

CHARACTERIZATION OF EQUINE BUTYRYLCHOLINESTERASE DISPOSITION IN THE MOUSE

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ABSTRACT:

Butyrylcholinesterase administration has been shown to block the effects of cocaine. However, even in model systems, the pharmacokinetics of the enzyme are only partly understood. Measurements of plasma enzyme concentration, antibody titer determinations, and measurement of cocaine-induced locomotor activity in mice were used to describe the disposition of butyrylcholinesterase. Clearance of the enzyme showed biexponential kinetics; the first component was sensitive to asialofetuin, suggesting a role for

the asialoglycoprotein receptor. Cocaine did not influence enzyme disposition. An antibody response to enzyme injection was seen; the role of this response is not clear. The antagonist effect of the enzyme was eliminated faster than the enzyme was eliminated from plasma; this may be due to a contribution of tissue esterases to cocaine metabolism. Intraperitoneal enzyme administration was not effective against cocaine, suggesting that the utility of the enzyme is route-dependent.

Butyrylcholinesterase (BChE¹) is an enzyme capable of hydrolyzing many substrates, including cocaine (Lockridge, 1990). Several laboratories have produced data showing that exogenous BChE is an effective cocaine antagonist in vivo. The enzyme has been shown to alter the metabolism of cocaine (Mattes et al., 1997; Carmona et al., 2000) as well as the behavioral effects (Lynch et al., 1997; Mattes et al., 1997; Carmona et al., 1998) and cardiovascular effects (Mattes et al., 1997). Finally, the neurological and lethal effects of cocaine are blocked by the enzyme (Hoffman et al., 1996; Lynch et al., 1997).

The clearance of both human and equine BChE from mouse plasma in vivo has been shown to proceed in a biexponential manner (Saxena et al., 1997, 1998). These biexponential kinetics have also been seen after administration of the enzyme to humans, with the rapid phase of elimination described as distribution (Østergaard et al., 1988). Following intravenous enzyme administration, biexponential kinetics have generally been seen whenever early samples are taken. However, there has been little discussion of how the enzyme is removed from plasma. In mice, desialylation of the enzyme accelerated the removal of the enzyme from circulation (Saxena et al., 1997, 1998), hinting at a role for the hepatic asialoglycoprotein receptor in the processing of the enzyme (Ashwell and Steer, 1981). Acetylcholinesterase has been studied using both desialylation and hypersialylation; the results suggest a linear relationship between clearance rate and the fraction of glycans ending in sialic acid (Kronman et al., 1995; Chitlaru et al., 1998). In addition, the asialoglycoprotein receptor ligand asialofetuin has been shown to inhibit the clearance of recombinant, partially

sialylated human acetylcholinesterase from plasma in mice (Kronman et al., 1995). Understanding the role of the asialoglycoprotein receptor in the different phases of butyrylcholinesterase clearance might allow development of longer acting forms of the enzyme. In addition, because asialoglycoprotein receptor-mediated clearance takes place in the liver, and because cocaine is hepatotoxic (Roth et al., 1992), it would be useful to know whether intercurrent administration of cocaine alters the clearance of the enzyme.

Another question raised by the literature is whether antibodies to the enzyme will be formed. Repeated i.m. administration of affinity-purified equine BChE to rabbits has been shown to stimulate an antibody response (Gentry et al., 1996). Intravenous administration of semipure equine BChE to monkeys twice has also been shown to stimulate an antibody response (Matzke et al., 1999). The importance of the antibody response is highlighted by the fact that the rate of clearance of a second dose of enzyme appeared faster than the corresponding rate for the first dose (Matzke et al., 1999). Although repeated i.m. injection of a protein in rabbits is a relatively standard protocol for antibody development (Harlow and Lane, 1988), the development of antibodies in monkeys after i.v. administration is surprising.

The final question raised by the literature is the effect of i.p. enzyme administration. One study found i.p. enzyme effective against cocaine in mice and reported high plasma BChE activity levels (V_D , 85 ml/kg, about three times plasma volume; Hoffman et al., 1996). However, another study has reported a peak plasma activity of 55 units/ml after i.p. administration of 5000 units to rats weighing about 320 g (V_D approximately 270 ml/kg, or approximately nine times plasma volume; Genovese and Doctor, 1995). It is not clear whether the differing pharmacokinetics in these studies reflect differences in experimental variables, variable uptake of the enzyme from the peritoneum, or simply the anatomical inconsistency of i.p. injection (Claassen, 1994).

The studies reported here measure the clearance of the enzyme

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¹ Abbreviations used are: BChE, butyrylcholinesterase; ELISA, enzyme-linked immunosorbent assay.

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TABLE 1
Sampling times

All times are given in hours.

Experiment	Group											
Long time course	25,000 U/kg	3	9	26	33	49	73	97	245	409		
Long time course	2500, 7900 U/kg	3	19	26	44	51	73	95	119	143	263	455
Short time course	1 (n = 4)	0.125			0.75							
	2 (n = 3)		0.25			1.5						
	3 (n = 4)			0.42			2.5					
Pharmacokinetic modification	Control	0.25	0.5	0.75	1	1.5	19.3	28	43	52	70	97
	Early asialofetuin	0.25	0.5	0.75	1	1.5	17.5	26	40	50	68	95
	Early cocaine	0.25	0.5	0.75	1	1.5	21.8	32	48	55	77	103
	Late asialofetuin						23.5	32.5	49	56	78	104
	Late cocaine						22.5	32.5	48	56	78	104

TABLE 2
BChE pharmacokinetic parameters

Estimated Parameters	25,000 U/kg	7,900 U/kg	2,500 U/kg	25,000 U/kg 7.5 to 150 min.
C_0 ($\mu\text{mol}/\text{min}/\text{ml}$)	120.9	41.5	13.0	55.5
k (h^{-1})	0.0282	0.0343	0.0316	1.3075
Baseline ($\mu\text{mol}/\text{min}/\text{ml}$)	1.6	1.7	1.6	174.8
$t_{1/2}$ (h)	24.5	20.2	21.9	0.53

Model used for regression: activity = baseline + ($C_0 \cdot e^{(-k \cdot \text{time})}$). All data are for observations taken from 2.5 to 400 h, except as noted.

from plasma and its antagonist effect on cocaine-induced locomotor activity. Development of antibodies was measured as well as the effect of asialofetuin and cocaine on BChE clearance.

Materials and Methods

Subjects. Male C3H mice (Harlan, Indianapolis, IN) weighing 25 to 40 g were used for these experiments. 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. 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.

Locomotor Activity Measurements. The locomotor activity response to repeated injections of cocaine was measured using automated counting equipment (Digiscan; Omnitech Electronics, Columbus, OH). Mice were placed in an acrylic bin ($8 \times 10 \times 22$ 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. 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).

Enzyme Activity Measurements. Butyrylcholinesterase activity was measured using 1 mM butyrylthiocholine and 0.3 mM dithio-bis-nitrobenzoic acid (Sigma-Aldrich) in a modification of published protocols (Ellman et al., 1961; Lockridge, 1990) for a reaction volume of 150 μl . The reaction was monitored via absorbance at 412 nm using a microplate spectrophotometer. For some experiments, butyrylcholinesterase activity was measured using 50 μM benzoylcholine (Sigma-Aldrich) in a protocol modified from published methods (Kalow and Lindsay, 1955; Lockridge, 1990) for a reaction volume of 150 μl . Benzoylcholine assays were conducted in ultraviolet-transparent microplates (Corning, Corning, NY); the reactions were monitored using absorbance at 240

nm. 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 background rate of absorbance change.

Blood 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 (Fort Dodge Laboratories, Fort Dodge, IA) anesthesia. 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. When multiple samples from the same individual were required (i.e., time course experiments), blood was collected from the tip of the tail; for each sample, mice were restrained, and approximately 1 mm was cut off the tip of the tail. This typically allowed for the collection of 40 to 50 μl of blood; if the tail continued to bleed, a monopolar cautery pen was used to stop the bleeding. Different sampling intervals were used for different experiments; these intervals are outlined in Table 1. For some experiments, blood was collected from the retro-orbital venous sinus using a microcapillary pipette. In all cases, blood was placed in tubes, which were chilled with ice to inhibit coagulation; plasma was separated by centrifugation at 4°C.

Antibody Measurements. The development of antibody titers over time were followed using enzyme-linked immunosorbent assay methods. A 20 $\mu\text{g}/\text{ml}$ solution of BChE was coated on microtiter plates (Corning) overnight at 4°C. Plates were blocked with 3% ovalbumin (Sigma-Aldrich) for 2 h at room temperature; before and after blocking, plates were washed twice with chilled phosphate-buffered saline. Plasma samples were diluted in 3% ovalbumin and incubated for 2 h at room temperature. Secondary antibodies diluted in 3% ovalbumin were also incubated for 2 h at room temperature; before and

TABLE 3
Modification of BChE pharmacokineticsModel used for regression: activity = ($C_0 \cdot e^{(-k \cdot \text{time})}$) + ($C' \cdot e^{(-k' \cdot \text{time})}$). Activity determined using butyrylthiocholine except as noted.

	k	$t_{1/2}$	k'	$t_{1/2}'$
		h		h
Control	0.943	0.73	0.029	23.9
Cocaine early	0.976	0.71	0.031	22.4
Cocaine late	N.D.		0.021	33
Asialofetuin early	0.348	1.99	0.024	28.9
Asialofetuin late	N.D.		0.024	28.9
Control (benzoylcholine)	1.659	0.42	0.027	25.7

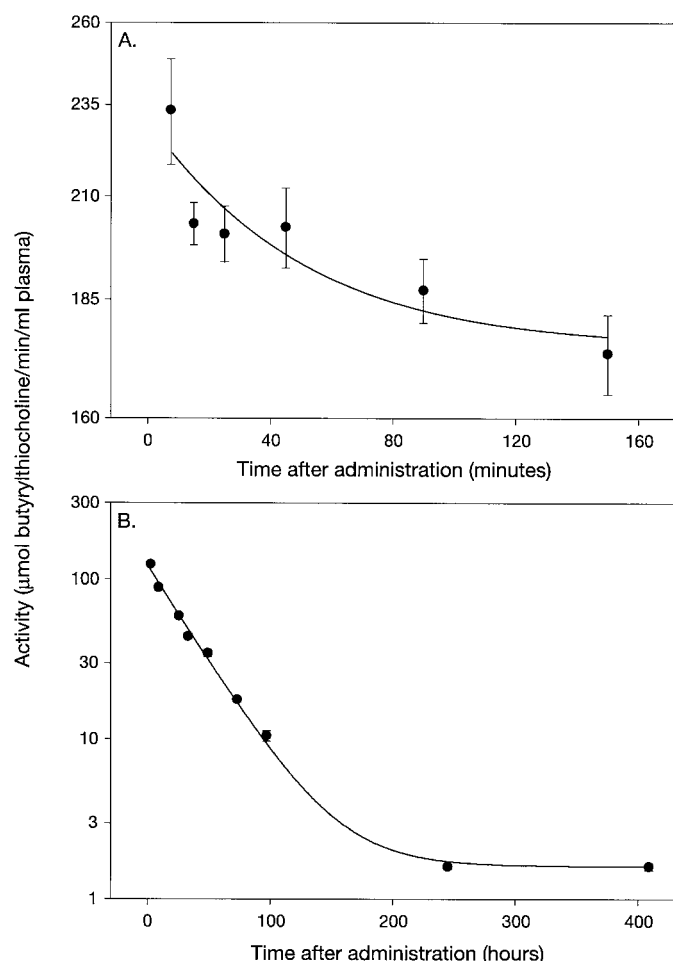


FIG. 1. Rapid and slow phase of BChE elimination.

A, rapid phase of BChE elimination; samples taken from 7.5 to 150 min after 25,000 U/kg BChE administration were tested for activity against butyrylthiocholine. B, slow phase of BChE elimination; samples taken from 2.5 to 400 h after BChE administration (25,000 U/kg) were tested for activity. Points represent mean observed enzyme activity \pm S.E.M.; the lines represent the fitted models (cf. Table 2).

after secondary antibody incubation, plates were washed three times with chilled phosphate-buffered saline. The secondary antibody solution used for these experiments was an alkaline phosphatase-coupled antibody against murine immunoglobulin G (Sigma-Aldrich) diluted 1:10,000. After plates were dried, activity was measured with *para*-nitrophenol phosphate (Sigma-Aldrich) and incubated for 25 min at room temperature; results were read as absorbance at 405 nm using a microplate reader (Molecular Devices, Sunnyvale, CA).

Data Analysis. Since previous research has described biexponential clearance (Østergaard et al., 1988; Saxena et al., 1997, 1998), we thought it likely that these data would also show two clearance rates. Initial experiments on two groups of mice, one sampled at short time intervals and one at longer intervals, revealed estimated elimination rate constants differing by a factor of 40 (Table 2). When these data were pooled, a biexponential model afforded a much better fit as indicated by both analysis of variance and the use of Akaike's Information Criterion (cf. McQuarrie and Tsai, 1998). This was also true for an analysis of the control mice sampled at both early and late time periods (Table 3). Nonlinear regression was conducted using Statistica for Windows (StatSoft, Tulsa, OK).

Results

Initial pharmacokinetic studies used two groups of mice, with blood sampled from one group at intervals up to 150 min to measure the rapid phase of enzyme elimination and blood sampled from the other

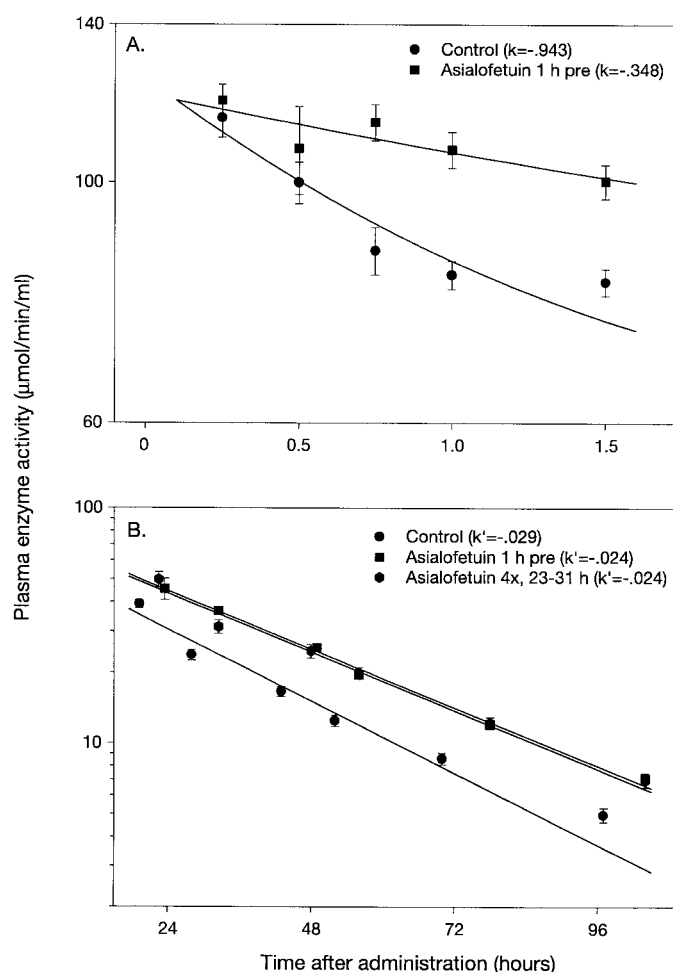


FIG. 2. Effect of asialofetuin on BChE elimination.

The disappearance of plasma enzyme activity was monitored in mice receiving no pretreatment, 200 mg/kg asialofetuin i.v. 1 h before enzyme administration, or 200 mg/kg asialofetuin four times during a period 1 day after enzyme administration. Points represent mean observed enzyme activity \pm S.E.M.; the lines represent the fitted models (cf. Table 3).

group at longer intervals to measure the slow phase. Samples taken from 7.5 to 150 min after i.v. injection of 25,000 U/kg showed an elimination $t_{1/2}$ of 31.8 min (Fig. 1A). The volume of distribution determined for the rapid component was 0.56 ml, or approximately 23 ml/kg; this is similar to the anticipated plasma volume (approximately 50–55% of a blood volume of 58 ml/kg). Plasma samples taken from a second group of mice from a starting time of 2.5 h after injection fit a model of a slow elimination component with a $t_{1/2}$ of about 1 day (24.6 h; Fig. 1B). This is very slightly longer than the estimates for lower doses (2500 and 7900 U/kg, 20.2 and 21.9 h; Table 2). Estimates of baseline taken from these later models closely matched plasma butyrylthiocholine hydrolysis rates in mice not treated with enzyme (data not shown).

Three groups of mice were prepared for an experiment designed to test the role of the asialoglycoprotein receptor in BChE elimination. Control mice were injected with 12500 U/kg BChE, and blood was collected by tail clip at short intervals during the first 90 min after injection and at longer intervals for the next 4 days. Another group received the competitive asialoglycoprotein receptor ligand asialofetuin (200 mg/kg, i.v.) 1 h before BChE injection; blood was sampled from these mice for both short and long time intervals. A third group received four injections of asialofetuin (200 mg/kg, i.v.) at 2.5 h

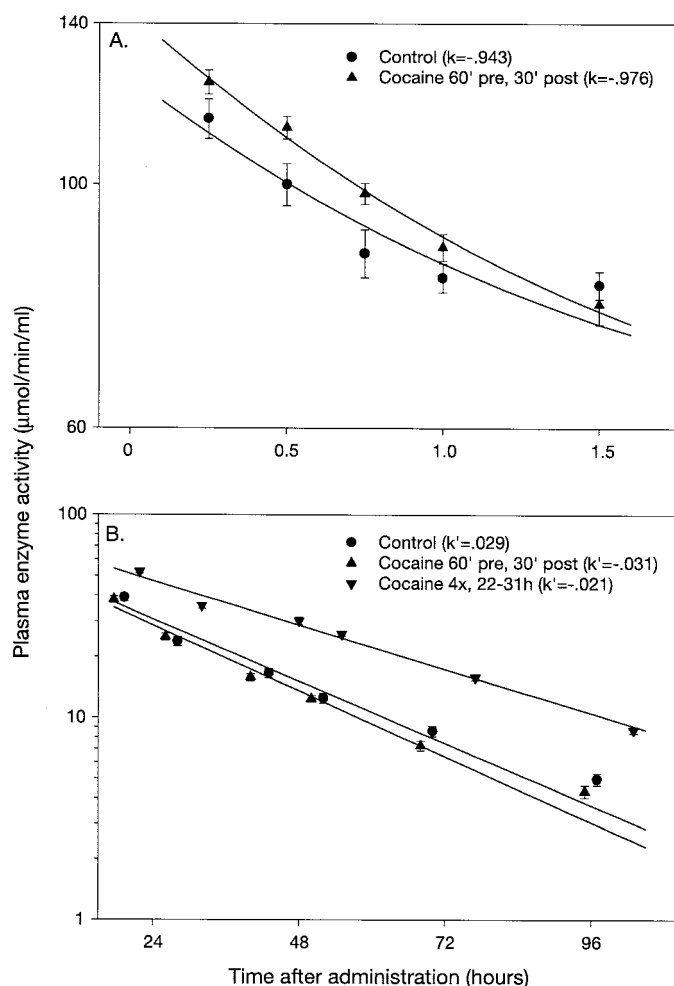


FIG. 3. Effect of cocaine on BChE elimination.

The disappearance of plasma enzyme activity was monitored in mice receiving 30 mg/kg cocaine i.p. 1 h before and 30 min after enzyme administration or 30 mg/kg cocaine four times during a period 1 day after enzyme administration; these groups were compared with the control group shown in Fig. 2. Points represent mean observed enzyme activity \pm S.E.M.; the lines represent the fitted models (cf. Table 3).

intervals 1 day after BChE injection; blood was sampled at long time intervals only. Asialofetuin pretreatment reduced the rate constant for the rapid phase of BChE elimination from plasma (0.348 h^{-1} versus controls, 0.943 h^{-1} ; Fig. 2; Table 3). However, there was no effect on the rate constant for the slow phase of BChE elimination (controls, 0.029 h^{-1} ; asialofetuin pretreatment, 0.024 h^{-1} ; and late asialofetuin, 0.024 h^{-1}). Of plasma enzyme activity, 33.8% was eliminated during the period of repeated asialofetuin injection; over the same period, control mice eliminated 38.4% of plasma enzyme activity.

To ensure that cocaine does not influence the elimination of BChE, the control mice from the previous experiment were compared with mice injected with the same dose of enzyme and cocaine (30 mg/kg, i.p.). One group received cocaine 60 min before and 30 min after enzyme; blood was sampled at short and long time intervals. A second group received four cocaine injections at 2.5 h intervals 1 day after BChE injection; blood was sampled at long time intervals only. Acute cocaine treatment did not change the rate constant for rapid elimination of BChE (controls, 0.943 h^{-1} and cocaine, 0.976 h^{-1} ; Fig. 3; Table 2). Repeated late cocaine injection decreased the rate constant for slow elimination of BChE slightly (0.021 h^{-1} versus controls, 0.029 h^{-1} and acute cocaine, 0.031 h^{-1}). However, the difference in

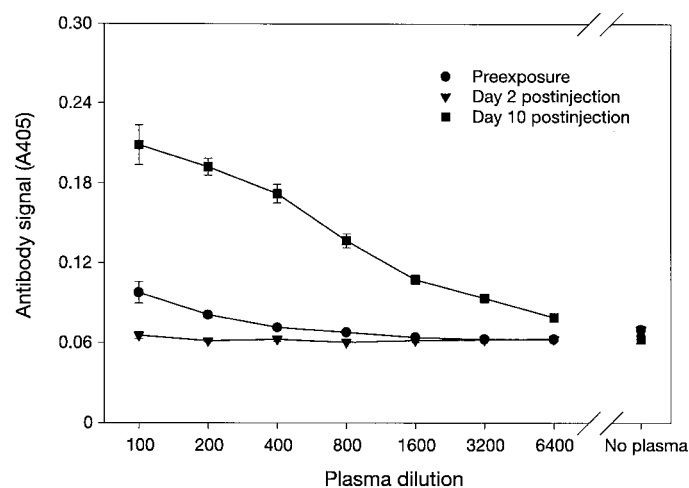


FIG. 4. Antigenicity of BChE injection.

Blood taken from mice before and 2 or 10 days after BChE injection was analyzed by ELISA for antibodies to the BChE solution.

parameter estimates for the two groups depends largely on the earliest observation; elimination of the 20-h observation from the control group and the early cocaine group results in estimates that are nearly identical (0.0234 h^{-1} versus 0.0236 h^{-1} , respectively). Of plasma enzyme activity, 31.7% was eliminated during the period of repeated cocaine injections; over the same period, control mice eliminated 38.4% of plasma enzyme activity.

Samples from mice before injection of 25,000 U/kg BChE as well as 2 and 10 days after injection were tested by ELISA for antibodies to the BChE solution. An elevation in immunoglobulin G signal could be seen 10 days after injection (Fig. 4). The samples taken 2 days after injection show an apparent loss of antibody signal when compared with preexposure samples. Specificity controls, including substitution of anti-BChE antibodies from another species, omission of secondary antibody, and preincubation of secondary antibody with 20 $\mu\text{g/ml}$ mouse IgG all eliminated antibody signal.

Based on the elimination of BChE from plasma, times were chosen so that the predicted remaining activity would be about one-half and one-fourth of C_0 , based on the model and parameters in Table 2. Mice were pretreated with 25,000 U/kg BChE and returned to their cages; one group was tested at 15.3 h after injection, whereas the other group was tested at 39 h after injection. Both groups responded to cocaine like mice administered no pretreatment (Fig. 5). Plasma from these animals after activity testing showed activity of $54.6 \mu\text{mol}$ of butyrylthiocholine/min/ml at 15.3 h and $31.0 \mu\text{mol/min/ml}$ at 39 h.

Plasma samples were tested with two test substrates to rule out the possibility that butyrylthiocholine yields aberrant data when used as a test substrate. Benzoylcholine is an aromatic substrate and is thought to produce the same benzoate-enzyme intermediate produced by the hydrolysis of cocaine. Activity against both substrates declined over time in parallel (Fig. 6); elimination rate constant estimates were similar (Table 2). When individual subject data points were compared, activity against one substrate predicted activity against the other (data not shown).

Locomotor activity stimulated by cocaine following intraperitoneal administration of 25,000 U/kg butyrylcholinesterase resembled the pattern seen after omission of enzyme pretreatment (Fig. 7). Plasma samples taken from these animals showed enzyme activity well above control levels ($36.7 \mu\text{mol}$ of butyrylthiocholine/min/ml of plasma). However, to rule out the possibility that residual enzyme present in the peritoneal cavity contaminated the sample as it was drawn from the

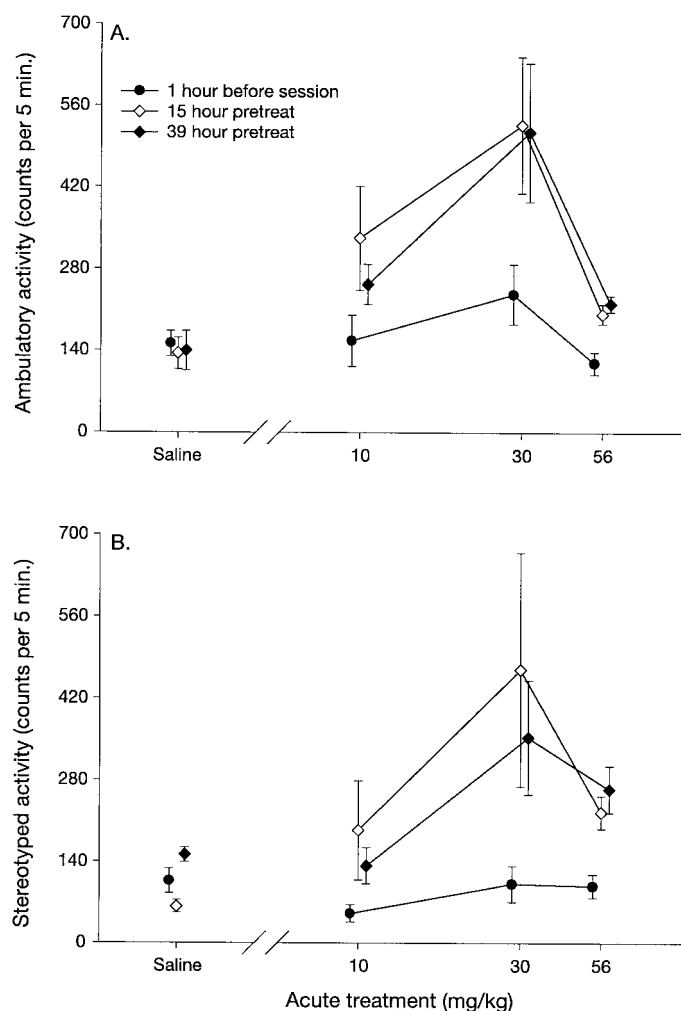


FIG. 5. Elimination of BChE antagonist effect.

The locomotor stimulant properties of i.p. cocaine were compared in mice pretreated with 25,000 U/kg BChE i.v. 1 h before the beginning of the test session and transferred to the testing room or pretreated 15 or 39 h before testing and returned to their home cages.

thorax, multiple samples were taken from a separate group of mice. About 2.5 h after intraperitoneal administration of 12,500 U/kg of enzyme, blood was taken from the retro-orbital sinus, tail vessels (tail clip), and from the heart (cardiac puncture, again by a caudal approach through the diaphragm). These samples all showed similar enzyme activity levels (6.9, 6.8, and 6.7 $\mu\text{mol}/\text{min}/\text{ml}$, respectively).

Discussion

Elimination from plasma proceeded in a biexponential fashion, with an early component having a $t_{1/2}$ of about 0.5 h (Fig. 1A; Table 2). Samples taken after 150 min (i.e., after six half-lives of the first component) showed a late component having a $t_{1/2}$ of about 24 h (Fig. 1B; Table 2), consistent with published reports (e.g., Saxena et al., 1997, 1998). The data expand on previous reports by showing that the elimination of the enzyme is independent of dose over the range tested (Table 1). In addition, the rate of the first component could be slowed by injection of the asialoglycoprotein receptor ligand asialofetuin. However, neither early nor repeated late injections of asialofetuin had any effect on the slow rate of elimination (Fig. 2; Table 3). By arguing for receptor-mediated clearance (Ashwell and Steer, 1981), these data also argue against simple distribution of the enzyme as the process

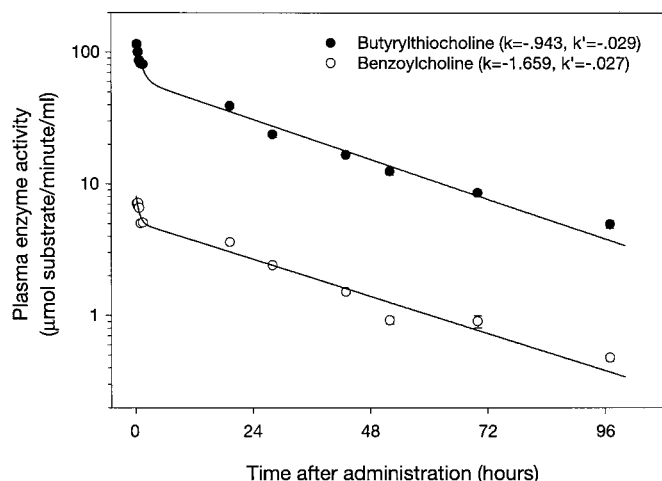


FIG. 6. Effect of substrate on elimination rate estimates.

Samples taken from a group of mice after BChE administration were tested for enzyme activity using both butyrylthiocholine and benzoylcholine as test substrates. Points represent mean observed enzyme activity \pm S.E.M.; the lines represent the fitted models (cf. Table 3).

responsible for rapid elimination of the enzyme from plasma. Although sensitivity of acetylcholinesterase elimination to asialofetuin injection has been reported (Kronman et al., 1995), this is the first study of BChE and the first to show a mechanistic distinction between the two kinetic components of elimination. There are similarities between the equine BChE and mouse subjects used in this experiment and the human enzyme and patients. The glycosylation of human and equine BChE, although not identical, is similar (Saxena et al., 1998); the asialoglycoprotein receptor is found in the liver of both rodents and humans (Ashwell and Steer, 1981).

Since these data suggest a role for the liver in the clearance of the enzyme, and since cocaine can be hepatotoxic (Roth et al., 1992), cocaine might alter clearance of the enzyme. In these experiments, cocaine had no effect on the elimination of BChE (Fig. 3; Table 3). Although there was a slight difference between the elimination rate calculated for mice receiving several cocaine injections 1 day after injection ("late cocaine") and the other groups, the difference was no greater than the variability in rate estimates when one point was omitted (data not shown).

The present data show that even after a single i.v. injection, mice will generate antibodies that recognize the enzyme preparation (Fig. 4). The ELISA procedure used here does not distinguish between antibodies recognizing the enzyme and antibodies recognizing contaminating proteins; however, it should be noted that rabbits have been shown to produce antibodies to affinity-purified equine BChE (Gentry et al., 1996). Even if the antibodies are directed toward the enzyme, it is possible that contaminating proteins will have an adjuvant effect (similar to that shown for impurities in insulin preparations; Schlichtkrull et al., 1974). This argues for the use of pure preparations of enzyme, ideally from the same species.

It is not clear what role antibodies and the reticuloendothelial system played in the elimination of enzyme in the present experiment. Whether baseline antibody signal represented conventional binding through complementarity-determining regions or nonspecific interactions, levels were diminished 2 days after enzyme injection (Fig. 4). It may be that the activity of the reticuloendothelial system in clearing blood-borne immune complexes (Klein and Horejsi, 1997) represents the part of the early elimination of enzyme not eliminated by asialofetuin (Fig. 2).

The rate of elimination of the antagonist effect did not match the

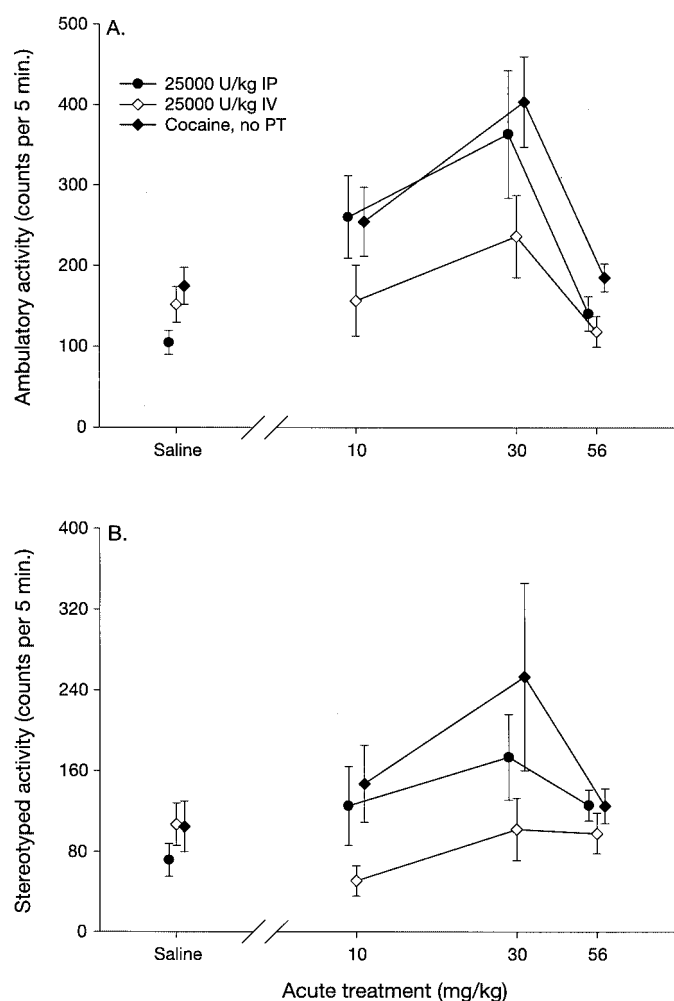


FIG. 7. Route dependence of BChE antagonist effect.

25,000 U/kg BChE was injected either i.p. or i.v. and compared with mice receiving no pretreatment.

rate of elimination of the enzyme. At 15 and 39 h after enzyme administration, about one-half and one-fourth of the injected dose should remain (calculated using the biexponential model and data in Table 2). However, even at 15 h after injection, the enzyme had no effect (Fig. 5). It is theoretically possible that the test substrate used for enzyme pharmacokinetic determinations, butyrylthiocholine, gave results that do not predict metabolism of other (e.g., aromatic) substrates. Benzoylcholine is thought to form the same enzyme/intermediate complex as cocaine but have a faster turnover (Xie et al., 1999). Accordingly, samples were tested in parallel using butyrylthiocholine and benzoylcholine; the results were very similar (Fig. 6), given the differences in turnover number (Xie et al., 1999). It is more likely that the measurement of plasma BChE activity does not accurately reflect the total body cocaine hydrolyzing activity of the subject. If other esterase activities contribute to the disposition of cocaine, then the effect of the exogenous enzyme will lose its significance when it drops below a certain multiple of the endogenous cocaine hydrolyzing activity. The importance of tissue esterases has already been established for succinylcholine metabolism in several species (Hobbiger and Peck, 1970, 1971).

Intraperitoneal administration of the enzyme was also studied. One publication showed i.p. BChE to be effective against cocaine and reported good bioavailability of the enzyme (terminal V_D , 85 ml/kg;

Hoffman et al., 1996). However, another publication reports much lower bioavailability (terminal V_D about 270 ml/kg; Genovese and Doctor, 1995). A full bioavailability study was beyond the scope of this project; however, some interesting data are available. Intraperitoneal BChE was ineffective in the present study (Fig. 7), although enzyme activity was found in plasma samples. As it is possible that these samples were contaminated with peritoneal fluid, a second set of experiments was conducted using i.p. administration of a lower dose of enzyme and sampling blood from three different sites. Enzyme activities were low, even from the cardiac puncture sample. Intraperitoneal and i.v. administration differ in terms of the plasma enzyme activity that is achieved; perhaps more importantly, however, the time in which maximum activity is achieved differs, with an absorption process required for i.p. activity (Claassen, 1994). Thus, there are several pharmacokinetic differences between routes, complicated by the erratic nature of absorption of material from the peritoneal cavity (Claassen, 1994). Whatever the reason for the present results, it seems clear that the i.p. route is not particularly suitable for BChE administration.

These experiments confirmed that BChE elimination proceeds in two kinetic components; the data also show a mechanistic difference between the components. Specifically, the asialoglycoprotein receptor is involved in the rapid component. Neither component was affected by cocaine. Further research will be necessary to determine a mechanism for the slow component of enzyme clearance and to establish the role of antibodies in enzyme clearance.

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