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INTERACTIONS OF COCAINE AND COCAINE CONGENERS WITH SODIUM CHANNELS

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Abstract—To assess the role that action on sodium channels may play in the physiological effects of cocaine and to obtain information on the structure–activity relationships of this action, cocaine, norcocaine, *N*-allynorcocaine, (+)-pseudococaine, (−)-pseudococaine, (±)-allococaine, (±)-allopseudococaine, ecgonine, ecgonine methyl ester, *O*-benzoylecgonine and atropine were tested for their effects on sodium channels. The method employed was a sodium channel specific equilibrium $^{22}Na^+$ uptake assay with rat brain membrane homogenates. All of the compounds listed with the exception of the ecgonines were found to be single affinity competitive inhibitors of veratridine activation of sodium channels. Ecgonine showed no inhibition at concentrations as high as 10^{-3} M while ecgonine methyl ester and *O*-benzoylecgonine showed inhibition only at very high concentrations. The order of inhibition potencies was found to (+)-pseudococaine ≃ norcocaine ≃ *N*-allynorcocaine > cocaine > (−)-pseudococaine ≃ (±)-allopseudococaine > (±)-allococaine > atropine > *O*-benzoylecgonine > ecgonine methyl ester > ecgonine. This ordering of potencies is in good agreement with published reports of the local anesthetic potencies of these agents.

Cocaine (I),† an alkaloid obtained from the leaves of *Erythroxylon coca,* is currently one of the most popular drugs of abuse. Cocaine exhibits a local anesthetic activity and is, in fact, the prototypical local anesthetic drug [1]. In addition to, or in conjunction with, its local anesthetic activity, cocaine exhibits a central nervous system stimulatory effect similar to that exhibited by amphetamine [2]. This central nervous system stimulation is manifested by, among other effects, increased locomotor activity and body temperature, reduced total and rapid eye movement sleep, reduced food intake, desynchronized electroencephalogram, and increased multiple unit activity in the reticular formation. Cocaine elicits stereotypical behavior and turning towards the lesioned side in animals with unilateral lesion of the dopaminergic nigro-striatal pathway [3]. Norcocaine (V), a metabolite of cocaine, and (+)-pseudococaine (IIa), the C_2 epimer of cocaine, both exhibit local anesthetic activity with greater potency than cocaine [4–6]. These two agents share a number of pharmacological properties with cocaine but their activity profiles are different than that found with cocaine [7–10]. (±)-Allococaine (III), the racemic C_3 epimer of cocaine, has been shown to exhibit a lower local anesthetic potency than cocaine [11]. In addition, other tropane derivatives with the C_3 constituent oriented in the axial configuration, as is true with (±)-allococaine, are reported to have lesser local anesthetic potency than cocaine [12]. The order of potencies for other pharmacological actions of cocaine and its congeners, such as their sympathomimetic activities [11, 13], is not the same as the order of potencies for local anesthetic activity.

A major component of the local anesthetic activity of cocaine is its ability to block sodium channels [14]. Sodium channels are the membrane components responsible for controlling sodium ion conductance in excitable cells such as neurons. To obtain information concerning the structure–activity relationships of the ability of cocaine to block sodium channels, we analyzed the sodium channel effects of cocaine and a number of its congeners using a previously described sodium channel specific $^{22}Na^+$ uptake assay employing rat brain membrane homogenates [15].

MATERIALS AND METHODS

Materials. Norcocaine–HCl and *N*-allynorcocaine–HCl were gifts from Dr. Ronald Borne, Department of Medicinal Chemistry, University of Mississippi School of Pharmacy. Ecgonine and *O*-benzoylecgonine were gifts from Dr. Mahmoud ElSohly, Research Institute of Pharmaceutical Sciences, University of Mississippi School of Pharmacy. (+)-Pseudococaine–HCl, (−)-pseudococaine–HCl, (±)-allococaine, (±)-allopseudococaine and ecgonine methyl ester–HCl were provided by the National Institute on Drug Abuse via the Research Triangle Institute, Research Triangle Park, NC. Cocaine–HCl was obtained from the Mallinckrodt Chemical Corp., St. Louis, MO, and atropine-sulfate was obtained from Merck & Co., Rahway, NJ. All other materials were as previously described [15].

$^{22}Na^+$ uptake assay. $^{22}Na^+$ uptake measurements were performed as described previously [15]. All steps were performed at 25°. Three microliters of a stock solution of cocaine, or a cocaine congener, or

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† The Roman numerals in the text refer to the numbering of compounds as listed in Table 1.

3 μl of solvent for control was added to an 0.3-ml aliquot of the rat brain membrane homogenate prepared in standard buffer (SB) (0.32 M sucrose, 10 mM Tris–HCl, 1 mM KN_3, pH 7.5) with 1 mM ouabain [the solvents used were SB (I, IIa, IIb and X) and ethanol (III–IX)]. This was incubated for 10 min, and then 3 μl of a veratridine stock solution in ethanol or 3 μl of ethanol for control was added and incubated for an additional 20 min. Next, 0.3 ml of 0.3 μCi/ml $^{22}Na^+$ as NaCl in SB was added and incubated for an additional 6 min. At this time the sample was passed through a 2-ml bed volume Dowex 50W-X8, 50–100 mesh cation exchange resin column equilibrated with SB. The brain membrane vesicles with their sequestered $^{22}Na^+$ pass freely through the column, while $^{22}Na^+$ in the bulk solution is quantitatively retained by the column resin. The $^{22}Na^+$ contents of the column effluents were determined with the use of a Packard model 500C autogamma counter.

Analyses. The following analyses were performed on cocaine and its congeners listed in Table 1: (1) dose-dependent analysis for effects on control and 5×10^{-6} M veratridine-stimulated $^{22}Na^+$ uptake, (2) time course for $^{22}Na^+$ uptake with control, veratridine and veratridine plus an approximate 50% inhibitory dose of inhibitor, and (3) double-reciprocal analysis utilizing four inhibitor concentrations versus each of five veratridine concentrations, in which the concentrations of inhibitor and veratridine were chosen from dose–response curves to lie within the range of maximum slope.

RESULTS

Compounds I–VI and X were found to inhibit veratridine-stimulated $^{22}Na^+$ uptake with different potencies as shown in Fig. 1 and Table 1. The inhibition dose curves were found to be parallel when analyzed by the method of Litchfield and Wilcoxon [16] (Fig. 1). Compounds I–VI and X exhibited no effects on control $^{22}Na^+$ uptake at concentrations $\leq 10^{-4}$ M. At concentrations greater than 10^{-4} M these compounds showed decreases in control uptake. The data for (−)-pseudococaine (IIa), which are representative of the data for the other compounds, are given in Fig. 2. Ecgonine and ecgonine methyl ester (VII and IX) had no effect on control $^{22}Na^+$ uptake at concentrations as high as 10^{-3} M. *O*-Benzoylecgonine (VIII) was not tested at concentrations above 10^{-4} M due to limits in its solubility. Compound IX had no effect on control uptake at concentrations $\leq 10^{-4}$ M. Compound VII produced no inhibition of veratridine-stimulated $^{22}Na^+$ uptake at $\leq 10^{-3}$ M, VIII produced approximately 40% inhibition at 10^{-3} M, and IX produced approximately 30% inhibition at 10^{-4} M. Further analyses with compounds VII–IX were not performed. The decreases in control uptake which were observed with compounds I–VI and X are believed to have been due to non-specific membrane disruptive effects as observed previously with propranolol [15], lidocaine and procaine [17].

The time courses for veratridine stimulated $^{22}Na^+$ uptake when inhibited approximately 50% by any one of compounds I–VI or X were parallel with control or veratridine-stimulated uptake in the absence of inhibitor. Data for compound X, which are representative of these data, are shown in Fig. 3. Parallel time courses demonstrate that the re-equilibration rates of the inhibitors with the sodium channel were slow in relation to the time required

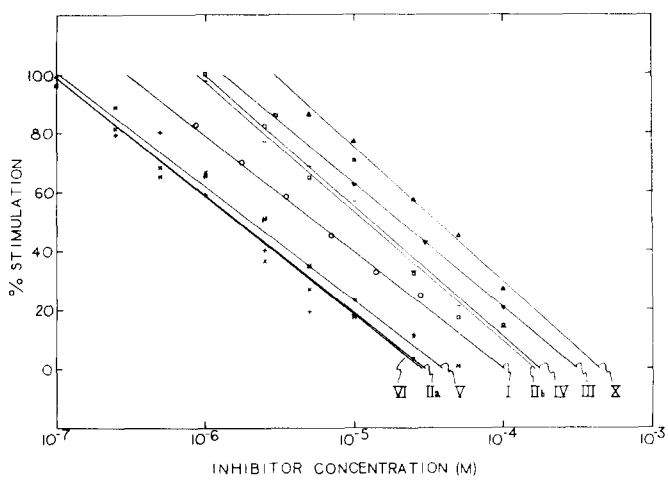
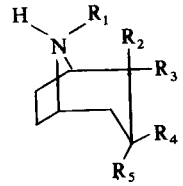


Fig. 1. Inhibition of 5 μM veratridine-stimulated $^{22}Na^+$ uptake by cocaine congeners. Key: (○) I; (+) IIa; (−) IIb; (▽) III; (□) IV; (X) V; (N) VI; and (△) X. The roman numerals refer to the various cocaine congeners as listed in Table 1. The data have been normalized to the 0–100% stimulation scale using the uptake level for no veratridine and no inhibitor (control), 0% stimulation, and the value for 5 μM veratridine and no inhibitor for 100% stimulation. The 0 and 100% stimulation values were independently determined for each inhibitor tested. These values in cpm were: I, 3300–7400; IIa, 4000–7900; IIb, 3450–8400; III, 3750–8200; IV, 3400–7600; V, 3650–8000; VI, 3650–8000; and X, 3600–8400. The data points represent the means of three independent determinations. The lines were fitted by the method of linear least squares using the mean value for each inhibitor concentration plotted.

Table 1. Cocaine congeners



No.	Trivial name	Group substituents R_1	R_2	R_3	R_4	R_5	$-\log IC_{50}$ ($^{22}Na^+$ uptake inhibition) Observed*	Expected for pure enantiomer
I.	Cocaine	$-CH_3$	$-CO_2CH_3$	$-H$	$-O-C(=O)-\phi$	$-H$	5.25 ± 0.57	
IIa.	(+)-Pseudococaine	$-CH_3$	$-H$	$-CO_2CH_3$	$-O-C(=O)-\phi$	$-H$	5.78 ± 0.56	
IIb.	(−)-Pseudococaine	$-CH_3$	$-H$	$-CO_2CH_3$	$-O-C(=O)-\phi$	$-H$	4.92 ± 0.57	
III.	(±)-Allococaine	$-CH_3$	$-CO_2CH_3$	$-H$	$-H$	$-O-C(=O)-\phi$	4.68 ± 0.76	4.96
IV.	(±)-Allopseudococaine	$-CH_3$	$-H$	$-CO_2CH_3$	$-H$	$-O-C(=O)-\phi$	4.88 ± 0.55	5.13
V.	Norcocaine	$-H$	$-CO_2CH_3$	$-H$	$-O-C(=O)-\phi$	$-H$	5.70 ± 0.57	
VI.	*N*-Allynorcocaine	$-C(=O)-CH=CH_2$	$-CO_2CH_3$	$-H$	$-O-C(=O)-\phi$	$-H$	5.79 ± 0.55	
VII.	Ecgonine	$-CH_3$	$-CO_2H$	$-H$	$-OH$	$-H$	<3	
VIII.	*O*-Benzoylecgonine	$-CH_3$	$-CO_2H$	$-H$	$-O-C(=O)-\phi$	$-H$	<4	
IX.	Ecgonine methyl ester	$-CH_3$	$-CO_2CH_3$	$-H$	$-OH$	$-H$	<3	
X.	Atropine	$-CH_3$	$-H$	$-H$	$-H$	$-O-C(=O)-CH(CH_2-OH)-CH_2-\phi$	4.44 ± 0.61	

* 95% Confidence limits for the data were determined by the method of Litchfield and Wilcoxon [16].

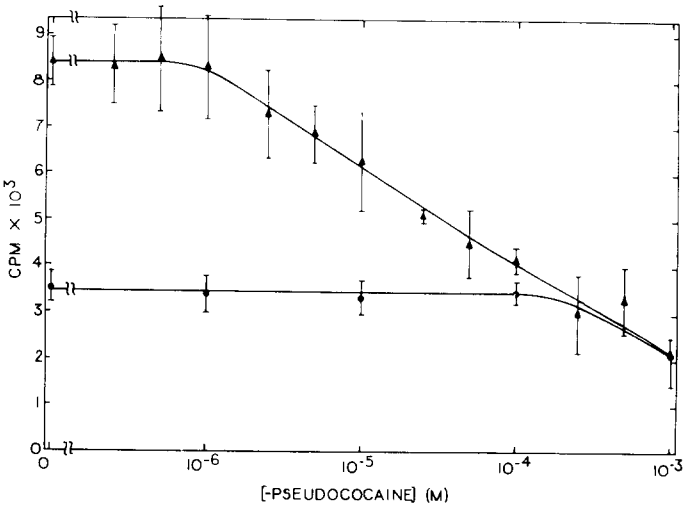


Fig. 2. Dose–response curve for (−)-pseudococaine inhibition of veratridine-stimulated $^{22}Na^+$ uptake. Key: (●) control; and (▲) 5 μM veratridine. The data points are the means ± S.D. of three independent determinations.

for uptake to reach completion. It has been shown previously that at low veratridine concentrations the equilibrium level of $^{22}Na^+$ uptake is dependent on veratridine concentration [17]. This occurs because the reequilibration rate of veratridine with the sodium channel is slow with respect to the time required for the contents of the veratridine-activated membrane vesicles to equilibrate with the $^{22}Na^+$ in the medium. This condition is an absolute necessity in order for analysis of equilibrium $^{22}Na^+$ uptake data to be valid [17].

Double-reciprocal analyses of compounds I–VI and X showed them all to be single-affinity competitive inhibitors of veratridine stimulation of $^{22}Na^+$ uptake. Data for compound I, which are representative of these data, are given in Fig. 4.

DISCUSSION

(+)-Pseudococaine and norcocaine have been reported to have greater local anesthetic potency than cocaine [4–6] while (±)-allococaine and *O*-benzoylecgonine have been reported to have lower local anesthetic potency than cocaine [11, 18]. Ecgonine and ecgonine methyl ester have been reported to be inactive as local anesthetics [1, 18]. Further, derivatives with the C_3 substituent in the axial position have, in general, lower local anesthetic potency than those with the C_3 substituent in the equatorial position [12]. This ordering of the local anesthetic potencies of the cocaine congeners correlates well with the ordering of potencies for sodium channel inhibitory activity reported here (Fig. 1). Other physiological activities of these compounds, such as their sympathomimetic activities, show entirely different ordering of potencies and do not correlate with sodium channel action [11, 13]. It is concluded that the sodium channel inhibitory activity of these compounds is a major contributor to their local anesthetic effect *in vivo*.

Veratridine binds to sodium channels and causes the channel gates to be held in an open configuration,

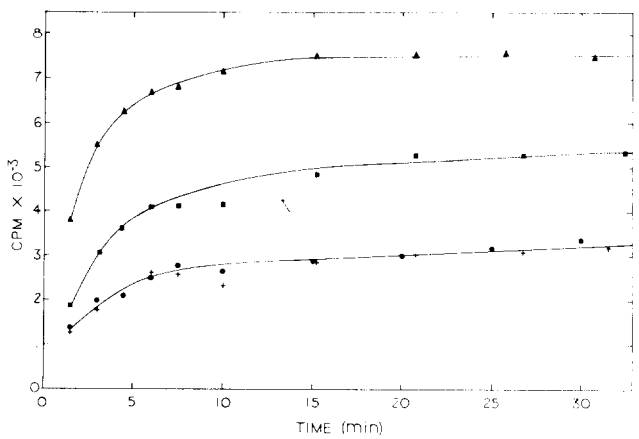


Fig. 3. Time course of veratridine-stimulated $^{22}Na^+$ uptake when partially inhibited by atropine. Key: (●) control; (+) 50 μM atropine; (■) 50 μM atropine + 5 μM veratridine; and (▲) 5 μM veratridine.

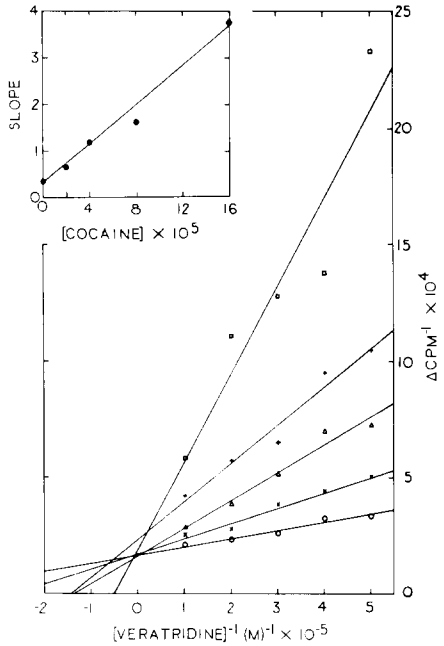


Fig. 4. Double-reciprocal plots of veratridine-stimulated $^{22}Na^+$ uptake and its inhibition by cocaine. Key: (○) no cocaine; (X) 20 μM cocaine; (△) 40 μM cocaine; (+) 80 μM cocaine; and (□) 160 μM cocaine. Data points are the reciprocals of the means from three independent determinations of the difference between the stimulated and control uptake. Data were fitted to the double-reciprocal plot by linear least squares. The inset is a replot of the slopes of the double-reciprocal lines versus the concentration of cocaine. Data were fitted using linear least squares. The units of the slope are $M \cdot cpm^{-1} \times 10^9$.

thus allowing sodium ions to pass through the channel. Compounds I–VI and X were found to be single-affinity competitive inhibitors of veratridine stimulation of $^{22}Na^+$ uptake in the brain membrane preparation. The inhibition dose curves for these inhibitors were all found to be parallel with one another. These findings indicate that the inhibitors in this group exhibit a common mechanism for inhibition of sodium channels. There are two basic possibilities for this type of competitive mechanism. The first is that the inhibitor binds at the veratridine binding site and prevents veratridine from binding without itself activating the channel. The second possibility is that the inhibitor binds to a site on the channel which exists only when the channel is closed, preventing the channel from opening, and that veratridine binds to a distinct site on the channel which exists only when the channel is open, preventing the channel from closing. Data presented here do not permit a distinction between these two possible mechanisms.

The finding of a common inhibition mechanism for the series of compounds tested here allows a structure–activity comparison. (+)-Pseudococaine (IIa), one of the most potent of the inhibitors reported here, is 7.1 times more potent than its enantiomer (−)-pseudococaine (IIb). This demonstrates a definite stereospecificity for interaction with the sodium channel. Pure enantiomers of allococaine (III) and allopseudococaine (IV) were not available for this study (the compounds used were racemic mixtures). If one assumes that the stereospecificity seen for IIa and IIb holds for III and IV as well, then the actual potencies of IIIa and IVa (the enantiomers corresponding to natural cocaine) should be nearly double the values found (expected IC_{50} = 0.5(observed IC_{50}) + 0.5(observed IC_{50})/7.1).

(+)-Pseudococaine (IIa) the C_2 epimer of (−)-cocaine (I) (the natural isomer) has both the C_2 and C_3 constituents of the tropane nucleus in equatorial positions (Table 1). Compound IIa was 3.3 times more potent than I where the C_2 constituent is in the axial position. C_3 epimerization of (+)-pseudococaine (IIa), yielding allopseudococaine (IV), reduced the potency of the molecule an estimated 4.4-fold (the observed change for the racemic mixture was 7.7-fold). Allococaine (III), in which both the C_2 and C_3 constituents are axial, was less potent than IV, having an estimated potency 6.5-fold lower than IIa (the observed change for the racemic mixture was 11.8-fold). Replacement of the C_2 constituent with H and the C_3 constituent with $-O-\overset{\overset{O}{\|}}{C}-C(C_6H_5)-CH_2OH$ in the axial position [atropine (X)] resulted in a molecule 20.6-fold less potent than (+)-pseudococaine (IIa). Removal of the *O*-benzoyl group from the C_3 position of cocaine, leaving the hydroxyl [ecgonine methyl ester (IX)], resulted in approximately a 1000-fold decrease in potency from IIa.

To summarize, placing the C_3 constituent in the axial position (IV) had a greater effect on reduction of potency than placing the C_2 constituent in the axial position (I). Placing both the C_2 and C_3 constituents in the axial positions (III) had a less than additive effect in reducing the potency of the molecule. Removal of the C_3 constituent (IX) resulted in a much greater reduction in potency than removal of the C_2 constituent (X). These findings demonstrate that, while the C_2 constituent is important, the C_3 constituent is the more important structural feature of the molecule for interaction with the sodium channel. This conclusion is supported by the fact that tropacocaine (C_2 = H) is a potent local anesthetic [1].

Removal of the methyl ester from the C_2 position of cocaine, leaving the highly polar ionizable acid group [*O*-benzoylecgonine (VIII)], reduced the potency of the molecule approximately 200-fold over that of IIa. This change had a much greater effect than replacing the C_2 constituent with a hydrogen (X), indicating that the reduction of potency was largely due to decreases in the lipophilicity of the molecule. Similarly, removal of the *O*-benzoyl group from C_3 (VIII) had a much greater effect than moving it to the axial position (III) or modifying it (X), again indicating that the reduction in lipophilicity of the molecule is very important in the reduction of potency. These effects of decreasing the lipophilicity of the molecule appear to be additive, as was seen with ecgonine (VII) which was virtually inactive.

Removal of the *N*-methyl group from cocaine [norcocaine (V)] or alteration of this group [*N*-allynorcocaine (VI)] resulted in increases of potency over that of cocaine of 3- to 3.5-fold. Removal of

the *N*-methyl group has been predicted to raise the pK_a [19], resulting in a higher proportion of the charged form at pH 7.5. This change was consequently expected to decrease the lipophilic character of the molecule. Substitution of the *N*-methyl group with an allyl group is predicted to have the opposite effects. One can speculate that the cationic form has a higher affinity for the sodium channel than the neutral form and that the more lipophilic the molecule the higher its affinity for the sodium channel, but beyond these generalizations no satisfactory explanation for this behavior can be offered.

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