Structural Requirements for Cocaine Congeners to Interact with [3H]Batrachotoxinin A 20-α-Benzozate Binding Sites on Sodium Channels in Mouse Brain Synaptosomes*

Maarten E. A. Reith1, Seung Soo Kim, and Abel Lajtha
From The Center for Neurochemistry, The Nathan S. Kline Institute for Psychiatric Research, Ward’s Island, New York, New York 10035

The present study examines the possible role of sodium channels in the behavioral effects of cocaine. Cocaine congeners are apparent competitive inhibitors of the scorpion toxin-enhanced binding of [3H]batrachotoxinin A 20-α-benzoate to sodium channels in mouse cerebrocortical synaptosomes. However, in agreement with the allosteric model for heterotropic cooperative interactions, the compounds produce a concentration-dependent increase in the rate of dissociation of binding. Concentrations that give a 2-fold increase of $K_d$ are close to $K_i$, values for inhibiting equilibrium binding of [3H]batrachotoxinin A 20-α-benzoate, suggesting that the inhibitory effect on binding results mostly from an increase of the apparent dissociation rate constant. The ester linkage between the tropone and benzoyl ring of cocaine is not essential for the inhibitory potency, and for both the C-2 and C-3 substituents the equatorial position results in a higher potency than the axial position. There is reasonable agreement between the rank order of potencies in blocking the sodium channel and in inhibiting locomotor behavior. The present results do not support a relationship between the capability of cocaine congeners in blocking sodium flux and in inhibiting uptake of dopamine into striatal synaptosomes. However, peak levels of cocaine in the brain of cocaine addicts could be high enough to interfere with sodium channel functioning, possibly contributing to some of cocaine’s actions.

Cocaine’s behavioral effects are usually interpreted in terms of its action on brain monoamines. However, it is clear that the local anesthetic property of cocaine can be involved in some of its behavioral effects. Examination of analogs showed that pigeons and rats trained to discriminate between the presence and absence of effects induced by cocaine generalize to some extent from cocaine to procaine (1, 2). Procaine and tetracaine reinforce the intravenous self-injection of cocaine in rhesus monkeys (3). Cocaine’s effect on kindled seizures in rats has a monoaminergic and a local anesthetic component (4). Cocaine, and also lidocaine, can produce amygdala hyperspindling, and cross-sensitization to cocaine occurs in rats chronically treated with lidocaine (5). Our work has indicated a local anesthetic component in the inhibitory effect of cocaine congeners on spontaneous locomotor behavior of mice (6, 7).

There is abundant electrophysiological evidence that the sodium channel is the site of local anesthetic action (8). With recently developed biochemical techniques it has been shown that cocaine and other local anesthetic drugs inhibit the veratridine-stimulated $\text{Na}^+$ uptake into rat brain homogenates (9, 10) and inhibit the binding of the alkaloid neurotoxin [3H]BTX-B to sodium channel preparations of rat brain (11, 12). This action involves a specific receptor, and a number of local anesthetics bind more tightly to the nonconducting state of the sodium channel than to the active state; the presence of the local anesthetic shifts the equilibrium between the two states towards the nonconducting blocked state (12). In the presence of scorpion toxin, which enhances alkald neurotoxin action, the affinity of [3H]BTX-B for the active state is higher than that for the inactive state (13, 14). Even though the inhibition of [3H]BTX-B binding by local anaesthetics appears to be competitive, the mechanism is best described by the allosteric model for heterotropic cooperative interactions (13, 15).

The present work was undertaken to 1) determine the structure-activity relationships for cocaine congeners in inhibiting [3H]BTX-B binding to sodium channels in mouse brain; 2) assess whether cocaine congeners are apparent competitive inhibitors of BTX-B binding, producing their effects according to the allosteric model for heterotropic cooperative interactions; 3) compare the potencies of cocaine congeners in blocking sodium channels with their potencies in inhibiting locomotor activity of mice; and 4) compare the channel blocking properties with the inhibition of uptake of dopamine into mouse striatal synaptosomes, since it has been suggested that a block of the Na$^+$ flux underlies the capability of cocaine structures to inhibit neuronal dopamine uptake (16). For all studies we used brain tissue from male BALB/cBy mice to facilitate comparisons.

**EXPERIMENTAL PROCEDURES**

**Materials**—The following drugs were the generous donations of the persons or companies indicated: benzoyltropine and benzoylpseudotropine, Dr. S. B. Ross, Astra; (+)-pseudococaine and (+)-nepseudococaine hydrogen tartrate, Merck; WIN compounds, Sterling-Winthrop Institute; norcocaine, (-)-pseudococaine, and ephedrine methylester, National Institute on Drug Abuse; N-allylnorcocaine hydrochloride, Dr. K. A. Nieforth, Storrs, CT; and veratridine, Dr. 

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†To whom reprint requests should be addressed: Center for Neurochemistry, Ward’s Island, New York, NY 10035.

‡The abbreviations used are: BTX-B, batrachotoxinin A 20-α-benzozate; $K_d$, equilibrium dissociation constant; $k_{on}$, association rate constant; $k_{off}$, dissociation rate constant; $E_C$, concentration that produces a 2-fold increase of the dissociation rate constant; $IC_{50}$, concentration required to inhibit binding or uptake by 50%; $K_i$, equilibrium dissociation constant of inhibiting drug.
G. B. Brown, Birmingham, AL. Cocaine hydrochloride was from Sigma Chemical Co. All other drugs were from commercial sources.

Animals—For all our experiments we used male BALB/cBy mice, 8–12 weeks of age, weighing 21–24 g, from the breeding colony of our Institute. The animals were kept on a 12-h light/dark cycle (7 a.m./7 p.m. light), with food and water available ad libitum.

[3H]BTX-B Binding Assays—Synaptosomes were prepared from cerebral cortex tissue by a modification of the method of Gray and Whittaker (17) as described by Postma and Catterall (12). Slowly frozen suspensions of synaptosomes (12) were stored at −70 °C. Specific high-affinity binding of 7 nM [3H]BTX-B (60 Ci/mmol; New England Nuclear) was measured at 36°C in the presence of tetrodotoxin and scorpion toxin for enhancement of binding as described previously (12) with the following exceptions. Binding reactions were started by the addition of 0.1 ml of synaptosome suspension (0.7 mg of protein) to 0.42 ml of standard incubation medium. The assays were terminated by addition of 5 ml of ice-cold wash medium (12) and filtration through Whatman GF/C glass-fiber filters with a cell harvester (Brandel); two 5-ml washes were used to rinse the filters. Radiolactivity and protein were estimated as described previously (18).

Non-specific binding of [3H]BTX-B was defined with a final concentration of 0.3 mM veratridine or aconitine added as concentrated stocks in ethanol; the final ethanol concentrations did not exceed 1% (+) and had little or no effect on total binding. Values for non-specific binding with veratridine and aconitine were averaged and amounted to approximately 13% of the total binding of 7 nM [3H] BTX-B. The cocaine congeners had no effect on non-specific binding. Filter binding of [3H]BTX-B in the absence of synaptosomes was negligible. In experiments aimed at measuring the off rate of [3H] BTX-B, the dissociation was initiated by addition of 3 μl of aconitine in ethanol, resulting in a final concentration of 0.2 mM aconitine, and 17–27 μl of a solution of cocaine congener in water as indicated. We also used 0.2 mM veratridine to initiate dissociation in some experiments and found the same results as with aconitine.

Locomotor Activity and Striatal Dopamine Uptake—Spontaneous locomotor activity of mice was measured as described by us previously (6). Additional animals were monitored to confirm the previous data for (+)-neopseudococaine and benzoylpseudotropine; all other data are from our previous publication (6). The densities of drugs in inhibiting the neuronal uptake of dopamine into mouse striatal synaptosomes are from our recent work (18).

Data Analysis—In general six different concentrations of a drug (three below and three above the IC50) were used to assess its potency and pseudo Hill coefficient in inhibiting [3H]BTX-B binding. Complete curves were run in four separate experiments, involving at least two different preparations of synaptosomes. IC50 values were computed with the ALLFIT program developed by DeLean et al. (19). Pseudo Hill coefficients were obtained from Hill plots by standard linear regression techniques. Since the Hill numbers were essentially unity, the IC50 values were transformed into KI values by the Cheng and Prusoff correction (20), with a KI value of 62 nM for [3H]BTX-B binding under our conditions for mouse cerebrocortical synaptosomes. For instance, previous studies with rat brain synaptosomes reported a half-time of 60 min and a KI of 82 nM (14), and, more recently, a half-time of 20 min and a KI of 116 nM (12). Such differences may result from different degrees of enhancement of BTX-B binding by scorpion toxin (14). At concentrations of 10 times the KI for inhibition of equilibrium binding cocaine, (+)-neopseudococaine, and benzoylpseudotropine enhanced the KI by a factor of 3 to 4 when added together with 0.2 mM aconitine to initiate the dissociation (Fig. 2). For comparison, a similar effect by the local anesthetic drug procaine is also shown in

RESULTS

Inhibition of Equilibrium Binding of [3H]BTX-B by Cocaine Congeners—Competition curves for the inhibition of [3H] BTX-B binding by cocaine congeners were steep (Fig. 1) with pseudo Hill coefficients close to unity (Table I). There was a 1000-fold difference in potency between cocaine and its de- benzylated derivative, cocaine methylester. Within the series of structures containing both the tropane and benzoyl ring, there was a 400-fold difference between (+)-neopseudococaine, the most active congener, and WIN 35,428, the weakest. Only moderate stereospecificity, a 3-fold difference, was observed in comparing the potencies of (+)-neopseudococaine and (−)-pseudococaine, of benzoylpseudotropine and benzo- ylpseudolactone, and of WIN 35,140 and WIN 35,065-3.

Effects of Cocaine Congeners on Dissociation of [3H]BTX-B Binding—When the measurement of dissociation was initiated by the addition of 0.2 mM aconitine, [3H]BTX-B dissociated from its binding sites with a dissociation rate constant, k−1 of 0.0068 ± 0.0005 min−1 (mean ± S.E. for 6 independent experiments involving at least 6 time points) or a half-time of 102 min (Fig. 2). This is somewhat slower than reported previously, probably resulting from the lower KI value, 62 nM, of [3H]BTX-B binding under our conditions for mouse cerebrocortical synaptosomes. For instance, previous studies with rat brain synaptosomes reported a half-time of 60 min and a KI of 82 nM (14), and, more recently, a half-time of 20 min and a KI of 116 nM (12). Such differences may result from different degrees of enhancement of BTX-B binding by scorpion toxin (14). At concentrations of 10 times the KI for inhibition of equilibrium binding cocaine, (+)-neopseudococaine, and benzoylpseudotropine enhanced the k−1 by a factor of 3 to 4 when added together with 0.2 mM aconitine to initiate the dissociation (Fig. 2). For comparison, a similar effect by the local anesthetic drug procaine is also shown in

FIG. 1. Inhibition of [3H]BTX-B binding by cocaine congeners. The concentration of [3H]BTX-B was 7 nM. Nonspecific binding as defined under "Experimental Procedures" has been subtracted. The average specific binding was 129 fmol/mg of protein. The number of the compounds is as in Table I. Points shown are those obtained in a single experiment, performed in triplicate, which was replicated three times.
Effect of cocaine congeners and local anesthetics on binding and dissociation of [3H]BTX-B

Equilibrium binding was determined as in Fig. 1 in four independent experiments involving five to seven different drug concentrations assayed in triplicate. Kᵢ values were computed from IC₅₀ estimates obtained by the ALLFIT program (see "Experimental Procedures"). Dissociation data were obtained as in Fig. 2 in two independent experiments involving four different drug concentrations.

| Drug            | Equilibrium binding | Dissociation |
|-----------------|----------------------|--------------|
|                 | Hill coefficient     | Kᵢ µM        | ¹Kᵢ µM      |
| 1. Cocaine      | 0.85 ± 0.04          | 40.3 ± 7.0   | 35.7 ± 4.1  |
| 2. WIN 35,428   | 0.91 ± 0.05          | 153.9 ± 8.4  | 94.5 ± 15.1 |
| 3. WIN 35,140   | 0.92 ± 0.03          | 41.2 ± 2.8   | 49.2 ± 4.1  |
| 4. WIN 35,004   | 0.90 ± 0.05          | 49.2 ± 4.1   | 49.2 ± 4.1  |
| 5. WIN 30,068-3 | 0.88 ± 0.05          | 122.2 ± 26.1 | 220.0 ± 62.0|
| 6. Norcocaine   | 0.90 ± 0.10          | 9.6 ± 2.0    | 14.2 ± 0.1  |
| 7. (+)-Pseudo-  | 0.98 ± 0.06          | 9.9 ± 2.5    | 12.9 ± 0.3  |
| cocaine         |                      |              |              |
| 8. (-)-Pseudo-  | 0.51 ± 0.07          | 31.7 ± 4.4   | 29.8 ± 10.3 |
| cocaine         |                      |              |              |
| 9. (+)-Neopseudo- | 0.22 ± 0.04        | 0.4 ± 0.1    | 1.3 ± 0.1   |
| cocaine         |                      |              |              |
| 10. Benzoylpseudo- | 0.99 ± 0.01      | 5.0 ± 0.4    | 22.3 ± 6.5  |
| dotropine       |                      |              |              |
| 11. Benzytropine| 1.01 ± 0.10          | 17.2 ± 2.6   | 20.3 ± 0.8  |
| 12. Ecgonine    | 0.82 ± 0.01          | 41,000.0     | 29,000.0    |
| methylester     |                      |              |              |
| 13. Atropine    | 0.97 ± 0.05          | 240.6 ± 39.2 | 47.3 ± 0.2  |
| 14. Tetracaine  | 0.93 ± 0.06          | 2.5 ± 0.6    | 4.4 ± 0.2   |
| 15. Procaine    | 1.03 ± 0.12          | 143.6 ± 43.8 | 216 ± 7.0   |

* Concentration that produces a 2-fold enhancement of the dissociation rate constant (see end of Experimental Procedures).
* Mean ± S.E.
* Mean ± range.
* Limited supply of this drug only allowed one complete concentration curve.

Fig. 2. Acceleration of dissociation of [3H]BTX-B binding by cocaine congeners and procaine: time course. Specific binding (SB) at zero time (SB₀) was, on the average, 117 fmol/mg of protein at 7 nM [3H]BTX-B. Measurement of dissociation was initiated by the addition of 0.2 mM aconitine with or without drug as indicated. There was no change in the nonspecific binding with time. ○, without drug; •, with drug; ●, with drug, 9.5 µM (+)-neopseudococaine; □, 50 µM benzoylpseudotropine; ●, 500 µM cocaine; and ▲, 1700 µM procaine. Data shown are from a single experiment assayed in triplicate, which was replicated once.

Fig. 3. Acceleration of dissociation of [3H]BTX-B binding by cocaine congeners: concentration dependency. Values of Kᵢ, in the presence and absence of drug were estimated in single time point studies by addition of 0.2 mM aconitine as described under "Experimental Procedures." The control Kᵢ was, on the average, 0.0068 min⁻¹. The numbering of the compounds is as in Table I. Points shown are from a single experiment assayed in triplicate, which was replicated once.
ent association rate constant, \(k_{-1}\). On the average, for concentrations of the drugs at the \(K_d\) value, the ratio of \(k_{-1}\) over that in the absence of the drugs was 1.7 ± 0.3 (S. E. for 9 drugs). Thus, in kinetic terms, the inhibition of equilibrium binding of \([3H]BTX-B\) by cocaine congeners can be viewed as resulting mostly from their effects on the apparent dissociation rate constant of \([3H]BTX-B\) binding. A direct measure of \(k_{-1}\) in the presence of a cocaine congener is difficult to obtain in an allosteric system. If the congener alters the apparent allosteric equilibrium constant as has been shown for local anesthetic drugs (12), the equilibrium is shifted toward the nonconducting state, which may have a different \(k_{-1}\) than the active state. The ratio of apparent \(k_{-1}\) over \(k_{-1}\) measured at early time points, therefore, does not necessarily determine the \(K_d\) observed after equilibration of the system. Our results indicate that the nonconducting state in fact has a smaller \(k_{-1}\) than the active state (Fig. 4). Thus, in the presence of 30 \(\mu M\) tetracaine, a concentration \((10 \times K_d)\) that effectively reduces the binding of \([3H]BTX-B\) to the active state (Table I), the association of \([3H]BTX-B\) binding was dramatically slowed down; the same effect was found with 5 \(\mu M\) \((+)\)-neopseudococaine \((10 \times K_d)\). The fact that the deceleration of association was concentration dependent, as shown by the smaller effect of 1 \(\mu M\) \((+)\)-neopseudococaine, is consonant with a shift of the allosteric system toward the nonconducting state with increasing concentrations of \((+)\)-neopseudococaine, thus diminishing the chance of \([3H]BTX-B\) molecules to bind to high-affinity sites. There was no effect of the drugs on the nonspecific binding of \([3H]BTX-B\), which remained constant during the time course of these experiments.

Comparison of Effects on Sodium Channels with Those on Locomotor Behavior and on Dopamine Uptake into Striatal Synaptosomes—The rank order of potencies of cocaine congeners and local anesthetic drugs in inhibiting \([3H]BTX-B\) binding corresponded reasonably well with the rank order of potencies in depressing spontaneous locomotor activity. There was a positive correlation between the BTX inhibitory potencies and the hypom OTIC potencies \((r = 0.52, n = 13,\) two-tailed \(p < 0.07\)) (Fig. 5). Benzylpseudotropine and \((+)\)-neopseudococaine were two notable exceptions, being much weaker in depressing locomotion in vivo than expected from their sodium channel blocking activities.

There was only a weak correlation between the potencies in inhibiting the \([3H]BTX-B\) binding to mouse brain synaptosomes and the \(IC_{50}\) values for inhibiting the neuronal uptake of dopamine into mouse striatal synaptosomes \((r = 0.18, n = 12,\) two-tailed \(p > 0.5\)) (Fig. 5). Cocaine and WIN 35,428 were much more potent in inhibiting dopamine uptake than BTX binding. The results, however, do not rule out the possibility that for some congeners a blockade of the sodium channel may underlie the inhibition of dopamine uptake. For instance, for \(N\)-allylnorcocaine, benzoylpseudotropine, benzoyltropine, \((+)\)-pseudococaine, and WIN 35,465-3 the absolute \(K_i\) values for BTX inhibition and \(IC_{50}\) values for dopamine uptake inhibition were reasonably comparable (Fig. 5); assuming all other matters equal, one would expect in principle the \(IC_{50}\) values to be somewhat higher than the \(K_i\) values since the concentration of \([3H]dopamine\) used to obtain the \(IC_{50}\) estimates in the uptake assays was 0.1 \(\mu M\) as compared with a \(K_i\) of 0.13 \(\mu M\) (21) for transport of dopamine into synaptosomes. An intriguing result is the strong potency of \((+)\)-neopseudococaine in inhibiting \([3H]BTX-B\) binding as compared with its effect on dopamine uptake (see "Discussion").

**FIG. 5.** Potencies of cocaine congeners and local anesthetics in inhibiting \([3H]BTX-B\) binding, dopamine uptake into striatal synaptosomes, and spontaneous locomotor behavior. All measures were obtained in BALB/cBy mice. \(IC_{50}\) values for inhibition of dopamine uptake are from our recent work (18). Doses required for a 2-fold inhibition of locomotion were determined by us previously (6); additional assays were run on 9 and 10. The numbering of the compounds is as in Table 1. Additional compounds shown: 16, \(N\)-allylnorcocaine; 17, lidocaine; and 18, prilocaine. The BTX inhibitory potency for 17 and 18 is from Ref. 23, and for 16 from Ref. 16 with cocaine as a reference value. For correlation coefficients see "Results."

**DISCUSSION**

The Allosteric Model—The present results indicate that cocaine congeners, like other local anesthetic drugs, are apparent competitive inhibitors of \([3H]BTX-B\) binding, producing their effects by an allosteric heterotropic cooperative mechanism (13, 15). In equilibrium experiments, the cocaine congeners appear to inhibit \([3H]BTX-B\) binding by a competitive mechanism with pseudo Hill numbers close to unity (Fig. 1 and Table I); in dissociation experiments, the compounds produce a concentration-dependent increase in the rate of dissociation of \([3H]BTX-B\) binding (Fig. 2). Even though the estimates of \(EC_{50}\) values by the use of single time points should be viewed with caution, the similarity between the \(K_i\) and \(EC_{50}\) values is striking. It suggests that the inhibitory effect on binding results mostly from an increase of the apparent dissociation rate constant associated with an increase in the apparent allosteric equilibrium constant (= ...
(nonconducting state)/[active state]). The association rate constant appears to be lower for the nonconducting than for the active state (Fig. 4). Any attempt at measuring association rate constants is compounded by the problem of distinguishing the association of $[3H]$BTX-B with low-affinity binding sites on the nonconducting state ($k_{1,2}$) from that with high-affinity sites on the active state ($k_{1,1}$). However, the data for the first 5 min in Fig. 4 allow the estimation of a maximal value for $k_{1,1}$ of 5.7 x 10^4 M^−1min^−1 if it is assumed that all of the associated radioactivity in the presence of 30 μM tetracaine represents the nonconducting state, and 0.023% of the sodium channels is in the active state (12). A minimal value for $k_{1,1}$ of 0.5 x 10^4 M^−1min^−1 can be arrived at if the radioactivity associated in the presence of 30 μM tetracaine is taken as the sum of radioactivity bound to the nonconducting state and to the active state; the latter radioactivity is equal to or less than (0.023/0.2) x radioactivity associated in the absence of tetracaine, when 0.2% of the channel is in the active state (12). The minimal value is subject to large experimental error since most of the decrease in associated radioactivity by 30 μM tetracaine is due to the reduced concentration of active channels. The above estimates, along with a $K_i$ of $[3H]$BTX-B for the nonconducting state of 15 μM (13), give a $k_{1,1}$ of 0.08 to 0.85 min^−1, very much higher than the $k_{1,2}$ observed in the absence of local anesthetic drug, 0.0068 min^−1 (Fig. 3). This is in agreement with the increase in $k_{1,1}$ observed when a greater proportion of the channels are drawn into the nonconducting state by cocaine congeners (Fig. 3).

Structure-Activity Relationships—Removal of the ester linkage between the tropane and phenyl rings of cocaine either does not reduce the potency of the molecule in inhibiting the binding of $[3H]$BTX-B (WIN 35,140 and WIN 35,004, Table I) or diminishes the potency (WIN 35,428 and WIN 35,065-3) (for structures see Refs. 6 and 10). N-Demethylation (nor-cocaine) increases the inhibitory activity. Moving the carbonmethoxy group on C-2 from an axial cocaine and WIN 35,428) to an equatorial (−)-pseudococaine and WIN 35,140) position increases the potency by a factor of 4; (−)-pseudococaine, which is the enantiomer of (+)-pseudococaine and has the C-2 and C-3 substituents also in the equatorial positions, is three times less potent than (+)-pseudococaine. Removal of the O-benzoyl group from C-3 (ecgonine methylester) had a greater effect in reducing the potency than modifying the group (atropine). So far, these relationships generally agree with those observed by Matthews and Collins (10) for the inhibition of veratridine-stimulated 22Na+ uptake into rat brain membrane homogenates. Moving the O-benzoyl group on C-3 from an equatorial (benzoylpseudotropine) to an axial (benzoyllactone) position reduces the BTX inhibitory activity (Table I), consonant with the reduction in the potency on Na+ uptake observed with the C-3 epimer of cocaine, allo cocaine (10). The differences observed in the present work between cocaine, tetracaine, and procaine agree completely with those reported previously by Creveling et al. (11) and by Postma and Catterall (12). A surprisingly high potency was found for (+)-neopseudococaine. The above structure-activity relationships are different from those reported for various central activities (6) and for the neuronal uptake of dopamine into striatal synaptosomes (Fig. 5).

There is reasonable agreement between the rank order of potencies in inhibiting $[3H]$BTX-B binding and in depressing spontaneous locomotor behavior (Fig. 5). Such a comparison has to be judged with caution, since drugs applied in vivo must be transported to their presumed sites of action, are susceptible to metabolizing enzymes, and may have multiple effects. Yet, the observed correlation is consonant with the implication of sodium channels in the inhibition of locomotor behavior by cocaine congeners. Since the properties of sodium channels are probably similar in different tissues (8), the present results do not indicate whether central sodium channels are involved. There is some evidence suggesting action at the peripheral level (6, 7).

The weak correlation between sodium channel activity and neuronal dopamine uptake blockade observed in the present experiments does not support the suggestion (16) that interference with Na+ uptake can block dopamine uptake. In addition, (+)-neopseudococaine has no effect on dopamine uptake at concentrations that inhibit $[3H]$BTX-B binding (Fig. 5). Many other drugs are known to have a weaker potency in blocking striatal dopamine uptake than in inhibiting $[3H]$BTX-B binding to sodium channels, such as desipramine, imipramine, doxepin, mianserin, and chlorpromazine (22, 23). We could consider the possibility that the sodium channel assays are more sensitive because of the required activation by veratridine (9, 10) or scorpion toxin (11, 12, 23, this paper); this seems unlikely in view of the similar potencies of local anesthetics in those assays and in inducing membrane depolarization (11) and electrophysiologically measured channel block at low frequency stimulation (24) in the absence of activators. The present result for (+)-neopseudococaine is in agreement with the report of Holz and Coyle (21) that the uptake of dopamine into striatal synaptosomes is the same in the presence and absence of tetrodotoxin. It appears that it is necessary to have the proper Na+ gradient for synaptosomal dopamine uptake but the sodium channels can be blocked; veratridine and batrachotoxinin inhibit in vitro dopamine uptake, presumably by reducing the Na+ gradient (21). However, it is obvious that for in vivo functioning of the synapse the sodium channels are required, and the present work shows that many cocaine congeners inhibit sodium channel functioning at concentrations that also inhibit neuronal dopamine uptake. It is therefore necessary to consider action at the level of the sodium channel for pharmacological effects of cocaine congeners, in addition to the always invoked inhibition of neuronal dopamine uptake blockade observed in the present results (11, 669-672).

In humans, peak plasma levels of 0.3 μg of cocaine/ml of plasma have been found after cocaine administration of 1.4 mg/kg intranasally or 0.45 mg/kg intravenously (26); 0.9 μg/ml plasma has been measured after unlimited smoking of cocaine paste (27). These plasma levels could correspond to brain levels as high as 2 to 10 μg/ml, or 7 to 33 μM, achieved by a dose of cocaine normal for a cocaine addict. Such brain levels, if present in the vicinity of sodium channels, can be expected to inhibit the passage of sodium. It is conceivable that this underlies the ability of both cocaine and lidocaine to produce angydala hyperspinding (4); at the present time, however, we have to extrapolate the effects on in vivo sodium channel functioning from results obtained in vitro.

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