male Wistar rats weighing 100–150 g, with one exception: for the treatment with PCA[1] (80 mg/kg) (see below) we used female rats weighing 250 g. Platelet membranes were prepared from human blood obtained from St. Luke's Hospital Center (New York, N. Y.).

*Treatment of rats with neurotoxins.* Experimental animals received

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[1] The abbreviations used are: PCA, *p*-chloroamphetamine hydrochloride; PCPA, *p*-chlorophenylalanine methylester hydrochloride; 5,7-DHT, 5,7-dihydroxytryptamine creatinine sulfate; 6-OHDA, 6-hydroxydopamine hydrobromide.

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i.p. injections of PCA (20 mg/kg) dissolved in 0.9% NaCl; control rats received equivalent volumes of 0.9% NaCl alone. Four days later, the animals were decapitated. In experiments with a high dose of PCA (80 mg/kg), the neurotoxin was injected 1 hr after pretreatment with chlorpromazine (10 mg/kg i.p.) to prevent hyperthermia and hyperactivity (14); control animals received the same treatment with 0.9% NaCl instead of PCA. The effect of PCPA was measured 4 days after a single i.p. injection of free base (270 mg/kg). 5,7-DHT was administered intracisternally in a volume of 20 μl of sterile 0.9% NaCl containing ascorbic acid (1 mg/ml) to rats under pentobarbital anesthesia (2 mg/rat i.p.) 1 hr after an i.p. injection of desipramine (25 mg/kg) (15); control animals were pretreated with desipramine but received 0.9% NaCl instead of 5,7-DHT. Animals initially received 75 μg of 5,7-DHT (free base) and another 75 μg 1 week later; they were killed 1 week after the second injection. Treatment with multiple intracisternal injections of 200 μg, 50 μg, and 50 μg of 6-OHDA in sterile 0.9% NaCl containing ascorbic acid (1 mg/ml) was performed as described by Bloom *et al.* (16); before injection, animals were anesthetized with chloral hydrate (380 mg/kg i.p.). The animals were killed 5 days after the last 6-OHDA injection.

*Uptake of [$^3$H]serotonin and [$^3$H]norepinephrine into slices of cerebral cortex.* Mice were decapitated, and the brains were rapidly removed. Slices (0.42 mm) from the cerebral cortex were prepared and incubated in oxygenated 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid-buffered medium as described by Sershen and Lajtha (17). The medium contained ascorbic acid (0.2 mg/ml) and nialamide ($10^{-5}$ M) (18). After a preincubation of 15 min, the test compound was added and the incubation was continued for 15 min. Subsequently, [1,2-$^3$H($N$)] serotonin binoxalate [30.1 Ci/mmole; New England Nuclear Corporation (Boston, Mass.); final concentration 3.3 nM] or L-[2,5,6-$^3$H]norepinephrine (46.5 Ci/mmole; New England Nuclear Corporation; final concentration 2.1 nM) was added, and after incubation for 10 min (serotonin) or 15 min (norepinephrine), the tissue was rapidly filtered from the medium and processed as described previously (17). Inhibition by the test compounds was computed as the percentage decrease in the radioactivity actively accumulated into intracellular fluid (17) above medium levels. Under the conditions of the assay, the active uptake of serotonin was linear for at least 10 min and that of norepinephrine for 15 min. A Lineweaver-Burk plot of data obtained with concentrations of [$^3$H]serotonin between 0.007 and 0.21 μM indicated a Michaelis constant of 0.4 μM, in agreement with values reported for the high-affinity transport system for serotonin in rat brain slices (18). Inhibition by test compounds was routinely assayed with 3.3 nM [$^3$H]serotonin in the medium. At this low concentration, predominantly the high-affinity transport was found to be measured (18); also, in experiments with inhibiting drugs, we observed inhibitions as high as 85%, indicating that at most 15% of the total serotonin transport measured was nonspecific uptake. In the experiments on uptake of norepinephrine, maximal inhibitions of 95% were recorded.

*Uptake of [$^3$H]serotonin and [$^3$H]norepinephrine into synaptosomes.* Fresh cerebral cortex and cerebellum from rat brain were weighed and homogenized in 8 volumes of ice-cold 0.32 M sucrose containing 0.1 mM EDTA and sufficient Tris-HCl to achieve pH 7.4 (3) with a motor-driven Teflon pestle (0.5-mm clearance) and glass homogenizer. The homogenate was centrifuged at 1000 × $g$ for 10 min at 0–4°, and the cloudy supernatant was used as the crude synaptosomal preparation (10). Uptake of serotonin in the cerebellum was measured in whole homogenates according to the method of Kuhar *et al.* (19), since cerebellar serotonin nerve endings tend to sediment with the nuclear fractions. Samples (0.1 ml) of the synaptosomal preparations were incubated for 5 min at 37° with 1 ml of incubation buffer [as described elsewhere (10) and gassed with 100% oxygen] containing 0.01 mM nialamide and 3 nM [$^3$H]serotonin or 14 nM [$^3$H]norepinephrine. The reaction was terminated by filtration through Millipore filters (0.8 μm). After washing twice with 5 ml of the above buffer (without ascorbic acid and nialamide), the filters were transferred to counting vials and radioactivity was measured (7). The protein content of the synaptosomal preparations was assayed by the method of Lowry *et al.* (20). Active uptake of [$^3$H]serotonin and [$^3$H]norepinephrine was defined as total uptake minus uptake in the presence of 45 μM chlorimipramine and 4.5 μM desipramine, respectively.

*Binding of [$^3$H]cocaine to preparations of brain membranes.* Brain tissues (fresh or kept frozen at −80°) were homogenized in 10 volumes of ice-cold 50 mM Tris-HCl (pH 7.4) (room temperature), with a Brinkmann Polytron (setting 6, 15 sec). Membranes were washed and resuspended in the above buffer as described previously (5); protein content was measured according to the method of Lowry *et al.* (20). Portions of the membrane preparations (0.5 ml; 0.65–1.0 mg of protein) were incubated at 21° for 20 min with L-(−)-[*benzoyl*-3,4-$^3$H($N$)]cocaine (35.2 Ci/mmole; New England Nuclear Corporation), at a final concentration of 15 nM, and (where indicated) varying amounts of unlabeled cocaine or other drugs in a final volume of 0.56 ml. Binding assays were terminated on poly-L-lysine-presoaked filters as described previously (8), and radioactivity was measured (7).

Saturable binding was defined as the total binding minus the nonspecific binding in the presence of 1 mM unlabeled cocaine. In a typical experiment with a preparation from mouse cerebral cortex incubated with 15 nM [$^3$H]cocaine, 0.4 ml of incubation mixture (0.6 mg of membrane protein) bound 785 dpm in the presence of 1 mM unlabeled cocaine and 3130 dpm in its absence. In the absence of membranes, approximately 75 and 180 dpm, respectively, were bound to the filters. Accordingly, the saturable binding in this typical experiment was (3130 − 180) − (785 − 75) = 2240 dpm.

In previous papers we have described kinetic experiments (8), the stability of [$^3$H]cocaine under the conditions of the binding assay (7, 8), the unlikelihood that cocaine binding sites are cocaine uptake sites (7), and the differences between central and peripheral (liver) binding (7). The binding constants for [$^3$H]cocaine in brain and liver obtained by filtration assays were very similar to those obtained by centrifugation assays, although the latter were subject to considerable error caused by high nonspecific binding (8). There was no significant decrease in saturable cocaine binding to membranes of the mouse cerebral cortex upon washing membrane-containing filters with one, two, three, or four successive portions of 5 ml of ice-cold buffer (8), indicating that there is no loss within the time scale of the rapid filtration procedure. In the present study this was confirmed for membranes of the rat cerebral cortex: with one, two, and three washes of the filters we found 1670 ± 162, 1615 ± 45, and 1573 ± 70 dpm saturable binding (average ± standard error of the mean; $n$ = 3) per assay under standard conditions (see above). To investigate whether the effects on cocaine binding in animals treated with PCA or 5,7-DHT could be due to residual neurotoxin in their brains, we treated homogenates of rat cerebral cortex *in vitro* with 100 μg of PCA per gram of cerebral cortex (i.e., 5 times the amount injected *in vivo*, 20 μg/g of body weight), or with 150 μg of 5,7-DHT per gram of cerebral cortex (i.e., the amount totally injected intracerebrally into a rat). Membranes prepared as above from these homogenates showed binding of [$^3$H]cocaine identical with that in untreated control membranes (data not shown).

*Binding of [$^3$H]cocaine to platelets.* The preparation of blood platelets was started within 2 hr after collecting 450–500 ml of venous blood in a pack with 63 ml of a solution containing 1.66 g of sodium citrate, 1.61 g of dextrose, 206 mg of citric acid, and 140 mg of sodium biphosphate. Platelets were prepared essentially as described by Briley *et al.* (21), with additional centrifugations for a better yield (22). Washed and lysed platelets were resuspended with the Polytron in ice-cold 50 mM Tris-HCl (pH 7.4) (room temperature) and centrifuged at 35,000 × $g$ for 10 min. The resulting pellets were either used immediately or frozen and stored at −80°. For binding assays, a pellet (derived from 150 ml of original whole blood) was resuspended with the Polytron in 20 ml of Tris-HCl buffer. Portions of this suspension (0.5 ml, 0.5–0.7 mg of protein) were assayed for binding of [$^3$H]cocaine as described above. No differences in the binding of [$^3$H]cocaine were found between freshly prepared and stored platelet preparations. At 15 nM [$^3$H]cocaine in the incubation mixture (0.6 mg of platelet protein), the radioactivity was distributed in the following manner: total binding, 2915 dpm; nonspecific binding, 532 dpm.

*Binding of [$^3$H]imipramine to membrane preparations of cerebral cortex and cerebellum.* Crude synaptosomes prepared as described above were used as the starting material for the [$^3$H]imipramine binding. A 2-ml sample of the synaptosomal preparation was diluted with 8 ml of an ice-cold solution that was 50 mM Tris-HCl, 100 mM NaCl, and 5 mM KCl (pH 7.4) (room temperature). The mixture was centrifuged at 35,000 × *g* for 10 min, and the resulting pellet was washed with and resuspended in the same buffer. Binding assays were performed as described previously by Langer *et al.* (11), with a final concentration of 4 nM [2,4,6,8-$^3$H]imipramine hydrochloride (24.4 Ci/mmole, New England Nuclear Corporation) and 0.4 μM imipramine for defining nonspecific binding.

*Estimation of $IC_{50}$ values of drugs in inhibiting uptake of [$^3$H]serotonin and [$^3$H]norepinephrine, and binding of [$^3$H]cocaine.* Inhibitions by four to five concentrations of each drug were assayed in triplicate in two or three separate tissue preparations. $IC_{50}$ values were estimated from linear regression analysis of log-probit plots.

*Materials.* The following drugs, listed alphabetically, were generously donated by the companies indicated: benztropine mesylate, Merck Sharp & Dohme Research Laboratories (Rahway, N. J.); chlorimipramine hydrochloride, Geigy Pharmaceuticals (Ardsley, N. Y.); *d*-chlorpheniramine, Schering Corporation (Kenilworth, N. J.); cocaine hydrochloride, Mallinckrodt (St. Louis, Mo.); desipramine hydrochloride, USV Laboratories (Tuckahoe, N. Y.); fluoxetine, Lilly Research Laboratories (Indianapolis, Ind.); fluvoxamine maleate, Duphar (Weesp, The Netherlands); imipramine hydrochloride, Geigy Pharmaceuticals; iprindole, Wyeth Laboratories (Philadelphia, Pa.); pseudococaine hydrogen tartrate, Merck; Ro 11-2465 hydrochloride, Hoffmann-La Roche (Nutley, N. J.); WIN 35,004, WIN 35,065-3, and WIN 35,428, Sterling-Winthrop Research Institute (Rensselaer, N. Y.); and zimelidine, Astra Pharmaceutical Products (Worcester, Mass.).

## RESULTS

*Correlation between inhibition of neuronal uptake of neurotransmitters and inhibition of binding of [$^3$H]cocaine.* We compared the potencies of a series of uptake blockers, tricyclic antidepressants, and cocaine analogues in inhibiting the saturable binding of [$^3$H]cocaine and in inhibiting the neuronal uptake of [$^3$H]norepinephrine and [$^3$H]serotonin in mouse brain (Fig. 1). There was no significant correlation between the inhibition of [$^3$H]cocaine binding to membranes and the inhibition of the uptake of [$^3$H]norepinephrine into slices ($r = 0.23$; $N = 14$; $p > 0.2$). In contrast, the correlation between the inhibition of [$^3$H] cocaine binding and the inhibition of [$^3$H]serotonin uptake was positive and significant ($r = 0.80$; $N = 17$; $p < 0.001$).

*Binding of [$^3$H]cocaine to platelets.* Since the human platelet is widely documented to be a model system for neuronal uptake of serotonin (12, 13), we investigated whether cocaine binding could be demonstrated in this system. Human platelet preparations indeed displayed saturable binding of [$^3$H]cocaine. The binding was equilibrated at 20 min and was linear with platelet concentrations up to 0.75 mg of platelet protein per assay mixture (data not shown). Graphical analysis (23) of Scatchard data (Fig. 2) was compatible with the existence of a high-affinity component ($K_d = 0.64$ μM, $\beta_{max} = 1.5$ pmoles/mg of protein) and a low-affinity component ($K_d \sim 50$ μM). The $K_d$ value of this high-affinity binding is in the range of the values for mouse cerebral cortex (5, 8), rat cerebral cortex (next section), and rat corpus striatum (9).

At low concentrations of [$^3$H]cocaine, the bulk of the radioactivity bound to platelets represents high-affinity binding, and under these conditions the potencies of drugs in inhibiting platelet binding were very similar to those in inhibiting the binding of cocaine in mouse brain (Fig. 3). In addition, the Hill numbers for these inhibitions were similar in platelets and in mouse brain: values ≤ 0.5 for Ro 11-2465 and serotonin, and values of 0.7–0.8 for all other compounds. The correlation between the

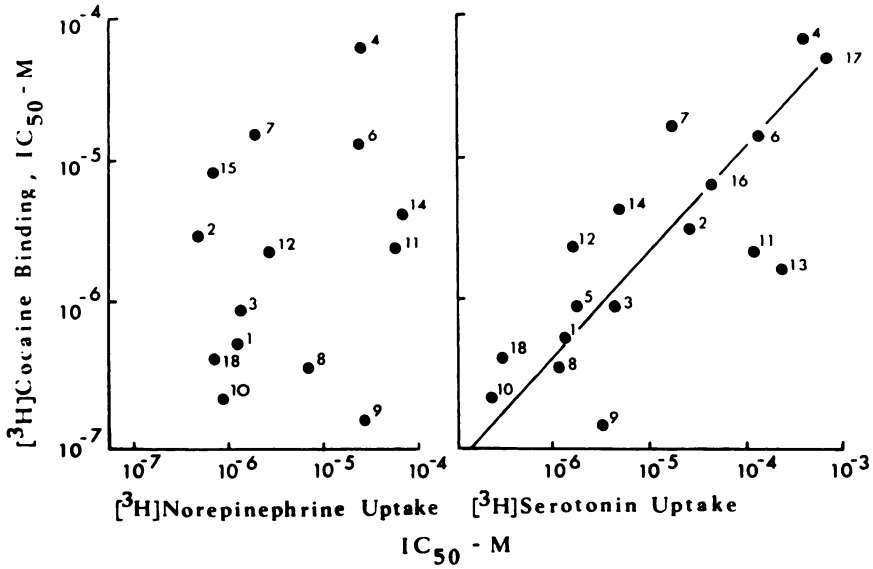


FIG. 1. *Drug potencies in inhibiting neuronal uptake of norepinephrine and serotonin, and in competing with cocaine binding in mouse cerebral cortex*

Assays were performed as described under Materials and Methods. *1*, Imipramine; *2*, desipramine; *3*, cocaine; *4*, WIN 35,004; *5*, WIN 35,428; *6*, pseudococaine; *7*, *d*-amphetamine; *8*, fluoxetine; *9*, fluvoxamine; *10*, Ro 11-2465; *11*, iprindole; *12*, *d*-chlorpheniramine; *13*, benztropine; *14*, zimelidine; *15*, methylphenidate; *16*, *N*-allylnorcocaine; *17*, WIN 35,065-3; *18*, chlorimipramine. The correlation between the inhibition of cocaine binding and norepinephrine uptake is statistically not significant ($r = 0.23$; $N = 14$; $p > 0.2$), in contrast to cocaine binding versus serotonin uptake ($r = 0.80$; $N = 17$; $p < 0.001$).

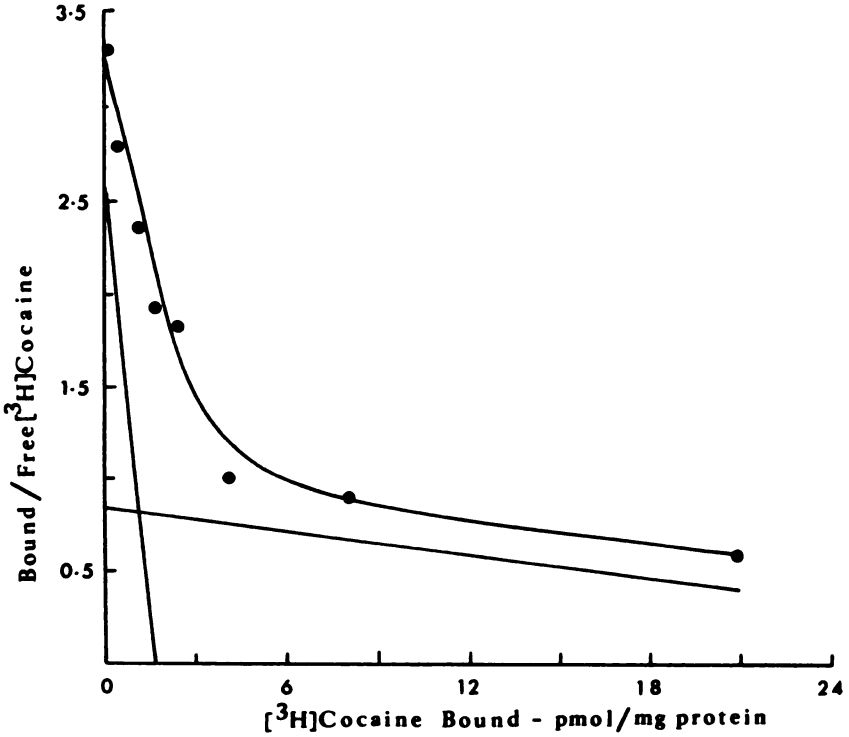


FIG. 2. *Scatchard analysis of [$^3$H]cocaine binding to membranes from human blood platelets*
The free cocaine concentrations ranged from 13 nM to 35 μM. Straight lines represent higher- and lower-affinity binding components derived from graphical analysis (23). The free [$^3$H]cocaine concentration in the Scatchard plot is expressed in micromolarity. Points shown are from a single experiment, performed in triplicate, which was replicated once with similar results.

inhibition of cocaine binding in human platelets and that in mouse cerebral cortex was highly significant ($r = 0.93$; $N = 8$; $p < 0.001$). Thus, brain and platelets appear to have binding sites for cocaine with a similar affinity and pharmacological profile.

*Effects of treatment with p-chloroamphetamine in cerebral cortex.* PCA was used to produce a long-term, selective destruction of serotonergic neurons. Since mice are much more resistant to the neurotoxic effect of PCA than rats (24), the following studies were performed on rats. Four days after injection of PCA (20 mg/kg i.p.) into rats, the neuronal uptake of serotonin into synaptosomes from the cerebral cortex was significantly ($p < 0.001$) reduced by 65–75% (Table 1). Concomitantly, the binding of [$^3$H]cocaine was reduced by 23–28% with a statistical significance of $p < 0.01$ in a single binding point analysis at 15 nM [$^3$H]cocaine with 1 mM cocaine for defining nonspecific binding. Similarly, a PCA dose of 80 mg/kg reduced serotonin uptake by 87% ($p < 0.001$) and cocaine binding by 32% ($p < 0.01$) (Table 1). Scatchard analysis of binding data obtained at various concentrations of cocaine, versus 1 mM cocaine as nonspecific binding (Fig. 4A), was compatible with the existence of high- and low-affinity binding in the cerebral cortex of both control and PCA-treated rats. In comparison, mouse cerebral cortex displayed considerably less low-affinity binding (Fig. 4B). Rats treated with PCA (80 mg/kg) showed a profound reduction in the high-affinity component of [$^3$H]cocaine binding, whereas the low-affinity component was similar to the one observed in control animals (Fig. 4A). The effect of PCA on the high-affinity component was estimated by defining the nonspecific binding with 10 μM cold cocaine, at which concentration the low-affinity binding sites are far from saturation. PCA-treated animals showed a dose-dependent reduction in high-affinity binding (Fig. 5). The lesion resulted in a decrease in the density of the high-affinity binding (0.8–1 pmole/mg of protein for control animals; 0.4 pmole/mg for rats treated with a PCA dose of 20 mg/kg;

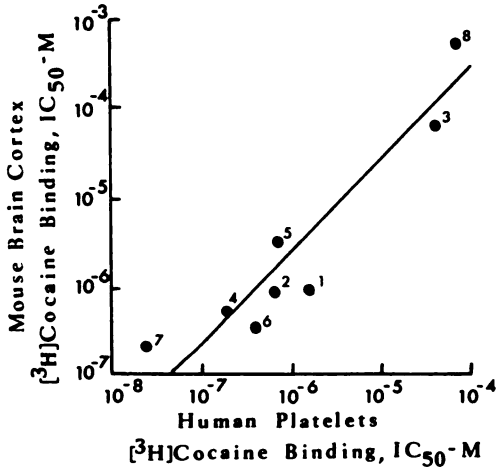


FIG. 3. *Drug potencies in inhibiting binding of [$^3$H]cocaine to membranes from mouse cerebral cortex and human blood platelets*
Assays were performed as described under Materials and Methods. *1*, Cocaine; *2*, WIN 35,428; *3*, WIN 35,004; *4*, imipramine; *5*, desipramine; *6*, fluoxetine; *7*, Ro 11-2465; *8*, serotonin. Incubations with serotonin, and respective controls, were carried out in the presence of ascorbic acid (1 mg/ml) and nialamide ($1.25 \times 10^{-5}$ M) to prevent degradation of serotonin. The correlation is significant ($r = 0.93$; $N = 8$; $p < 0.001$).

TABLE 1

*Effect of neurotoxins on neuronal uptake of [$^3$H]serotonin, binding of [$^3$H]cocaine, and binding of [$^3$H]imipramine in rat cerebral cortex*

Treatments with neurotoxins and assays for uptake and binding were as described under Materials and Methods. Cocaine binding was performed at 15 nM [$^3$H]cocaine with 1 mM unlabeled cocaine for defining nonspecific binding. The statistical significance of the changes (given in percentages) was determined with the two-tailed Student's *t*-test. Values are means ± standard error of the mean.

| Treatment | No. of animals | Serotonin uptake | Cocaine binding | Imipramine binding |
|---|---|---|---|---|
| | | *fmoles/mg protein/min* | *fmoles/mg protein* | *fmoles/mg protein* |
| 0.9% NaCl | 4 | 21.1 ± 1.2 | 46.1 ± 2.2 | |
| PCA (20 mg/kg) | 4 | 5.4 ± 0.2 −74%[a] | 33.0 ± 1.7 −28%[b] | |
| 0.9% NaCl | 5 | 25.4 ± 0.8 | 50.3 ± 2.1 | 125.7 ± 12.7 |
| PCA (20 mg/kg) | 5 | 8.7 ± 1.0 −66%[a] | 38.6 ± 1.7 −23%[b] | 72.2 ± 7.4 −43[c] |
| 0.9% NaCl | 5 | 14.9 ± 0.7 | 42.4 ± 1.9 | 115.1 ± 13.3 |
| PCA (80 mg/kg) | 3 | 2.0 ± 0.2 −87%[a] | 28.8 ± 2.1 −32%[b] | 42.3 ± 6.6 −64%[b] |
| 0.9% NaCl | 3 | 25.4 ± 2.1 | 44.0 ± 1.3 | 121.8 ± 2.8 |
| 5,7-DHT (2 × 75 μg) | 4 | 11.4 ± 1.8 −55%[b] | 33.6 ± 1.7 −24%[b] | 69.7 ± 7.9 −43%[b] |
| 0.9% NaCl | 4 | 19.3 ± 0.7 | 52.2 ± 2.4 | 119.0 ± 5.1 |
| PCPA (270 mg/kg) | 4 | 19.3 ± 0.7 0% | 52.5 ± 2.2 +1% | 118.9 ± 2.8 0% |
| 0.9% NaCl | 5 | 19.1 ± 1.4 | 37.7 ± 2.2 | |
| 6-OHDA (200 + 50 + 50 (μg) | 5 | 19.4 ± 0.7[d] +2% | 37.5 ± 0.5 0% | |

[a] $p < 0.001$.
[b] $p < 0.01$.
[c] $p < 0.05$.
[d] The effect on neuronal uptake of [$^3$H]norepinephrine was −57% ($p < 0.001$)

0.2 pmole/mg for a PCA dose of 80 mg/kg) rather than in the affinity ($K_d$ = 0.4–0.6 μM for all animals).

*Effects of treatment with 5,7-DHT, PCPA, and 6-OHDA in cerebral cortex.* Intracisternal injections of 5,7-DHT can be used to damage serotonergic neurons selectively in the brains of rats pretreated with desipramine, which prevents the neurotoxin from being taken up into noradrenergic neurons and thus protects the noradrenergic system (15). This neurotoxin reduced both the serotonin uptake and the cocaine binding (defined with 1 mM cocaine as nonspecific binding) in the cerebral cortex in a statistically significant manner (Table 1).

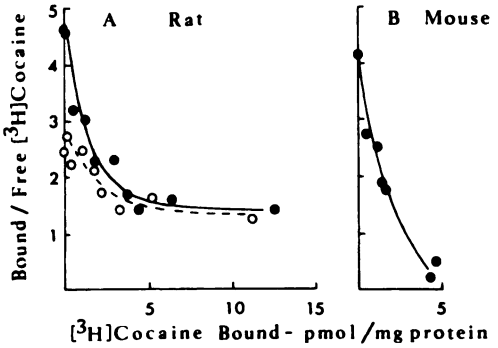


FIG. 4. *Scatchard analysis of binding of [$^3$H]cocaine to membranes of cerebral cortex of rats and mice*

The free [$^3$H] cocaine concentration is expressed in micromolarity. A. Rats treated with PCA (80 mg/kg) (○---○) or 0.9% NaCl (●——●). Aliquots of membranes from each animal of each group were pooled; i.e., each membrane fraction is a pool of tissue from all animals in the control ($n$ = 4) or PCA-treated ($n$ = 4) group. The whole experiment was repeated once with similar results. B. Mice. This experiment was repeated many times with similar results (8). The values shown are averages of triplicate determinations. The free [$^3$H] cocaine concentrations ranged from 7 nM to 9 μM in A, and from 10 nM to 29 μM in B.

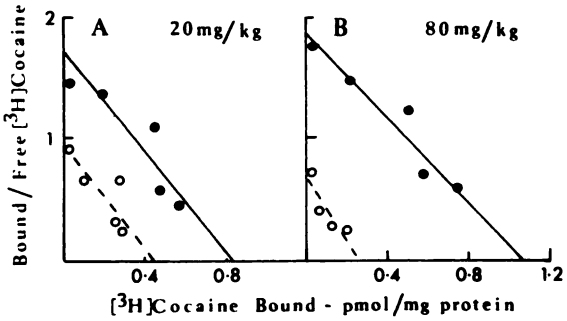


FIG. 5. *Scatchard analysis of high-affinity binding of [$^3$H]cocaine to membranes from cerebral cortex of rats treated with PCA (open symbols) or 0.9% NaCl alone (closed symbols)*

High-affinity binding was estimated by defining nonspecific binding at 10 μM unlabeled cocaine. Each membrane preparation is a pool of tissue from all animals in the respective treatment group. A. ●——●, four control rats; ○---○, four PCA (20 mg/kg)-treated rats. Results from single binding point assays are given in the first two lines of Table 1. B. ●——●, five control rats, ○---○, three PCA (80 mg/kg)-treated rats. See Table 1 for single binding point assays.

It is known that PCPA, an inhibitor of tryptophan hydroxylase, produces a profound, reversible decrease in the level of serotonin in rat brain, and also affects to some extent the level of norepinephrine (25). Our results indicated that the neuronal uptake of serotonin in cerebral cortex was not affected by PCPA. In addition, the cocaine binding was similar in control and PCPA-treated animals (Table 1).

6-OHDA, a neurotoxin directed toward noradrenergic and dopaminergic neurons (16), reduced the synaptosomal uptake of [$^3$H]norepinephrine in cerebral cortex by 57% ($p < 0.001$, data not shown), indicating the effectiveness of the multiple-injection schedule. In contrast, the treatment had no effect on either serotonin uptake or cocaine binding.

*Effects of treatment with PCA in cerebellum.* In cerebellum, which contains few serotonergic terminals, serotonin uptake was about 4 times lower than in the cerebral cortex (data not shown). PCA (20 mg/kg) further reduced this uptake by 59% ($p < 0.001$). In contrast, cocaine binding (defined with 1 mM cocaine as nonspecific binding) in the cerebellum was comparable to or only slightly lower than that found in the cerebral cortex and was not affected in a statistically significant manner by PCA at a dose of 20 mg/kg or 80 mg/kg (data not shown).

## DISCUSSION

*Correlation between inhibition of monoamine uptake and inhibition of cocaine binding.* Since cocaine binding had been characterized previously in mouse cerebral cortex (5–8), this tissue was used to study the inhibition of cocaine binding and the inhibition of neurotransmitter uptake. In mouse cerebral cortex, the binding of cocaine (with 1 mM cocaine for defining nonspecific binding) was

mostly of the high-affinity type, in contrast to that in rat cerebral cortex (Fig. 4). The binding of 15 nM [$^3$H]cocaine (as defined above) in mouse cerebral cortex was inhibited by cocaine and the drugs depicted in Fig. 1 with Hill coefficients close to 0.8, with the exception of Ro 11-2465 (0.5). There was a significant correlation between the inhibition of cocaine binding in mouse cerebral cortex and the inhibition of neuronal uptake of serotonin with drug potencies for the latter activity taken from our own experiments (Fig. 1) or from the literature (3, 10, 11). In contrast, no correlation was found between the inhibition of cocaine binding in mouse cerebral cortex and the inhibition of norepinephrine uptake with values for uptake inhibition observed in the present experiments (Fig. 1) or values reported in the literature (3, 10, 11). There was no correlation between the inhibition of cocaine binding in cerebral cortex and the uptake of dopamine into synaptosomes from corpus striatum (3, 10). This lack of correlation in itself does not exclude the possibility that cocaine binds to noradrenergic and dopaminergic nerve terminals. For instance, cocaine may bind to these terminals at sites distinct from the norepinephrine and dopamine uptake recognition sites, and the binding may not be inhibited by norepinephrine and dopamine uptake blockers. Alternatively, a correlation between inhibition of cocaine binding and, for example, dopamine uptake may exist in striatal tissue in which dopaminergic terminals predominate.

*Binding sites for cocaine on platelet membranes.* Blood platelets accumulate, store, and release serotonin in a manner analogous to that of central serotonergic neurons (12) and, as elegantly shown by Stahl and Meltzer (13), can serve as a model for serotonergic neurons. Platelets also accumulate norepinephrine, but at a much lower rate than the uptake of serotonin (12). To our knowledge the only study available on dopamine uptake by platelets indicates a lower initial uptake rate for dopamine than for serotonin; in addition, dopamine is lost rapidly from platelets (for citations see ref. 12). In the present study, human platelet membranes were found to possess binding sites for cocaine with affinity for cocaine similar to that displayed by the cocaine binding sites on membranes from cerebral cortex. In addition, the pharmacological profiles of the platelet and brain sites are similar (Fig. 3). This is analogous to the similarities found for the sites in platelet and brain that bind [$^3$H] imipramine (26). The densities of high-affinity cocaine binding sites in human platelets and rat brain cortex are approximately 1.5 pmoles/mg of protein (Fig. 2) and 1 pmole/mg of protein (Fig. 5), respectively; the density of high-affinity imipramine binding sites in both cases is approximately 3 times lower (26). The finding of cocaine binding sites on platelet membranes is consonant with a possible association of cocaine binding with serotonergic nerve terminals.

*Lesion studies.* The measurements of the effects of lesions in rats were complicated by the binding of a sizable amount of [$^3$H]cocaine to low-affinity sites in the cerebral cortex that were exposed in our routine binding assay with 1 mM unlabeled cocaine for defining nonspecific binding. Cerebral cortex tissue from rats showed more low-affinity binding than that from mice (Fig. 4); in agreement with this, preliminary experiments indicated Hill coefficients of 0.4–0.7 for inhibition of cocaine binding (with 1 mM cocaine for defining nonspecific binding) in rat cerebral cortex by cocaine, imipramine, fluoxetine, and *d*-chlorpheniramine, whereas the same drugs inhibited binding in mouse cerebral cortex with Hill coefficients of 0.8. These low-affinity sites in rat cerebral cortex resist treatment with PCA (Fig. 4A) and can explain the discrepancy between the effects on serotonin uptake and high- plus low-affinity binding of [$^3$H]cocaine (Table 1). In comparing effects of lesions on the neuronal uptake of serotonin and on cocaine binding, one should also consider that cocaine might bind to glial cells, which in turn might infiltrate lesioned brain areas.

In order to obtain another measure of the extent of the lesions, we monitored the binding of [$^3$H]imipramine, which has been shown to be associated with serotonergic nerve terminals and to be sensitive to serotonin neurotoxins (27). Indeed, damage to the serotonergic neurons resulted in a reduction in binding of [$^3$H]imipramine (Table 1) due to a decrease in $B_{max}$ (data not shown), in agreement with effects found by others (27). It is of interest that the effects of PCA on imipramine binding (43% and 64%; Table 1) are quantitatively comparable to those on high-affinity binding of cocaine versus 10 $\mu$M cocaine (48% and 76%, respectively; Fig. 5A and B). It is noteworthy that PCPA, which depletes serotonin but leaves the terminals intact, as shown by the normal serotonin uptake in the cerebral cortex (Table 1), has no effect on the binding of either imipramine or cocaine in that region. In the present experiments, the imipramine binding was decreased to a lower extent than the neuronal uptake of serotonin, indicating that some of this binding is not serotonin-related. The binding of [$^3$H] imipramine in the cerebellum was about 2.5 times lower than that in the cerebral cortex, and was decreased by 33% ($0.05 < p < 0.10$) as a result of PCA (20 mg/kg). In contrast, single-point binding of cocaine in the cerebellum was similar to that in the cortex and was not affected by PCA (20 and 80 mg/kg), indicating that serotonin-related binding in the cerebellum is a minor component of the total binding. This is in line with the finding that there is more low-affinity binding of cocaine in the cerebellum than in the cortex of rat brain (data not shown).

*On the possible nature and role of binding sites for cocaine.* The present results suggest an association of some of the cocaine binding (of the high-affinity type) in the cerebral cortex with serotonergic nerve terminals. Recently it was shown (9) that $Na^+$-independent cocaine binding sites are not localized on dopaminergic terminals in the striatum. At present, we can only speculate on the nature of the high-affinity binding sites for cocaine on serotonergic nerve endings. From the potencies of serotonin and imipramine in inhibiting the high-affinity cocaine binding (Fig. 3), it appears unlikely that this binding represents the serotonin uptake recognition sites or imipramine binding sites themselves. In addition, serotonin uptake and imipramine binding are $Na^+$-sensitive, whereas cocaine binding is not, in areas other than the striatum (7, 9). However, $Na^+$ may not be necessary for the binding of inhibitory substances at sites distinct from the serotonin binding site on the carrier, and $Na^+$ de-

pendency should not be regarded as a prerequisite for binding sites related to serotonin uptake recognition sites.

The localization of some of the cocaine binding sites on serotonergic nerve terminals is interesting in view of reports implicating serotonergic systems in the stimulatory effect of cocaine. For instance, cocaine-induced locomotor activity is not blocked by α-methyl-*p*-tyrosine or haloperidol (28), and both the increase in motor activity and the reduction in the serotonin content of the pons-medulla as a result of cocaine are attenuated by concomitant administration of 5-hydroxytryptophan plus decarboxylase inhibitor (29). However, the locomotor stimulation produced by the cocaine analogues WIN 35,065 and WIN 35,428 can be blocked by haloperidol and pimozide, implicating synaptically released dopamine (30). Cocaine administration can produce an array of effects, including locomotor stimulation, mood elevation, stereotypy, and hallucinations. Probably, there is not a single mechanism that underlies this complex pattern of behavioral activities. Binding sites for cocaine on serotonergic nerve terminals may only mediate some of its effects. In view of the similarities observed in the present study between the high-affinity binding sites for cocaine and those for the antidepressant drug imipramine, and in view of the implication of indoleamines in depression (26), it appears that the binding sites for cocaine on serotonergic neurons may be involved in the mood-elevating properties of cocaine.

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**Send reprint requests to:** Dr. Maarten E. A. Reith, Center for Neurochemistry, Ward's Island, New York, N. Y. 10035.