CHARACTERIZATION OF BUTYRYLCHOLINESTERASE ANTAGONISM OF COCAINE-INDUCED HYPERACTIVITY

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ABSTRACT:

Although there are several published demonstrations that exogenous butyrylcholinesterase (EC 3.1.1.8) works to antagonize cocaine in vivo, a systematic characterization of the enzyme-drug interaction is lacking as is confirmation of the mechanism of effect. This has been addressed using cocaine-induced locomotor activity in mice as a behavioral endpoint. The enzyme was effective, but the enzyme dose-antagonist effect relationship revealed an asymptotic partial maximum effect. This effect was not due to dose-dependent enzyme pharmacokinetics or to a stimulant effect of the cocaine metabolites but rather to partial metabolism of cocaine.

Since neither metabolite of cocaine inhibited enzyme activity as potently as cocaine, partial metabolism is not likely due to end-product inhibition. The enzyme reduced the maximum effect of cocaine on locomotor activity. The mechanistic data are generally consistent: the enzyme was inactive against the nonester dopamine/norepinephrine uptake inhibitor, nomifensine, and a paraxoxon-inactivated sample of enzyme was ineffective. However, the enzyme was effective against bupropion, a nonester dopamine uptake inhibitor.

Butyrylcholinesterase (BChE) is an enzyme capable of hydrolyzing many substrates, including cocaine (Lockridge, 1990). The role of the enzyme in cocaine metabolism was first established by Werner Kalow and his colleagues (Stewart et al., 1977), who showed that human plasma metabolized cocaine. This metabolism could be blocked by cholinesterase inhibitors and could be reproduced by reconstitution of purified enzyme (Stewart et al., 1977). Metabolism was seen despite the fact that the turnover number is much lower for (−)-cocaine, the naturally occurring isomer, than for (+)-cocaine (Gatley, 1991). Recently, catalytic parameters have been published for BChE from a number of species. Equine BChE (Km = 45 μM, Kat = 7.5 min−1) and human BChE (Km = 14 μM, Kat = 3.9 min−1) appear to be the best catalysts of cocaine hydrolysis (Xie et al., 1999).

At the same time, a number of groups have reported data showing that BChE can reduce the behavioral and physiological effects of a dose of cocaine and can alter the metabolism of cocaine. In mice, highly purified BChE administered via the i.p. route reduced the incidence of convulsions and lethality following i.p. administration of cocaine; pretreatment with a larger dose of enzyme further reduced the effect of the highest dose of cocaine (Hoffman et al., 1996). In rats, the same preparation of enzyme given via an i.v. route reduced the hypertensive effect of i.v. cocaine (Lynch et al., 1997). Pretreatment with BChE increased the LD50 of cocaine; administration of BChE after i.p. cocaine reduced the incidence of convulsions and lethality (Lynch et al., 1997). In a companion report, BChE reduced the amount of cocaine in plasma or brain samples from rats given i.p. cocaine and reduced the locomotor stimulant effect of i.v. cocaine (Mattes et al., 1997). Administration of BChE to cats after i.v. cocaine reduced the hypertension induced by cocaine; administration of enzyme after cocaine reduced the effect of cocaine on heart rate and QRS interval (Mattes et al., 1997). A less pure preparation of equine BChE given i.v. to rats reduced the locomotor stimulant effects of i.p. cocaine (Carmona et al., 1998). The same enzyme preparation given i.v. to squirrel monkeys was able to reduce the concentration of cocaine and slightly increase the concentration of ecgonine methyl ester seen in plasma after i.v. administration of cocaine (Carmona et al., 2000).

The published studies on in vivo interactions between cocaine and exogenous BChE present a diverse collection of data. Two species of enzyme are used in 11 doses, given i.v. or i.p.; most involve pretreatment, but in several cases, BChE was given after cocaine. Four species of experimental subjects were used, with pharmacokinetic, behavioral, and cardiovascular endpoints, in addition to lethality. Cocaine doses from 1 to 25 mg/kg (i.v.) and 17 to 150 mg/kg (i.p.) were used. Although this diversity of experimental protocols adds strength to the argument that exogenous BChE is an effective catalyst in vivo, it does not show a systematic dose-response curve for the enzyme effect. BChE could shift the cocaine dose-response curve as a competitive antagonist might, or suppress it as if a noncompetitive antagonist had been given.

The work reported to date has generally assumed that BChE is the source of the antagonism. However, even the best available preparations of the enzyme are frequently contaminated with one or more other enzymatic activities (Checler et al., 1990). Because BChE preparations are given in large doses designed to overcome the inef-
ficiency of (−)-cocaine as a substrate, the likelihood of administration of a substantial quantity of an impurity is increased. If impurities in the preparation produce the antagonist effect, then the mechanism of the effect should not resemble BChE. However, no published studies of exogenous BChE have tested the effect of preinactivation with a cholinesterase inhibitor; only one study has made any attempt to show pharmacological specificity of the antagonist effect (Lynch et al., 1997).

The studies reported here address the question of what the dose-response curves for cocaine and BChE are in vivo. As the BChE dose-response curve shows an asymptotic partial effect, studies address the mechanism of this effect. The effect of the enzyme on the time course of cocaine action is investigated. The mechanism of the antagonist effect is addressed using both tests of specificity (i.e., using nonster drugs with similar neurochemistry) and paraoxon-inactivated BChE.

Materials and Methods

Subjects. Male C3H mice (Harlan, Indianapolis, IN) weighing 25 to 40 g were used for these experiments in groups of 8 to 12 per condition. Enzyme and control pretreatments were made by injection into the lateral marginal tail vein except as noted. Intravenous infusions were made over 5 to 10 s in a volume of 0.5 ml.

Drugs. The BChE used for these experiments was a commercial preparation from horse plasma by potassium phosphate precipitation (Sigma-Aldrich, St. Louis, MO). Enzyme doses are in terms of nominal units, as labeled by the vendor, per kilogram of animal weight. Drug dosages are reported in terms of milligrams of their respective salt forms per kilogram of animal weight (bupropion HCl, GlaxoSmithKline, Uxbridge, Middlesex, UK; cocaine HCl, NIDA Research Technology Branch, Rockville, MD; and nomifensine maleate, Aventis, Strasbourg, France).

Locomotor Activity Measurements. The locomotor activity response to repeated injections of cocaine was measured using automated counting equipment (Digiscan; Omnitec Electronics, Columbus, OH). Mice were placed in an acrylic bin (8 × 10 × 22 inches) with an array of 16 infrared sensors along the long axis of the bin, 1 inch above the floor of the bin. Consecutive breaks of adjacent infrared beams were recorded as ambulatory activity; consecutive breaks of the same beam were recorded as stereotyped activity. Pilot data indicated that both types of activity are initially high but habituate with repeated injections of saline and that sensitivity to a given dose of cocaine is highest in the morning. Accordingly, all sessions were run in the morning; standard sessions began with a habituation period. After a saline injection (intraperitoneal), habituation continued for 10 min. Saline was injected again, and the activity was recorded for 15 min. Injections of drug (or saline) were followed by 15 min of recording. Thus, animals were injected once during habituation and four times during recording. Activity after these injections was analyzed as a repeated measure. The dose reported is the individual injection; thus, a mouse received a total of 96 (10 + 30 + 56) mg/kg cocaine in most experiments. For cocaine time course experiments, activity was recorded for 20 min after saline and 40 min after cocaine injection. When enzyme or control pretreatments were given, they were administered intravenously 1 h before the beginning of the initial habituation period of the test session. Blood samples were taken approximately 1 h after the end of behavioral testing (approximately 2.5 h after injection of pretreatments).

Enzyme Activity Measurements. Activity was measured using 1 mM butyrylthiocholine and 0.3 mM dithio-bis-nitrobenzoic acid (Sigma-Aldrich) in a modification of published protocols (Elliott et al., 1961; Locke, 1990) for a reaction volume of 150 μl. The reaction was monitored via absorbance at 412 nm using a microplate spectrophotometer. Assays were conducted at 25°C in 0.067 M potassium phosphate (pH 7.4). Sample volumes were adjusted to produce absorbance changes that were linear over several minutes but still at least 30 times the greatest observed background rate of absorbance change.

Paraoxon Inactivation. Inactivation reactions were carried out for 3 days at room temperature with 1 to 2 mM paraoxon added twice. This incubation duration was necessary to ensure that bound paraoxon was irreversibly bound (Mason et al., 1993). Free paraoxon was removed from the enzyme by three cycles of ultrafiltration (centrifugal concentrators with a nominal 100-kDa cutoff; Millipore Corp., Bedford, MA) and buffer exchange. The resulting enzyme preparation did not contain sufficient free paraoxon to inhibit the activity of a fresh enzyme preparation. Before injection, paraoxon-inactivated enzyme hydrolyzed butyrylthiocholine at about 0.02% its original rate.

Blood and Brain Sampling. For determination of plasma butyrylcholinesterase activity at a single time (i.e., after a locomotor activity test session), blood was collected by cardiac puncture under deep ketamine anesthesia (Fort Dodge Laboratories, Fort Dodge, IA). Heparin (500 U; Elkins-Sinn, Cherry Hill, NJ) was injected i.p. 15 min before blood collection. Blood was collected using a needle and syringe via an approach to the heart through the diaphragm and transferred to a microcentrifuge tube. Blood was placed in tubes that were chilled with ice to inhibit coagulation; plasma was separated by centrifugation at 4°C. For some experiments, brains were harvested, frozen in chilled 2-methylbutane, and stored on dry ice at ~80°C. In time-sensitive experiments (Fig. 7), injections were made on a staggered schedule so that samples could be taken within 1 min of the indicated time.

Cocaine Measurements. The protocol used in the current study was adapted from published methods (Jatlow and Nadim, 1990). The HPLC system used for determination of plasma cocaine levels was comprised of a solvent delivery system (Waters 600E; Waters, Milford, MA), ultraviolet detector (Waters 486), and injector fitted with a 20-μl injection loop (Waters 717+). The HPLC system was fitted with a 3.9 × 150-mm Waters Symmetry C8 column (5-μm particle size) and was controlled with Waters Millennium software. Solvents were purchased in HPLC grade from Fisher Scientific (Fair Lawn, NJ) except as noted. The mobile phase consisted of a solution of 333 ml of acetonitrile and 1 liter of 50 mM phosphate buffer (6.9 g of KH2PO4 in 1 liter of distilled water, adjusted to a pH of 3.0 with ortho-phosphoric acid) containing 1.88 g of sodium hexanesulfonate as an ion pairing reagent. The mobile phase was vacuum filtered (47-μm nylon 0.22-μm filter membrane; Micron Separations, Westboro, MA) and degassed under vacuum for 20 min. A carbonate buffer (700 mg of a dry mixture of 20 g of Na2CO3 and 17.5 g of NaHCO3, constituted in 10 ml of deionized water) was used to extract samples. Chromatography was performed with a mobile phase flow rate of 1.0 ml/min, and the effluent was monitored at 235 nm with sensitivity and rise time set at 0.01 absorbance units full scale and 1 s, respectively. Aliquots of 100 μl of plasma (with sodium fluoride added to blood before centrifugation to inhibit enzymatic hydrolysis of cocaine) or halves of brains frozen at ~80°C with the cerebellum removed were extracted for measurement of cocaine. These samples and standards were prepared for extraction by the addition of 25 μl of carbonate buffer, 100 μl of 40 mg/ml sodium fluoride (for brain samples), and 1.2 ml of an isoamyl alcohol/hexane (20 ml:1.0 liter) solution. Samples were then agitated for 15 min (plasma) or 45 min (brain samples, after homogenization) and centrifuged at 3100 g for 15 min. The upper (organic) layer was transferred to a new tube containing 40 μl of 0.1 N HCl, agitated for 5 min, and centrifuged for 5 min. The organic layer was discarded, and the lower (aqueous) phase was injected into the HPLC. Standard cocaine solutions (100,000, 10,000, 1,000, and 100 ng/ml concentrations) were prepared from a stock solution of 1 mg/ml cocaine (calculated as the salt) mixed in deionized water and extracted in the same manner as the unknown samples.

Results

Consecutive cocaine injections of 10 and 30 mg/kg resulted in increased ambulatory activity counts; 56 mg/kg is associated with a lower level of activity (Fig. 1). This is consistent with previous reports (e.g., Reith et al., 1985, 1986). Although structured observations were not made, hindlimb abduction and muscle tension were noted; these may explain the reduction in activity at 56 mg/kg. Repeated injections of saline result in low activity counts, with a small degree of habituation over the course of the experiment. Pretreatment with BChE reduced the activity counts following 10 mg/kg and enzyme approached levels seen after saline injection or 56 mg/kg (Fig. 1). Although activity counts following 10 mg/kg and enzyme approached levels seen after saline injection or 56 mg/kg (Fig. 1).
counts following saline at each injection. These data were analyzed by repeated-measures analysis of variance (RM ANOVA); treatment with cocaine or repeated saline and treatment with enzyme or no pretreatment were used as group factors, and injection (dose) was used as the repeated measure. All three main effects were significant: enzyme pretreatment or control \( F(1,35) = 13.47, p < 0.001 \), treatment with cocaine or saline \( F(1,35) = 50.04, p < 0.00001 \), and injection \( F(3,105) = 11.20, p < 0.00001 \). In addition, the interaction between cocaine or saline treatment and injection or dose was significant \( F(3,105) = 13.07, p < 0.00001 \).

Stereotyped behavior shows stimulation by cocaine similar to ambulatory activity; 10 and 30 mg/kg injections result in substantial increases in activity counts, whereas saline and 56 mg/kg injections are followed by low counts (Fig. 2). Enzyme pretreatments are slightly more effective in reducing stereotyped activity as the counts more nearly approach those following repeated saline injections (Fig. 2). The significance of effects, determined by RM ANOVA, was identical to that seen for ambulatory behavior. All three main effects were significant: enzyme pretreatment or control \( F(1,35) = 6.23, p < 0.05 \), treatment with cocaine or saline \( F(1,35) = 20.05, p < 0.0001 \), and injection \( F(3,105) = 4.84, p < 0.005 \). In addition, the interaction between cocaine or saline treatment and injection or dose was significant \( F(3,105) = 7.37, p < 0.0005 \).

Enzyme doses from 250 to 25,000 U/kg were tested. Pretreatment with doses of 2500 U/kg and above were effective (Fig. 3). There is some variability in the effect of enzyme pretreatments against different cocaine doses. Since the effects of cocaine and saline without pretreatment differ most at a cocaine dose of 30 mg/kg, this cocaine dose was chosen to highlight the effects of enzyme dose. The activity seen after the 30 mg/kg cocaine treatment was plotted for all enzyme pretreatment doses and shown with data from animals given either cocaine or saline after no pretreatment (Fig. 4). The data show that the antagonist effect of the enzyme reaches an asymptotic maximum with a pretreatment dose of 7900 U/kg and does not increase when the enzyme dose is increased.

To address concerns about enzyme bioavailability, samples of plasma taken following locomotor activity test sessions were tested for butyrylcholinesterase activity. Figure 5 shows the plasma enzyme activity for the mice tested in the locomotor activity experiments for Fig. 4; treatment groups are the same. In all groups, pretreatment with increasing doses of butyrylcholinesterase increased plasma enzyme activity.

To answer the question of whether the metabolites of cocaine could stimulate locomotor activity, doses of ecgonine methyl ester and benzoic acid equimolar to 10, 30, and 56 mg/kg cocaine (30, 89, and 167 \( \mu \)mol/kg) were injected during locomotor activity testing. The results are similar to saline: neither ecgonine methyl ester nor benzoic acid stimulated ambulatory or stereotyped activity at any dose (Fig. 6).

Samples of plasma and brain taken from mice treated with 30 mg/kg cocaine and pretreated with either no enzyme or one of four effective doses of enzyme were analyzed for cocaine. Enzyme pretreatment had a statistically significant effect on plasma cocaine concentration \( F(4,33) = 6.14, p < 0.001 \) and on brain cocaine...
Plasma cholinesterase activity

![Graph showing plasma cholinesterase activity](image)

**Fig. 5.** BChE pretreatment dose and plasma enzyme activity.

Blood samples were taken from BChE-pretreated mice and reference groups 1 h after the conclusion of locomotor activity testing.

Cocaine, ecgonine methyl ester, and benzoic acid were tested for their ability to inhibit butyrylcholinesterase activity. Using hydrolysis of 1 mM butyrylthiocholine as a baseline, cocaine was able to fully inhibit the enzyme; the EC₅₀ for inhibition was approximately 50 μM (Fig. 8). Benzoic acid was ineffective as an inhibitor at concentrations to 1 mM, whereas ecgonine methyl ester inhibited enzyme activity by about 25% at a concentration of 1 mM (Fig. 8). Ecgonine methyl ester is therefore at least 30-fold less potent than cocaine.

Mice treated with 10 or 30 mg/kg bupropion show increases in locomotor activity; 56 mg/kg also increases activity, but less than 30 mg/kg. The effect of all three doses is reduced by pretreatment with 25,000 U/kg of BChE (data not shown). Analysis of the ambulatory activity data by RM ANOVA showed significant effects of acute treatment [doses of bupropion and saline; \( F(3,42) = 8.15, p < 0.001 \)] and of pretreatment [enzyme versus no pretreatment; \( F(1,14) = 11.25, p < 0.005 \)] and a significant interaction term [\( F(3,42) = 3.14, p < 0.05 \)]. The plasma cholinesterase activities of mice in enzyme and no pretreatment groups (71.5 and 1.0 μmol of butyrylthiocholine/min/ml plasma, respectively) were similar to those of the corresponding groups in the cocaine studies. Bupropion was able to inhibit butyrylthiocholine hydrolysis with an EC₅₀ of approximately 200 μM.

Mice treated with 10, 30, or 56 mg/kg nomifensine show increased locomotor activity, which decreases with increasing doses. Informal observation suggests that the highest dose is associated with hindlimb tension and abduction and the presence of Straub tail. These phenomena and the increases in locomotor activity are still seen after pretreatment with the highest dose of enzyme used against cocaine (Fig. 9). Plasma cholinesterase activity in animals not receiving BChE was 0.6 μmol/min/ml; in animals pretreated with the enzyme, activity was 50.9 μmol/min/ml. RM ANOVA showed a significant effect of acute...
treatment on ambulatory activity [nomifensine doses and saline; \( F(3,42) = 9.99, p < 0.001 \) but no significant effect of pretreatment \( F(1,14) = 0.0007, p = 0.979 \) and no significant interaction \( F(3,42) = 1.01, p = 0.40 \). To assess the activity of butyrylcholinesterase against lower doses of nomifensine, mice were treated with 1, 3, or 10 mg/kg nomifensine with or without pretreatment of 25,000 U/kg BChE. Mice pretreated with the enzyme showed lower activity levels, even after saline injection; accordingly, activity counts were normalized by dividing drug counts by saline counts for each mouse. Normalized locomotor activity data showed a slightly greater degree of stimulation with enzyme than without (data not shown); no difference in stereotyped activity was seen. Both nomifensine dose-response curves show a peak at 10 mg/kg. Cholinesterase activity in animals not receiving BChE was 0.9 \( \mu \text{mol/min/ml} \); in animals pretreated with the enzyme, activity was 87.2 \( \mu \text{mol/min/ml} \).

As a further specificity control, experiments were conducted to test the sensitivity of the enzyme effect to a mechanism-based inhibitor of butyrylcholinesterase. An aliquot of enzyme was treated with paraoxon to inactivate the enzyme and filtered to remove free paraoxon. Mice treated with the inactivated enzyme preparation responded to 10 and 30 mg/kg cocaine with increased locomotor activity (Fig. 10).

Injections of saline and 56 mg/kg cocaine in these animals were each followed by lower activity counts. Although the activity level seen after 10 mg/kg was high, mice treated with the paraoxon-inactivated cholinesterase otherwise responded to cocaine like mice not pretreated with the enzyme. Although no residual paraoxon could be demonstrated in the inactivated enzyme preparation (either by inhibition of untreated enzyme or by color reaction on addition of 10 M NaOH), a solution of paraoxon without enzyme was filtered to remove paraoxon as the inactivated enzyme had been filtered. Mice pretreated with this solution showed the same pattern of response to cocaine as mice given no pretreatment (Fig. 10). Mice pretreated with the paraoxon-inactivated enzyme showed a 5-fold increase in plasma cholinesterase activity over previous control mice (2.4 \( \mu \text{mol} \) of butyrylthiocholine/ min/ml plasma); mice pretreated with the paraoxon control solution...
showed no change in plasma cholinesterase activity (0.5 μmol/min/ml). RM ANOVA was used to assess the effects of acute treatment and pretreatment (25,000 U/kg BChE, inactivated BChE, paraoxon control, and no pretreatment). The effect of acute treatment was significant \[ F(3,105) = 33.99, p < 0.001 \]. The effect of pretreatment was of marginal significance \[ F(3,35) = 2.85, p = 0.051 \]. The interaction term clearly was not significant \[ F(9,105) = 1.36, p = 0.22 \]. The data were further analyzed by \( t \) tests of ambulatory activity counts; since the data for 30 mg/kg showed the greatest cocaine effect without pretreatment, analysis focused on these data. However, these tests only showed the difference between activity after active enzyme (25,000 U/kg) and no pretreatment to be of marginal significance \( t(21) = 2.04, p = 0.054 \). Therefore, it is not surprising that neither the difference between activity after active enzyme and inactivated enzyme \( t(17) = 0.70, p = 0.496 \) nor the difference between activity without pretreatment and with inactivated enzyme \( t(18) = 1.03, p = 0.318 \) was statistically significant. Likewise, the difference between inactivated enzyme and paraoxon control was not significant \( t(14) = 0.29, p = 0.779 \).

An analysis of the effect of butyrylcholinesterase on the time course of cocaine stimulation of locomotor activity was conducted using shorter time intervals (2 min instead of 5). Pretreatment with 7900 U/kg BChE i.v. or no injection; activity was monitored in 2-min intervals instead of the usual 5-min intervals.

Discussion

BChE is an effective antagonist of cocaine. Administration of equine BChE was able to reduce the ambulatory activity stimulated by cocaine in mice (Fig. 1). Despite the fact that stereotyped activity is mediated by different brain regions (Cooper and Dourish, 1990) and has different neurochemistry than ambulatory activity (Arnt et al., 1988), enzyme pretreatment also reduced cocaine-induced stereotyped activity (Fig. 2). This suggests that the enzyme acts against the injected dose of cocaine and is not regionally or behaviorally specific. These data confirm and extend the published work on the subject (Mattes et al., 1997; Carmona et al., 1998). Despite the large injection volume, no overt toxic effect of enzyme administration was observed;
this is also consistent with published data (Genovese and Doctor, 1995; Lynch et al., 1997; Matzke et al., 1999).

The effect of BChE was to lower the dose-response curve for cocaine, not to reduce potency as a competitive antagonist would. This has not been reported previously. There is only one published report of different doses of cocaine in the presence of different doses of BChE; the study mentions that the LD$_{50}$ of i.v. cocaine is increased by administration of BChE to rats (Lynch et al., 1997). It is interesting to note that the effect of BChE on cocaine LD$_{50}$ reached an asymptote (i.e., did not fully eliminate the cocaine effect; Lynch et al., 1997). This has been reported with other substrates. Apnea following i.v. succinylcholine can be shortened but not eliminated by BChE administration (Borders et al., 1955). Recovery from neuromuscular blockade in patients treated with i.v. mivacurium is accelerated, but the effect of mivacurium is not eliminated by any dose of BChE (Naugib et al., 1995). When the present data are included, the literature shows an asymptotic partial effect in mice, rats, and humans; with equine or human enzyme; with cocaine, succinylcholine or mivacurium; and with i.p. or i.v. substrate dosing. However, no data on a mechanism for such an asymptote have ever been discussed.

There are several possible explanations for an asymptotic partial effect of the enzyme. Dose-dependent bioavailability (e.g., clumping of the enzyme solution at high concentrations followed by rapid elimination of the particulate matter) would explain the data. However, blood taken from the animals after behavioral testing showed that this does not happen (Fig. 5). A partial effect could also be explained by a weak, partial agonist-type effect of a metabolite; however, neither metabolite had any effect on locomotor activity (Fig. 6). The remaining possibility is that not all of the cocaine is metabolized. Based on the amount of cocaine found in plasma and brain at a time corresponding to the midpoint of activity testing, this appears to be true (Fig. 7).

Although the data explain the asymptotic partial effect on behavior, an explanation for a partial effect on cocaine metabolism remains elusive. It seems unlikely that end-product inhibition is responsible (Fig. 8). Availability of the substrate might limit activity; the explanation may involve the kinetics of cocaine as a substrate. As the concentration of cocaine decreases, the activity of the enzyme ($V / V_{\text{max}}$) will decrease; data consistent with this have been reported. The half-life of cocaine in an in vitro system decreases with increasing BChE activity but appears to reach an asymptote (Brownie et al., 1998). After pretreatment with one dose of BChE, plasma and brain cocaine levels were reduced by a lesser extent in rats receiving a smaller dose of cocaine (Mates et al., 1997). In these experiments, the plasma cocaine concentration determined after administration of 30 mg/kg, 3 to 4 $\mu$g/ml as the salt form, corresponds to a concentration of about 10 $\mu$M. This is below the Michaelis-Menten coefficient for cocaine at equine BChE (Xie et al., 1999).

The present study used efficacy against nonester stimulants and inhibition by a mechanism-based cholinesterase inhibitor to verify a cholinesterase mechanism for the antagonist effect. Published reports suggest that even affinity-purified enzyme contains contaminating enzymatic activities (Checler et al., 1990); the preparation used for these experiments is not affinity purified and probably contains substantial impurities (data not shown). Nomifensine, an inhibitor of norepinephrine and dopamine uptake, was not affected by BChE pretreatment at high doses (Fig. 9) or low doses (data not shown), despite the presence of plasma enzyme activity levels that were effective against cocaine.

Preincubation of the enzyme with paraaxon essentially eliminated the antagonist effect of the enzyme (Fig. 10). Although there was some elevation of plasma enzyme activity in mice pretreated with inactivated enzyme, this is probably due to the fact that some BChE can reactivate after binding organophosphates (Mason et al., 1993).

The effect of BChE on bupropion, a dopamine uptake inhibitor, was surprising. Although bupropion does not contain an ester, the enzyme was an effective bupropion antagonist (data not shown). It is possible that a bupropion metabolite is an ester; this would explain the finding that meta-chlorobenzoic acid is produced as a major bupropion metabolite (Schroeder, 1983). It is also possible that bupropion interacts directly with the enzyme; bupropion was found to inhibit butyrylcholine hydrolysis in vitro (data not shown). Taken together with published data on the specificity of the enzyme effect for cocaine relative to phenylephrine (Mates et al., 1997), the data argue that BChE is responsible for cocaine antagonist effects, even in an impure enzyme preparation.

The last parametric detail investigated in these experiments was the action of the enzyme on the maximum behavioral effect of cocaine and the decay of the cocaine effect. A decrease in maximum effect would imply that the enzyme has an effect on cocaine as the drug distributes through the body of the subject; an increase in the rate of decay of cocaine effect would imply that the enzyme is able to metabolize cocaine and possibly force the redistribution of the drug.

In summary, the data from these experiments support the hypothesis that BChE is an effective cocaine antagonist. However, several important questions were raised. The asymptotic partial effect of the enzyme on behavior can be explained in terms of limited enzyme effects on the drug. Further experiments will be necessary to explain the asymptotic partial metabolism of cocaine. It is also not clear what role tissue esterases and other esterases play in cocaine disposition.

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