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ACTIVITIES OF THE ENANTIOMERS OF COCAINE AND SOME RELATED COMPOUNDS AS SUBSTRATES AND INHIBITORS OF PLASMA BUTYRYLCHOLINESTERASE

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Abstract—The behaviors of the enantiomers of cocaine (benzoylecgonine methyl ester) and related compounds with butyrylcholinesterase (BChE; EC 3.1.1.8) were investigated spectrophotometrically at 235 nm. The unnatural enantiomer, (+)-cocaine, was hydrolyzed by BChE (extinction coefficient 6.7 $L \cdot mmol^{-1} \cdot cm^{-1}$) at about half the rate of benzoylcholine, but over 2000 times faster than naturally occurring (−)-cocaine. This rapid hydrolysis of (+)-cocaine may account, in part, for its pharmacological inactivity. (+)-Norcocaine, (+)-benzoylecgonine, (−)-ψ-cocaine and tropacocaine were also substrates for BChE. Hydrolysis of (+)-cocaine was sensitive to several standard inhibitors of BChE, including those of competitive, carbamate and organophosphorus classes. Although (−)-cocaine was a poor substrate for debenzoylation, it was a fairly good competitive inhibitor ($K_i \sim 10\ \mu M$) of the hydrolysis of other substrates. The cocaine metabolites (−)-norcocaine, (−)-benzoylecgonine and (−)-ecgonine methyl ester inhibited BChE with K_i values of 15, 76 and 1300 μM, respectively. (+)-ψ-Cocaine had $K_i = 3\ \mu M$. *p*-Nitro and *p*-fluoro derivatives of cocaine and analogs with phenyl and *p*-fluorophenyl groups in place of the benzoyl ester linkage (WIN 35,065-2 and WIN 35,428) inhibited BChE comparably to (−)-cocaine itself. Both cocaine enantiomers were weak inhibitors of acetylcholinesterase (AChE; EC 3.1.1.7) from human erythrocytes with similar K_i values (160–170 μM). Although it is unlikely that the inhibition of BChE is an important factor in the subjective effects of cocaine, it may have implications for the toxicity of cocaine to the fetus, since BChE appears in the development of the central nervous system before AChE, and has been suggested to function as an embryonic acetylcholinesterase.

Cocaine abuse has become a major public health problem, and improved understanding of both the mechanism of addiction and its treatment is urgently required. Cocaine exhibits a complex pharmacology [1]. Its mood-altering effects and addictive nature are thought to involve binding to the presynaptic reuptake sites on dopaminergic neurons [2–5]. Cocaine, however, also interacts with sigma [6], muscarinic cholinergic [7] and glutaminergic [8] receptors, while its local anesthetic effects are mediated via neuronal sodium channels [9]. Natural cocaine, extracted from the leaves of *Eryroxylon coca*, is exclusively the levo enantiomer, (−)-cocaine, which has the absolute stereochemistry 1*R*, 2*S*, 3*S*, 5*S* [10]. Synthetic (+)-cocaine lacks the behavioral properties of the levo-rotatory isomer [11], and is also reported to be a much weaker inhibitor of presynaptic monoamine uptake [5, 12].

In conjunction with positron emission tomographic (PET) studies of the binding of ^{11}C labeled cocaine in the human brain [13], we prepared ^{11}C-labeled (+)-cocaine. Our intention was to use the unnatural enantiomer to better define the relative contributions of specific and non-specific binding of [^{11}C]cocaine to the brain uptake. However, label from (+)-cocaine did not enter the brain. *In vitro* experiments with ^{11}C-labeled compounds revealed extremely rapid stereoselective hydrolysis of (+)-cocaine in the blood plasma [14], which could be inhibited by physostigmine. Natural cocaine was also hydrolyzed in plasma, as has been reported previously for human plasma [15, 16], but several orders of magnitude more slowly than (+)-cocaine. Cleavage of the benzoyl ester linkage of (−)-cocaine has been ascribed to butyrylcholinesterase (BChE,* EC 3.1.1.8) which is important in the catabolism of other drugs such as heroin [17] and aspirin [18]. Our observations with the ^{11}C compounds prompted an *in vitro* examination of the behavior of isolated BChE. This new study has resulted in determination of K_m and V_{max} values and/or K_i values for the cocaine enantiomers and for several cocaine metabolites and related compounds.

MATERIALS AND METHODS

WIN 35,065-2 and WIN 35,428 were supplied by Sterling Drug Inc., Rensselaer, NY. Cocaine enantiomers and related compounds were obtained from the National Institute on Drug Abuse. (+)-Cocaine [19] was N-demethylated with α-chloroethyl chloroformate [20] to yield (+)-norcocaine, and was O-demethylated to give (+)-benzoylecgonine by heating an aqueous solution at 120° for 2 hr in a sealed vial. Preparations of *p*-nitro- and *p*-fluorococaine derivatives involved esterification of ecgonine methyl ester with the corresponding benzoyl chlorides.† Enzymes [acetylcholinesterase (AChE), EC 3.1.1.7, type XIII from human

* Abbreviations: BChE, butyrylcholinesterase (EC 3.1.1.8); AChE, acetylcholinesterase (EC 3.1.1.7); WIN 35,428, 3β-(4-fluorophenyl)-2β-carboxylic acid methyl ester; WIN 35,065-2, 3β-phenyl-2β-carboxylic acid methyl ester; and iso-OMPA, tetraisopropyl pyrophosphoramide.

† Yu D-W, unpublished data, cited with permission.

erythrocytes; BChE, highly purified, from horse serum, and BChE from human serum] and other reagents were obtained from the Sigma Chemical Co., St. Louis, MO.

Enzyme activities were measured at 30°. AChE was assayed with acetylthiocholine as substrate, in 50 mM potassium phosphate buffer containing 0.125 mM 5,5′-dithiobis(4-nitrophenol) at pH 7.2 [21]. Optical densities for calculation of initial rates were measured at 412 nm. In some experiments, BChE was assayed in a similar manner with butyrylthiocholine as substrate [22]. BChE was also assayed directly at 235 nm with benzoylcholine [23, 24] and other benzoyl esters as substrates. A change in extinction coefficient of 6.7 $L \cdot mmol^{-1} \cdot cm^{-1}$ was found for hydrolysis of (+)-cocaine, in good agreement with the data of Kalow for benzoylcholine and for piperocaine [23]. Enzyme kinetic parameters were calculated using the Cricket Graph program on a Macintosh IIcx computer from initial rates measured with substrate concentrations within the range 5–150 μM, where double-reciprocal plots are linear. At high substrate concentrations, plots for butyrylthiocholine as substrate curve downwards [25]. Enzyme concentrations were 20–40 μg/mL for human BChE, and 0.1–0.2 μg/mL for horse BChE. For examination of the effects of cholinesterase inhibitors, reaction was initiated by the addition of (+)-cocaine (final concentration 14.2 μM) at 30°. The percent inhibition was calculated as $100(C\text{-}I)/C$, where C and I are the rates in the absence and presence of inhibitor, respectively.

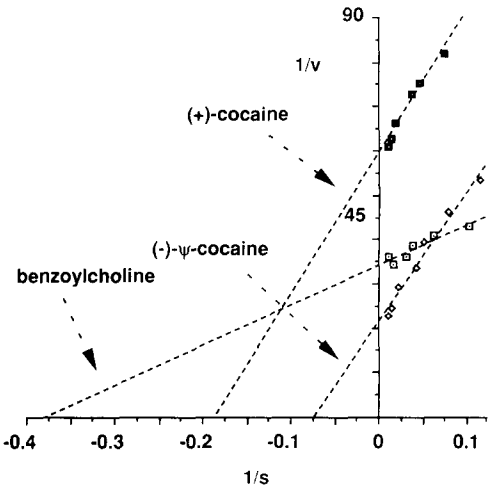


Fig. 1. Double-reciprocal plots for human BChE. Initial rates (O.D./min) were measured at 30° and pH 7.4 in the same session for incubations of human BChE with benzoylcholine, (+)-cocaine and (−)-ψ-cocaine. Regression lines are least-squared fits.

RESULTS

The effects of several compounds which are known to inhibit BChE on hydrolysis of (+)-cocaine by the human plasma enzyme are shown in Table 1. These data are consistent with the literature on anticholinesterase compounds [26], including greater inhibition by *R*-propranolol than the racemic compound [27] and inhibition by ethopropazine, a poor inhibitor of AChE [28], and by imipramine [29]. Reaction of 50 μM benzoylcholine with the human enzyme was inhibited 68% by 10 μM dibucaine and 30% by 50 μM fluoride under standard clinical conditions [30, 31] (data not shown).

Representative double-reciprocal plots from a single experiment where the rates of human BChE-catalyzed hydrolysis of benzoylcholine, (+)-cocaine and (−)-ψ-cocaine were compared are shown in Fig. 1. The calculated kinetic parameters for substrates and inhibitors of human BChE related to cocaine are shown in Table 2. Both (+)-cocaine and tropacocaine were substrates that showed less activity than the commonly employed substrates, benzoylcholine [23] and butyrylthiocholine [22]. (+)-Norcocaine was a weaker substrate than (+)-cocaine. (−)-ψ-Cocaine [the C2 epimer of (+)-cocaine] exhibited both higher K_m and V_{max} values than benzoylcholine. The V_{max} and K_m of the commercially available human BChE for (−)-cocaine could not be satisfactorily determined with the spectrophotometric assay; previous work [14] suggested that the baboon plasma enzyme was 1000 times less active

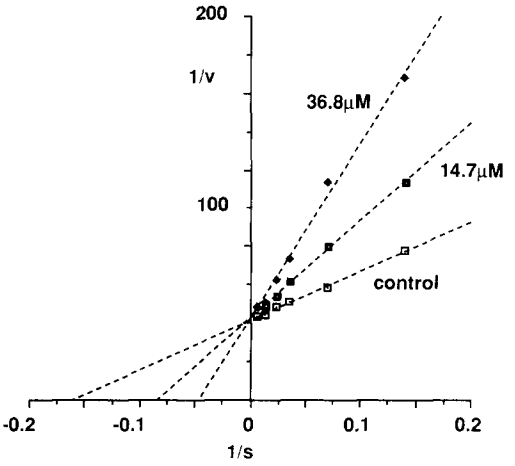


Fig. 2. Competitive inhibition of BChE by (−)-norcocaine. Initial rates were measured for (+)-cocaine alone and in the presence of the indicated concentrations of (−)-norcocaine. Conditions were the same as in Fig. 1.

with (−)- than with (+)-cocaine. However, (−)-cocaine inhibited the hydrolysis of other substrates, with a K_i value of about 10 μM. In all cases examined the inhibition was competitive, as seen for (−)-norcocaine ($K_i = 15$ μM) with (+)-cocaine as substrate in Fig. 2. (+)-ψ-Cocaine [related to (−)-cocaine] had the lowest K_i (3 μM) of the compounds tested. *O*-Demethylation of cocaine to give benzoylecgonine resulted in 10-fold weaker inhibition, while the product of debenzoylation of cocaine, (−)-ecgonine methyl ester, inhibited very weakly (Table 2). Several analogs of cocaine were also inhibitors of human BChE with similar potency (Table 3).

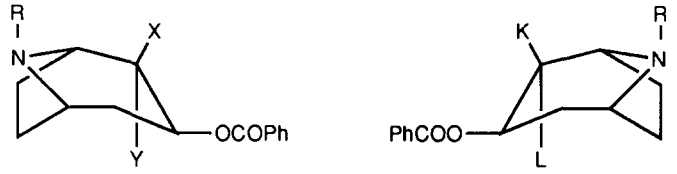
The more active horse serum BChE did allow

Table 1. Effects of standard BChE inhibitors on (+)-cocaine hydrolysis

Compound	Concentration (μM)	% Inhibition
R,*S*-Propranolol	1	53
R,*S*-Propranolol	20	92
R-Propranolol	1	70
Imipramine	20	71
Edrophonium	20	4
Ethopropazine	0.5	59
Ethopropazine	20	100
Neostigmine	1	30
iso-OMPA	1	10
iso-OMPA	20	76
1,5-bis(4-Allyldimethyl-amino)pentan-3-one	1	76
Physostigmine	1.45	79
EDTA	5000	11
Diisopropylfluorophosphate	0.004	7
Diisopropylfluorophosphate	0.03	100

Values are the means of duplicate or triplicate measurements. Control rats were 0.4 μmol/min/mg protein.

Table 2. Structure–activity relationships of cocaine and analogs with human plasma butyrylcholinesterase



Compound	R	X	Y	K	L	K_m (μM)	V_{max}*	K_i (μM)
(+)-Cocaine	CH_3	H	H	CO_2CH_3	H	6.2	0.59	—
(+)-Norcocaine	H	H	H	CO_2CH_3	H	5	0.17	—
Tropacocaine	CH_3	H	H	H	H	2.3	0.58	—
(−)-ψ-Cocaine	CH_3	H	H	H	CO_2CH_3	13	1.6	—
(+)-Benzoylecgonine	CH_3	H	H	CO_2H	H	100	0.063	—
(−)-Cocaine	CH_3	CO_2CH_3	H	H	H	—	—	7.5†,9‡
(−)-Norcocaine	H	CO_2CH_3	H	H	H	—	—	15†
(−)-Benzoylecgonine	CH_3	CO_2H	H	H	H	—	—	76‡
(−)-Norbenzoylecgonine	H	CO_2H	H	H	H	—	—	100‡
(+)-ψ-Cocaine	CH_3	H	CO_2CH_3	H	H	—	—	3‡
(−)-Ecgonine methyl ester						—	—	1300†
Benzoylcholine						3.4	1	—
Butyryl thiocholine						24	2.3	—

* Relative to V_{max} (benzoylcholine) = 1.
† (+)-Cocaine as substrate.
‡ Benzoylcholine as substrate.

determination of Michaelis–Menten parameters for (−)-cocaine (Table 4). The (+) enantiomer was hydrolyzed about 2300 times more rapidly. The K_m and the K_i values of horse BChE for the two cocaine enantiomers were quite similar. The K_m of human BChE for (+)-cocaine was lower than for the horse enzyme. The lower K_m for benzoylcholine than for butyrylthiocholine agrees with previous reports [23]. Neither cocaine enantiomer was hydrolyzed by human erythrocyte AChE, but both were weak inhibitors (K_i values 160–170 μM) of the hydrolysis of acetylthiocholine (data not shown).

DISCUSSION

This study extends the previous observation of rapid, physostigmine-inhibitable debenzoylation of (+)-cocaine in plasma [14] which may contribute to

Table 3. Inhibition of BChE by (−)-cocaine and analogs

Compound	% Inhibition
Cocaine	42
Norcocaine	33
p-Fluorococaine	40
p-Nitrococaine	56
WIN 35,065-2	25
WIN 35,428	29

Values are the means of duplicate measurements. Control rates were 0.4 μmol/min/mg protein.

the pharmacological inactivity of (+)-cocaine relative to the naturally occurring isomer. Similar considerations may apply to tropacocaine and (−)-ψ-cocaine which are also BChE substrates (Fig. 1, Table 2). The results may also be discussed in terms of previous studies of the substrate specificity of BChE, and in terms of the subjective and toxic effects of cocaine.

Measurements of debenzoylation of (−)-cocaine and norcocaine in crude human blood serum have been made by Stewart *et al.* [16] with tritiated compounds. They found a K_m of about 50 μM for cocaine, which is considerably higher than the K_i values (expected to be equal to the K_m if Michaelis–Menten kinetics are followed) of 7.5–9 μM found in the present study for purified human BChE (Table 2). A contributor to the discrepancy could be partial hydrolysis of [^{3}H]cocaine to [^{3}H]benzoylecgonine (K_i = 100 μM; Table 2) during incubation with plasma [16]. The interaction of BChE with local anesthetics structurally related to cocaine is also well known; for example, procaineamide affinity columns have been used to prepare electrophoretically pure BChE [32]. Kalow [23] investigated the action of human BChE on several local anesthetics, only one of which, piperocaine, contained an unsubstituted benzoyl group. It was hydrolyzed at a tenth the rate of benzoylcholine. Procaine and several other local anesthetics were substrates and inhibitors of BChE, but cocaine was not examined [23]. Kalow and Genest [30] later introduced the use of "dibucaine numbers" (percentage inhibition by 10 μM dibucaine of benzoylcholine hydrolysis under standard conditions) for detection of patients with atypical BChE, who may be more susceptible to succinylcholine-induced apnea during anesthesia [31]. Procaine and tetracaine were investigated more recently by Valentino *et al.* [18]; they exhibited low K_m values (6.6 and 0.14 μM, respectively) but were poor substrates in terms of V_{max} (1.7 and 0.5% that of benzoylcholine).

In the present work, (−)- and (+)-cocaine had very similar affinities for BChE, as shown by their K_i and K_m values, but binding led to rapid reaction only for the (+) enantiomer. Becket and coworkers [33, 34] found that the V_{max} of BChE for *S*-butyryl-β-methylcholine was about 5% of that for butyrylcholine, whereas the *R*-isomer had no detectable activity. Similar results for bovine erythrocyte AChE were reported over 50 years ago by Glick [35]. The C-methyl group of *R*-butyryl-β-methylcholine and the carboxymethyl group of (−)-cocaine occupy the same relative stereochemical positions. While later work on cholinesterase substrates which are asymetric in the acyl moiety has been reported [36], we are not aware of any recent work dealing with substrates possessing an optically active choline analog. The alterations in kinetic parameters caused by demethylation and epimerization (Table 2) suggest that more detailed examination of substrates and inhibitors possessing the tropane skeleton may be useful in mechanistic studies of cholinesterases.

AChE and BChE are serine hydrolases which are closely related in terms both of homology of ligand binding sites and the ways in which their catalytic subunits may be linked to generate a range of molecular forms [37–40]. Both cholinesterases cleave acetylcholine, but BChE accepts a much wider range of acylcholines than AChE. It is unclear whether BChE plays, either wholly or in part, the same role as AChE in nervous transmission. Its distribution in the adult brain differs from that of AChE [41, 42], and individuals are known who lack active plasma BChE. Although this can have adverse pharmacological consequences, such as increased sensitivity to succinylcholine used as a muscle relaxant during anesthesia, these individuals seem to be otherwise unaffected [26]. During development, however, BChE appears earlier than AChE, and BChE is suggested to function as an embryonic acetylcholinesterase, later replaced by "true" AChE [37]. If this is correct, our demonstration that (−)-cocaine inhibits BChE could bear on problems experienced by babies born to cocaine-addicted mothers. Plasma BChE levels are depressed in pregnant women, which may also increase fetal exposure to cocaine [43, 44]. Of the major metabolites of cocaine, norcocaine, which shares the rewarding effects of cocaine [45], also had a similar

Table 4. Kinetic parameters of horse plasma BChE for (+)- and (−)-cocaine

Substrate	V_{max} (μmol/min/mg protein)	K_m (μM)	K_i (μM)	
			(−)-Cocaine	(+)-Cocaine
(−)-Cocaine	0.0042	38		
(+)-Cocaine	9.8	25	25	
Butyrylthiocholine	59.6	60	29	14

K_i for BChE, while that of benzoylecgonine was 10-fold higher. Ecgonine methyl ester was quite inactive; this was expected, since choline is only a weak inhibitor of cholinesterases [46].

In the absence of an understanding of the role of BChE in normal brain function [47], the possible consequences of its inhibition by cocaine cannot be assessed. Inhibition of BChE is probably not involved in the mood-altering and addictive qualities of cocaine (unless one of the molecular forms of BChE in brain, or a related serine hydrolase, is more sensitive to cocaine than is plasma BChE) which require approximately 1 μM plasma concentrations of cocaine [48]. Also the two phenyltropane analogs (WIN 35,065-2 and WIN 35,428) were slightly weaker inhibitors of BChE than cocaine and several ester analogs (Table 3), although they are considerably more potent than cocaine in behavioral studies, and in blocking uptake of dopamine or epinephrine [11, 49]. However, although subjective effects parallel blood levels, the concentration of cocaine in brain is higher than that in intravenous blood immediately after cocaine is injected or "smoked". Peak levels of 0.01% of the injected dose/cc of striatum were found in volunteers given [^{11}C]cocaine [13], corresponding to 10 μM for administration of 34 mg (0.1 mmol) of cocaine. One must consider not only the euphoric action of cocaine, but also the subsequent dysphoria caused by a single administration, and longer term effects such as addiction and toxicity [50], as possibly involving BChE. It is conceivable, also, that cocaine inhibits another reaction of BChE, such as processing of substance P [51–53], more strongly than the hydrolysis of acylcholines and their analogs, although in one study it did not alter substance P levels [54]. Any cholinomimetic action of cocaine due to its anticholinesterase activity would tend to be offset by its recently described antagonistic muscarinic M2 receptor binding [7], which has a K_i of 19 μM, and which has been suggested to be involved in the toxicity of cocaine.

CONCLUSIONS

(−)-Cocaine and some stereochemically related compounds are moderately strong inhibitors of BChE, while (+)-cocaine and congeners are substrates of this enzyme, with a (+)/(−) ratio of >2000 for the cocaine enantiomers. Rapid hydrolysis of (+)-cocaine in primate blood plasma and possibly in tissue preparations may make advisable re-evaluation of some previous behavioral and *in vitro* studies with (+)-cocaine [14], since inactivity may have been due, in part, to destruction of the compound. A role for the previously undescribed inhibition of BChE by (−)-cocaine in the behavioral or toxicological profiles of cocaine remains to be established.

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