Neurochemistry of Aging. 2. Design, Synthesis, and Biological Evaluation of Halomethyl Analogues of Choline with High Affinity Choline Transport Inhibitory Activity

Jehangir S. Mistry,[†,∥] Donald J. Abraham,[*,†,⊥] Alan P. Kozikowski,[‡] and Israel Hanin[§]

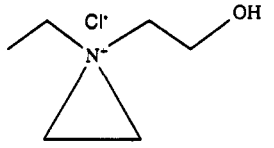
Department of Medicinal Chemistry, School of Pharmacy, University of Pittsburgh, Pittsburgh, Pennsylvania 15261, Department of Medicinal Chemistry, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298-0540, Research Department, Mayo Clinic Jacksonville, 4500 San Pablo Road, Jacksonville, Florida 32224, and Department of Pharmacology and Experimental Therapeutics, Loyola University of Chicago, Stritch School of Medicine, Maywood, Illinois 60153. *Received September 28, 1990*

The design, synthesis, and testing of several halomethyl analogues of choline and acetylcholine as potential cholinotoxins is described. The compounds were evaluated for their ability to inhibit high-affinity choline transport and their affinity toward postsynaptic muscarinic receptors. Among the analogues tested, bromomethyl and iodomethyl analogues of choline were found to be the most potent inhibitors of the high affinity choline transport system. Introduction of a β-methyl group in the halomethyl analogues drastically reduced their potencies. The bromomethyl and iodomethyl analogues were further investigated for their effects on choline acetyltransferase activity, acetylcholinesterase activity and QNB binding. Neither compound possesses significant ability to alter any of the above cholinergic markers, except at very high concentrations. These results suggest that the bromomethyl and iodomethyl choline analogues may be used as specific inhibitors of the presynaptic high-affinity choline transport system.

Introduction

A hypoactivity of the central cholinergic nervous system has been suggested in several neuropsychiatric disorders, including Alzheimer's disease.[1-4] The central cholinergic nervous system is also believed to be involved in learning and memory.[5-7] It is therefore conceivable that a *selective* cholinotoxin might be useful as a pharmacological tool to create an animal model that would simulate the cholinergic deficits observed in humans. The development of such a compound could prove to be extremely valuable, not only in understanding the cholinergic mechanisms in the brain, but also in devising successful treatment approaches for the diseases in which the cholinergic nervous system is compromised.

Several lines of evidence suggest that the presynaptic high affinity choline transport (HAChT) system may regulate the synthesis of acetylcholine by controlling the availability of choline, and that HAChT may even be the rate-determining step in the cascade of acetylcholine metabolism and synthesis.[8-12] Thus, the HAChT system represents a unique target for the development of putative cholinotoxins. Originally, our efforts were focused on designing neurotoxic analogues of choline that could specifically inhibit the transport system and eventually block the synthesis and release of acetylcholine. From these efforts, several alkyl aziridinium analogues of choline[13] emerged as potential cholinotoxins, with the most promising being AF64A, 1-ethyl-1-(2-hydroxyethyl)aziridinium chloride (1).[14,15] In vivo, compound 1 irreversibly inhibits



(1)

the HAChT system, causing a persistent central cholinergic hypofunction of presynaptic origin.[16-18] A comparison of the cholinergic deficits observed in patients with Alzheimer's disease and those induced by compound 1 in experimental animals showed a remarkable similarity. This finding led Hanin and his associates to suggest that 1 could be utilized as a potential pharmacological tool for the development of an animal model of Alzheimer's disease.[19,20]

[†] University of Pittsburgh.
[‡] Mayo Clinic Jacksonville.
[§] Loyola University of Chicago.
[⊥] Virginia Commonwealth University.
[∥] Present address: E1312 Biomedical Science Tower, Department of Pharmacology, School of Medicine, University of Pittsburgh, Pittsburgh, PA 15261.

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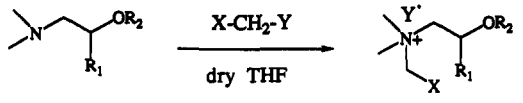
Scheme I



R_1 = H or CH_3
R_2 = H or $COCH_3$

compound	R_1	R_2	X	Y
2	H	H	Cl	I
3	H	H	Br	Br
4	H	H	I	I
5	CH_3	H	Cl	I
6	CH_3	H	Br	Br
7	CH_3	H	I	I
8	H	$COCH_3$	Cl	I
9	H	$COCH_3$	Br	Br
10	H	$COCH_3$	I	I
11	CH_3	$COCH_3$	I	I

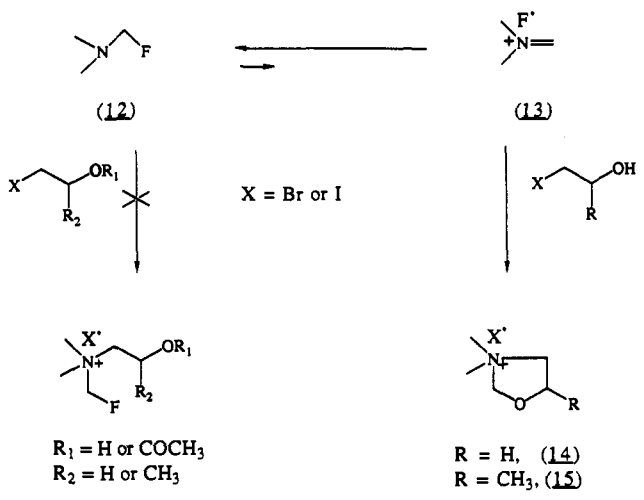
Although compound 1 and other aziridinium analogues of choline have great potential as useful pharmacological tools to study the cholinergic mechanisms, the risk of nonspecificity is always associated with such molecules due to the extremely reactive aziridinium moiety. One possible approach to reduce the nonspecific reactions is to replace the aziridinium group with a less reactive moiety such as a halomethyl group. Accordingly, we synthesized several halogenated derivatives of choline and acetylcholine that contain a halomethyl group in place of one of the methyl groups of the parent structures.

We anticipated that once electrostatic interaction between the cationic head of the ligand and an anionic site on the HAChT receptor takes place, an appropriately situated nucleophile in the vicinity of the halomethyl group could possibly displace the "albeit hindered" halogen atom, resulting in a covalent interaction which may ultimately lead to inhibition of the receptor function (e.g. inhibition of choline transport into the nerve terminal). Diminution of the charge density on nitrogen through the electrostatic interaction with the anionic site of the receptor should, of course, facilitate the displacement reaction. Enhancement in the alkylating ability of the choline analogues subsequent to the binding event thus provides a possible means for insuring their selectivity of action.

It was expected that the displacement reaction, if it occurs, would be most facile with the substrates containing a halogenated leaving group, i.e. a CH_2I, CH_2Br, or CH_2Cl group (in decreasing order of reactivity), and that it may be difficult with a substrate containing a CH_2F group. Nevertheless, we thought that the fluoromethyl analogue might prove valuable because it might best fit the stereochemical and electrostatic requirements of the HAChT receptor and, therefore, may compete with the natural substrate choline, for transport into the presynaptic nerve terminal. We also wished to test the action of these halogenated substrates as false transmitters that would eventually cause disruption of cholinergic function by covalent binding to the HAChT receptor.

This paper describes the synthesis and in vitro biological evaluation of several choline and acetylcholine analogues in terms of their ability to act as potential inhibitors of the cholinergic system. An attempt has also been made to identify optimal structural features required for inhibition of the HAChT system.

Scheme II



Chemistry

The halomethyl analogues of choline and acetylcholine were prepared as shown in Scheme I. Compounds 2–7 were prepared by reaction of either 2-(dimethylamino)-ethanol or 1-(dimethylamino)-2-propanol with the appropriate dihalomethane in the presence of anhydrous THF. The acetylcholine analogues were prepared by O-acetylation of 2-(dimethylamino)ethanol or 1-(dimethylamino)-2-propanol, followed by reaction with the appropriate dihalomethane.

The products from each reaction precipitated out as solids, whereas the unreacted starting materials remained in solution. Washing the precipitates with anhydrous THF gave the desired compounds. Higher yields were obtained when the leaving group Y was iodine in the dihalomethane and also when R_2 = H. The compounds containing Y = Br as the counterion were found to be more hygroscopic than the compounds containing Y = I.

The fluoromethyl analogues could not be prepared by using Scheme I because the starting material, fluoroiodomethane, was not commercially available. Therefore, dimethyl(fluoromethyl)amine (12) was prepared by a reported literature procedure.[21] When 12 was allowed to react with iodoethanol or 1-bromo-2-propanol, the desired fluoromethyl analogues were not obtained. Instead, compounds 14 and 15 were isolated as the only products. Protection of OH function of iodoethanol or 1-bromo-2-propanol as $OCOCH_3$, OTHP, or $OCONH_2$ and subsequent reaction of the OH-protected reagents with 12 under various reaction conditions also failed to yield the desired fluoromethyl compounds, and the starting materials were recovered unchanged. Moreover, attempts to react ethylene oxide with 12 under various reaction conditions, did not produce the desired products. When EtOH was used as a solvent for reaction of 12 with ethylene oxide, a vigorous reaction pursued upon addition of EtOH to 12 and the resulting compound showed a ^1H NMR characteristic of $(CH_3)_2NCH_2OCH_2CH_3$. An explanation of these observations is that 12 is in equilibrium with sufficient amounts of its highly reactive iminium salt 13 to allow for reaction with the above halo alcohols with the creation of a new C–O bond. Subsequent halide displacement then gives rise to 14 or 15. The iminium salt like reactivity of 12 would also explain why the electrophilic reagents such as ICH_2CH_2OAc, ICH_2CH_2OTHP, $ICH_2CH_2O(CO)NH_2$,

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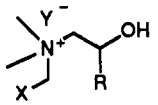
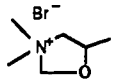
Table I. Effect of the Halomethyl and Rigid Analogues of Choline on the HAChT System



comp	structure X	Y	R	IC_{50} (μM)[a] ± SEM
2	Cl	I	H	0.92 ± 0.02
3	Br	Br	H	0.87 ± 0.03
4	I	I	H	0.49 ± 0.02
5	Cl	I	CH_3	130 ± 2.47
6	Br	Br	CH_3	56 ± 0.84
7	I	I	CH_3	50 ± 1.05
14				78 ± 4.76
15				200 ± 9.4

[a] IC_{50} value for compound 1 = 1.5 μM.

or ethylene oxide failed to react.

Biological Results and Discussion

A. High-Affinity Choline Transport. The in vitro inhibitory potencies on the HAChT of halomethyl analogues 2–7 as well as the rigid analogues (14 and 15) were determined by using rat hippocampal synaptosomes. The IC_{50} values (the concentration required to inhibit 50% of the transport of [^{3}H]choline into synaptosomes) for each of the above compounds were determined. The results are presented in Table I.

It is clear (Table I) that compounds 2–4 are potent inhibitors of HAChT. Furthermore, the inhibition caused by compounds 3 and 4 was found to be enhanced in a dose-dependent manner when various concentrations (10^{-6} to 10^{-4} M) of these compounds were preincubated with synaptosomes for 7 min followed by washing the inhibitors off the synaptosomes. For example, IC_{50} values for the two compounds following preincubation experiments were found to be 0.24 μM and 0.072 μM, respectively. This suggests that compounds 3 and 4 may be *irreversible* inhibitors of the system. Alternatively, the reduction in IC_{50} values after preincubation could be the result of nonspecific interaction of the compounds with the HAChT protein to alter its affinity for choline. In contrast, the inhibitory effect of compound 2 was not altered following the preincubation experiment.

A comparison between the IC_{50} values of 2–4 and 5–7 (Table I) indicates that introduction of the β-methyl group sharply reduces potency. Moreover, the rigid, oxazolidinium analogues 14 and 15 were also found to be weak inhibitors of the transport system, probably due to the absence of free OH group in their structures.

B. Guinea Pig Ileum Studies. Guinea pig ileum serves as a very useful preparation for measuring postsynaptic muscarinic activity of pharmacological agents.[22] The ability of the choline and acetylcholine analogues to contract the isolated guinea pig ileum preparations at 3–7 different concentrations was measured and compared with that of contractile responses to various concentrations of choline or acetylcholine. A concentration-dependent increase in the ileum contractile response was observed with choline, acetylcholine, and the halomethyl analogues. Atropine was found to be able to inhibit ~75% of the contractile responses exerted by the various analogues tested.

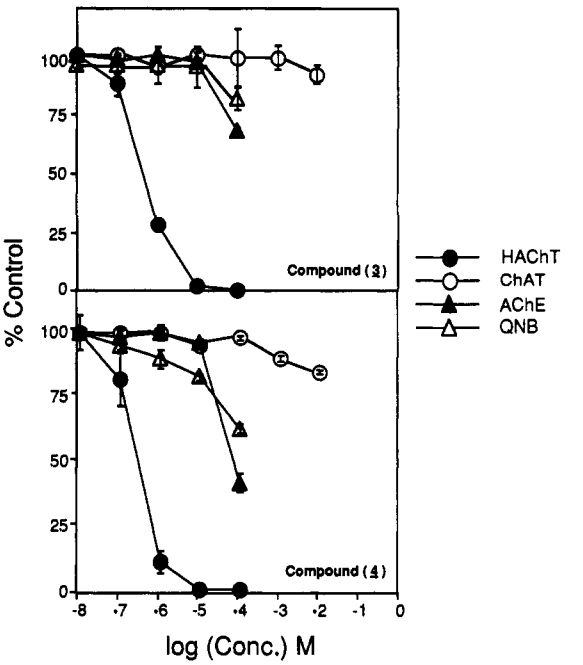


Figure 1. Effect of bromomethyl and iodomethyl analogues of choline (compounds 3 and 4, respectively) on high-affinity choline transport (HAChT) (●), choline acetyl transferase (ChAT) (○), acetylcholinesterase (AChE) (▲), and QNB binding (QNB) (△). The standard deviation is included within the symbols unless noted by bars.

Table II compares the relative potencies of the various analogues with that of choline or acetylcholine, in the same tissue preparation. It is evident from Table II that all the β-methyl analogues (5–7 and 11) are less potent than the corresponding choline and acetylcholine analogues (2–4 and 10), which themselves are less potent than their parent molecules choline and acetylcholine, with the exception of the rigid oxazolidinium compounds 14 and 15. The iodomethyl analogues of choline and acetylcholine are slightly more active than the corresponding chloromethyl or bromomethyl analogues. However, it is apparent that none of the compounds tested possessed any significantly higher muscarinic activity.

C. Effect of Bromomethyl and Iodomethyl Analogues (3 and 4, Respectively) of Choline on Choline Acetyltransferase (ChAT) Activity, Acetylcholinesterase (AChE) Activity, and Quinuclidinyl Benzilate (QNB) Binding. Since compounds 3 and 4 were found to be the most potent inhibitors of the HAChT system, we further evaluated their effect on ChAT activity, AChE activity, and QNB binding. Figure 1 compares the ability of compounds 3 and 4 to inhibit the HAChT system vis-à-vis their ability to affect the above cholinergic markers. Thus, when 3 and 4 were tested for their ability to inhibit ChAT activity, no significant inhibition (<15%) was observed at concentrations as high as 10^{-2} M. AChE activity was inhibited approximately 32% and 58% at 10^{-4} M by 3 and 4, respectively. [^{3}H]QNB binding was inhibited only 18% and 37% at 10^{-4} M by 3 and 4, respectively. These results clearly indicate that both 3 and 4 selectively inhibit only the presynaptic HAChT system.

Conclusions

The HAChT study with the halogenated analogues indicates that the chloro-, bromo-, and iodomethyl analogues of choline are potent inhibitors of the system in increasing order of activity, respectively. Furthermore, the bromo-

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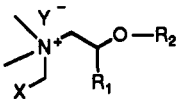
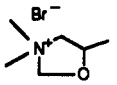
Table II. Data for Guinea Pig Ileum Bioassay Experiments



comp	structure X	Y	R_1	R_2	concentration, M[a]	relative potency with respect to choline	relative potency with respect to acetylcholine
choline	H	I	H	H	0.2×10^{-3}	1.0	
2	Cl	I	H	H	1.53×10^{-3}	0.13	
3	Br	Br	H	H	1.43×10^{-3}	0.14	
4	I	I	H	H	0.66×10^{-3}	0.33	
5	Cl	I	CH_3	H	4.0×10^{-3}	0.05	
6	Br	Br	CH_3	H	5.0×10^{-3}	0.04	
7	I	I	CH_3	H	2.0×10^{-3}	0.1	
acetylcholine	H	I	H	$COCH_3$	14×10^{-9}		1.0
8	Cl	I	H	$COCH_3$	155×10^{-9}		0.09
9	Br	Br	H	$COCH_3$	280×10^{-9}		0.05
10	I	I	H	$COCH_3$	127×10^{-9}		0.11
11	I	I	CH_3	$COCH_3$	700×10^{-9}		0.02
14					0.047×10^{-3}	4.25	
15					0.1×10^{-3}	2.0	

[a] Concentration of an analogue that exerts maximum contractile response equivalent to that elicited by the indicated concentrations of choline or acetylcholine.

and iodomethyl choline analogues appear to be irreversible inhibitors of the system. The iodomethyl choline analogue was found to be the most potent inhibitor of the transport system. Introduction of a β-methyl group is not advantageous for inhibition of the HAChT system. Moreover, the observations that the oxazolidinium analogues were poor inhibitors of the transport system emphasizes the importance of a free OH group in the structure of an inhibitor. On the other hand, the oxazolidinium analogues were found to be more potent than choline in terms of their ability to contract the guinea pig ileum. All the other compounds were significantly less active than their corresponding parent molecules in their ability to interact at the postsynaptic muscarinic receptors.

Further evaluation of the effects of bromomethyl and iodomethyl choline analogues on ChAT, AChE, and QNB binding indicate that very high concentrations (2–4 orders of magnitude higher than that required for inhibition of the presynaptic HAChT system) are required for these compounds to significantly affect the above cholinergic parameters. Thus, these studies suggest that the bromomethyl and iodomethyl choline analogues exert their actions *presynaptically*, by selectively blocking the HAChT system. Therefore, these compounds may prove to be valuable pharmacological tools for studying the etiology of various neuropsychiatric disorders including Alzheimer's disease, in which the cholinergic hypofunction is of *presynaptic* origin (hypofunction is a result of *presynaptic* nerve terminal degeneration). It is hoped that our previous studies[13] as well as the work described herein will provide a basis for producing more specific cholinotoxins and will shed light on the involvement of cholinergic mechanism(s) in a variety of disorders such as Alzheimer's disease, tardive dyskinesia,[23,24] Huntington's chorea,[25-27] and Down's syndrome.[28]

Experimental Section

No attempts were made to maximize yields. Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were obtained with a JEOL FX90Q Fourier transform spectrometer and are reported in parts per million (δ) downfield from the internal standard sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS). Fast atomic bombardment (FAB) mass spectral analyses were conducted on a VG 70-G double-focusing mass spectrometer. Elemental analyses were performed on unknown compounds by Galbraith Laboratories, Inc., Knoxville, TN, and unless otherwise stated, they are within 0.4% of calculated values. THF was dried over $LiAlH_4$ and stored over Na wires.

Biological Test Procedures. 1. HAChT-Assay. High-affinity choline uptake was determined as described by Sherman et al.[29] This procedure is a modification of that originally described by Yamamura and Snyder,[30] utilizing a crude mitochondrial preparation enriched with synaptosomes prepared by the method of Gray and Whittaker.[31] The assay was performed as follows: Rats were sacrificed by decapitation. Brains were extirpated rapidly. The hippocampus was dissected out on ice according to the method of Glowinski and Iversen.[32] Samples were then homogenized in ice-cold 0.32 M sucrose. Homogenates were next centrifuged at 1000*g* for 10 min to remove cell nuclei and heavy debris. The supernatant was aspirated off and spun again (12000*g*) for 20 min to form a pellet (Whittaker's P_2 fraction) containing synaptosomes and mitochondria.[31] The pellet was next suspended in 0.32 M sucrose. A portion of this synaptosome-rich fraction was added in triplicate to ice-cold pH 7.4 Krebs–Ringer medium.

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(32) Glowinski, J.; Iversen, L. L. *J. Neurochem.* **1966**, *13*, 655–699.

Tritiated choline was next added to this medium at a concentration of 1.0 μM, in order to approximate the K_T for the high affinity component of choline accumulation (0.9 μM[30]). This concentration is well below the apparent K_T for low-affinity uptake of choline (94 μM[30]), and changes in the velocity of choline accumulation at this concentration should reflect changes in either the K_T or the V_{max} of the high-affinity system. At the same time, the concentration is intentionally higher than the 0.04 μM choline used by Atweh et al.[33] and Kuczenski et al.[34] in their studies on HAChT to avoid any significant dilution of endogenously released, unlabeled choline, which could amount to as much as 0.04 μM choline within the time period of incubation of the synaptosome.[35]

Increasing concentrations of the toxins were next added to the medium. The "control" samples did not contain any toxin. All the samples were then incubated in a thermostatically controlled water bath at 30 ± 0.2 °C for 8 min. The samples were subsequently poured over a Brandel filter apparatus to remove the medium. The filters were then washed three times with 3.0 mL of the medium of the same ionic composition and temperature as that used in the incubation. Synaptosomes were next lysed by incubating the filter in distilled H_2O, after which scintillation fluid was added and total sample tritium counted by using a liquid scintillation spectrometer.

The method of Lowry et al.[36] was used to measure the protein contained in the P_2 suspension, with bovine serum albumin used as a standard. The rate of HAChT uptake was then defined as the net accumulation of tritium (pmol (mg of protein)$^{-1}$ min^{-1}) when a standard medium was used minus accumulation when a sodium-free medium was used. The percent inhibition of tritiated choline transported by the high-affinity system at a particular concentration of the toxin tested = 100 − % tritiated choline transported by the system, at that particular concentration of the toxin.

2. Guinea Pig Ileum Assay. Guinea pigs were killed by a blow on the head and exsanguination. The abdomen was opened and a short segment of ileum was removed and placed in a dish containing Tyrode's solution (NaCl 137 mM, KCl 2.68 mM, $MgCl_2$ 1.05 mM, $CaCl_2$ 1.80 mM, NaH_2PO_4 0.42 mM, $NaHCO_3$ 11.90 mM, and glucose 5.55 mM). Contents of the ileum were washed by placing one end of the gut over the tip of a pipet containing Tyrode's solution and applying a very small head pressure (1–2 cm) by tilting the pipet. The gut (2–3 cm) was tied with a thread at each end, taking care that the ileum was left open and the threads did not close the lumen. Each piece was then mounted in an organ-bath in Tyrode's solution and bubbled with air at 37 °C. One end of the tissue was connected to the tissue holder and the other end to a transducer connected to an electrical amplifier and recorder. Contractile response of the ileum to various concentrations of the halomethyl analogues was recorded, by using a polygraph, and compared with that of the contractile response to various concentrations of the standards, choline, and acetylcholine.

3. ChAT and AChE Activities. ChAT activity was measured by a radiochemical assay by using the Spyker et al.[37] modification of the method developed by McCaman and Hunt.[38] Briefly, rat cortex was homogenized in 75 mM phosphate buffer (pH = 7.4, 4 °C). Homogenates were incubated in a final volume of 20 μL, containing 75 mM sodium phosphate buffer (pH = 7.4), 300 mM NaCl, 20 mM $MgCl_2$, 1 mM physostigmine, 0.05% bovine serum albumin, 3.3 mM choline iodide, 0.3 mM [1-^{14}C]acetyl CoA and various concentrations of compound **3** or **4** for 30 min at 38 °C. Samples were then placed on ice and 150 μL of 3-heptanone containing 75 mg/mL of tetraphenylboron was added. After vortexing, the samples were centrifuged and a 100-μL aliquot of the top (organic) layer was assayed for radioactivity. The amount of radioactivity extracted from buffer incubated in parallel, without tissue, was subtracted as a blank value.

For the determination of AChE activity, rat cortex homogenate was incubated for 20 min at 30 °C in a final volume of 20 μL containing 75 mM sodium phosphate buffer (pH = 7.0), 10 mM [^{3}H]acetylcholine iodide and various concentrations of compound **3** or **4**. Samples were placed on ice and 25 μL of distilled H_2O (4 °C) was added to each sample. Next, 150 μL of sodium tetraphenylboron/3-heptanone was added to each sample, the samples were vortexed and then centrifuged. The bottom (aqueous) layer was quickly frozen in a dry ice–acetone bath. After the top (organic) layer was removed by aspiration, the aqueous layer was thawed and a 25-μL aliquot was assayed for the amount of [^{3}H]acetate formed.

4. QNB Receptor Binding. The assay procedure used to measure the affinity of cholinotoxins toward brain muscarinic receptors was an adaptation of the procedure developed by Yamamura and Snyder[39] and has been described previously.[13]

Preparation of (Chloromethyl)(2-hydroxyethyl)dimethylammonium Iodide (2). General Procedure. To a magnetically stirred solution of (*N,N*-dimethylamino)ethanol (3.0 g, 33.7 × 10^{-3} mol) in anhydrous THF (10 mL) was added chloroiodomethane (5.93 g, 33.7 × 10^{-3} mol). The reaction mixture was stirred in the dark for 24 h at room temperature. The THF solution was decanted from the crude gummy product which was washed with anhydrous THF (8 × 20 mL). The desired product **2** was obtained upon removal of THF, as a white solid: mp 101 °C; yield 7.32 g (82%); ^{1}H NMR (D_2O) δ 5.25 (s, 2 H, NCH_2Cl), 4.25–3.90 (m, 2 H, CH_2OH), 3.83–3.50 (m, 2 H, NCH_2CH_2), 3.30 (s, 6 H, $N(CH_3)_2$); ^{13}C NMR (D_2O) δ 72.3 (NCH_2Cl), 66.8 (CH_2OH), 57.5 (NCH_2CH_2), 52.9 ($N(CH_3)_2$); FAB MS *m/z* (relative intensity) 140 (33, M$^+$), 138 (100, M$^+$). Anal. ($C_5H_{13}ClINO$) C, H, N, Cl.

(Bromomethyl)(2-hydroxyethyl)dimethylammonium bromide (3), mp 107 °C [lit.[40] mp 109 °C], was prepared by the same general procedure in 76% yield: ^{1}H NMR (D_2O) δ 5.25 (s, 2 H, NCH_2Br), 4.25–3.90 (m, 2 H, CH_2OH), 3.83–3.50 (m, 2 H, NCH_2CH_2), 3.30 (s, 6 H, $N(CH_3)_2$); ^{13}C NMR (D_2O) δ 67.8 (CH_2OH), 61.1 (NCH_2Br), 57.8 (NCH_2CH_2), 54.0 ($N(CH_3)_2$); FAB MS *m/z* (relative intensity) 184 (97, M$^+$), 182 (100, M$^+$).

(Iodomethyl)(2-hydroxyethyl)dimethylammonium iodide (4), mp 96–97 °C [lit.[40] mp 95 °C], was similarly prepared in 88% yield: ^{1}H NMR (D_2O) δ 5.25 (s, 2 H, NCH_2I), 4.25–3.85 (m, 2 H, CH_2OH), 3.83–3.50 (m, 2 H, $-NCH_2CH_2-$), 3.33 (s, 6 H, $N(CH_3)_2$); ^{13}C NMR (D_2O) δ 69.0 (CH_2OH), 58.1 ($-NCH_2CH_2-$), 55.5 ($N(CH_3)_2$), 35.4 (NCH_2I); FAB MS *m/z* (relative intensity) 230 (100, M$^+$).

(Chloromethyl)(2-hydroxypropyl)dimethylammonium iodide (5), mp 85–86 °C, was similarly prepared in 74% yield: ^{1}H NMR (D_2O) δ 5.27 (s, 2 H, NCH_2Cl), 4.65–4.25 (m, 1 H, $CH_2CH(CH_3)OH$), 3.50 (d, 2 H, $-NCH_2CH-$), 3.30 (s, 6 H, $N(CH_3)_2$), 1.25 (d, 3 H, $CHCH_3$); ^{13}C NMR (D_2O) δ 72.3 (NCH_2Cl), 70.5 ($-CH-$), 63.9 ($-NCH_2CH-$), 53.0 ($N(CH_3)_2$), 23.4 ($CHCH_3$); FAB MS *m/z* (relative intensity) 154 (35, M$^+$), 152 (100, M$^+$). Anal. ($C_6H_{15}ClINO$) C, H, N, Cl.

(Bromomethyl)(2-hydroxypropyl)dimethylammonium bromide (6), mp 68–69 °C, was similarly prepared in 68% yield: ^{1}H NMR (D_2O) δ 5.25 (s, 2 H, NCH_2Br), 4.70–4.27 (m, 1 H, $CH_2CH(CH_3)OH$), 3.53 (d, 2 H, $-NCH_2CH-$), 3.30 (s, 6 H, $N(CH_3)_2$), 1.25 (d, 3 H, $CHCH_3$); ^{13}C NMR (D_2O) δ 70.5 ($-CH-$), 64.0 ($-NCH_2CH-$), 60.8 (NCH_2Br), 54.0 (NCH_3), 53.4 (NCH_3), 23.3 ($CHCH_3$); FAB MS *m/z* (relative intensity) 198 (94, M$^+$), 196 (100, M$^+$). Anal. ($C_6H_{15}Br_2NO$) C, H, N, Br.

(Iodomethyl)(2-hydroxypropyl)dimethylammonium iodide (7), mp 94–95 °C, was similarly prepared in 79% yield: ^{1}H NMR (D_2O) δ 5.27 (s, 2 H, NCH_2I), 4.60–4.20 (m, 1 H, $CH_2CH(CH_3)OH$), 3.55 (d, 2 H, $-NCH_2CH-$), 3.35 (s, 6 H, $N(CH_3)_2$), 1.25 (d, 3 H, $CHCH_3$); ^{13}C NMR (D_2O) δ 72.5 ($-CH-$), 64.3 ($-NCH_2CH-$), 55.5 ($N(CH_3)_2$), 36.4 (NCH_2I), 23.4 ($CHCH_3$); FAB MS *m/z* (relative

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intensity) 244 (100, M^+). Anal. ($C_6H_{15}I_2NO$) H, N, I; C: calcd, 19.41; found, 19.83.

(Chloromethyl)(2-acetoxyethyl)dimethylammonium iodide (8), mp 66 °C, was similarly prepared in 70% yield: ^1H NMR (D_2O) δ 5.25 (s, 2 H, NCH_2Cl), 4.57 (t, 2 H, CH_2OAc), 3.87 (t, 2 H, $-NCH_2CH_2-$), 3.30 (s, 6 H, $N(CH_3)_2$), 2.15 (s, 3 H, $OCOH_3$); ^{13}C NMR (D_2O) δ 175.2 ($OCOCH_3$), 72.3 (NCH_2Cl), 63.9 (CH_2OAc), 53.0 ($N(CH_3)_2$), 23.0 ($OCOCH_3$); FAB MS m/z (relative intensity) 182 (33, M^+), 180 (100, M^+). Anal. ($C_7H_{15}ClINO_2$) C, H, N, Cl.

(Bromomethyl)(2-acetoxyethyl)dimethylammonium bromide (9), mp 58 °C, was similarly prepared in 66% yield: ^1H NMR (D_2O) δ 5.25 (s, 2 H, NCH_2Br), 4.55 (t, 2 H, CH_2OAc), 3.90 (t, 2 H, $-NCH_2CH_2-$), 3.35 (s, 6 H, $N(CH_3)_2$), 2.15 (s, 3 H, $OCOCH_3$); ^{13}C NMR (D_2O) δ 175.8 ($OCOCH_3$), 65.0 (CH_2OAc), 61.0 (NCH_2Br), 60.7 ($-NCH_2CH_2-$), 54.1 ($N(CH_3)_2$), 23.0 ($OCOCH_3$); FAB MS m/z (relative intensity) 226 (37, M^+), 224 (37, M^+), 146 (100, M^+ - Br). Anal. ($C_7H_{15}Br_2NO_2$) C, H, N, Br.

(Iodomethyl)(2-acetoxyethyl)dimethylammonium iodide (10), mp 100 °C, was similarly prepared in 76% yield: ^1H NMR (D_2O) δ 5.30 (s, 2 H, NCH_2I), 4.55 (t, 2 H, CH_2OAc), 3.90 (t, 2 H, NCH_2CH_2), 3.35 (s, 6 H, $N(CH_3)_2$), 2.15 (s, 3 H, $OCOCH_3$); ^{13}C NMR (D_2O) δ 175.5 ($OCOCH_3$), 65.9 (CH_2OAc), 60.7 ($N(CH_2CH_2)$, 55.4 ($N(CH_3)_2$), 35.3 (NCH_2I), 23.0 ($OCOCH_3$); FAB MS m/z (relative intensity) 272 (100, M^+). Anal. ($C_7H_{15}I_2NO_2$) C, H, N, I.

(Iodomethyl)(2-acetoxypropyl)dimethylammonium iodide (11), mp 143-144 °C, was similarly prepared in 17% yield: ^1H NMR (D_2O) δ 5.60-5.05 (m, 3 H, $CH(CH_3)O$, NCH_2I), 4.20-3.45 (m, 2 H, NCH_2CH), 3.30 (s, 6 H, $N(CH_3)_2$), 2.10 (s, 3 H, $OCOCH_3$), 1.32 (d, 3 H, $CHCH_3$); ^{13}C NMR (D_2O) δ 178.5 ($OCOCH_3$), 73.1 ($CH(CH_3)OAc$), 71.8 ($-NCH_2CH-$), 58.5 ($N(CH_3)_2$), 38.0 (NCH_2I), 26.5 ($OCOCH_3$), 23.4 ($CHCH_3$); FAB MS m/z (relative intensity) 286 (100, M^+). Anal. ($C_8H_{17}I_2NO_2$) C, H, N; I: calcd, 61.50; found, 61.07.

Preparation of 3,3-Dimethyloxazolidinium Bromide (14). To a magnetically stirred and ice-cooled solution of dimethylfluoromethylamine (12) (1.37 g, 17.8×10^{-3} mol) in dry THF (5 mL) was added bromoethanol (2.22 g, 17.8×10^{-3} mol). The solution was stirred at 0-4 °C for 15 min. The white solid was filtered and washed several times with dry THF. Removal of THF on a rotary evaporator from the solid gave the product 14: mp 198-200 °C; yield 2.77 g (86%); ^1H NMR (D_2O) δ 4.85 (s, 2 H, $-NCH_2O-$), 4.40 (t, 2 H, $-NCH_2CH_2O-$), 3.75 (t, 2 H, $-NCH_2CH_2O-$), 3.25 (s, 6 H, $N(CH_3)_2$); ^{13}C NMR (D_2O) δ 96.8 ($-NCH_2O-$), 68.5 ($-NCH_2CH_2O$), 64.0 ($-NCH_2CH_2O-$), 52.7 ($N(CH_3)_2$); FAB MS m/z (relative intensity) 102 (100 M^+). Anal. ($C_5H_{12}BrNO$) C, H, N.

3,3,5-Trimethyloxazolidinium bromide (15), mp 158 °C, was prepared by the same procedure in 63% yield: ^1H NMR (D_2O) δ 5.15-4.40 (m, 3 H, $-OCH(CH_3)CH_2-$, $-NCH_2O-$), 4.15-3.75 (d of d, 2 H, $-NCH_2CH-$), 3.33 (s, 6 H, $N(CH_3)_2$), 1.43 (d, 3 H, $CHCH_3$); ^{13}C NMR (D_2O) δ 95.6 (NCH_2O), 77.9 ($-OCH(CH_3)CH_2-$), 70.2 ($-NCH_2CH-$), 54.3 (NCH_3), 52.5 (NCH_3), 20.0 ($CHCH_3$); ^{13}C NMR-OFR (D_2O) δ 95.0 (t, NCH_2O), 77.7 (d, $-OCH(CH_3)CH_2-$), 70.2 (t, $-NCH_2CH-$), 54.34-50.33 (2 quartets, $N(CH_3)_2$), 19.7 (q, $CHCH_3$); FAB MS m/z (relative intensity) 116 (100, M^+). Anal. ($C_6H_{14}BrNO$) C, H, N.

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An NMR and Theoretical Study of the Conformation and Internal Flexibility of Butaclamol Hydrochloride

Marco G. Casarotto, David J. Craik, Edward J. Lloyd,* and Ashton C. Partridge†

School of Pharmaceutical Chemistry, Victorian College of Pharmacy Ltd., 381 Royal Parade, Parkville, Victoria, Australia 3052. *Received September 7, 1990*

A theoretical (MM2) and experimental (^1H and ^{13}C NMR) study of butaclamol hydrochloride in $CDCl_3$ has been done in order to determine preferred conformations and internal molecular flexibility of this molecule. The theoretical calculations suggest the presence of four low-energy conformations, two of which involve a trans junction of the D and E rings, with the other two involving a cis I ring junction. An alternative cis junction (cis II) was excluded on energetic grounds. The ^1H NMR data strongly suggest the presence of a trans D-E ring junction and are consistent with a chair conformation of the E ring. ^{13}C spin-lattice relaxation time measurements show that most of the molecule is rigid, although there is some degree of mobility in the seven-membered B ring, associated with rapid flipping of the bridging C8 and C9 carbons between two skewed conformations, which have previously been referred to as conformer A and conformer B (Laus et al. *Heterocycles* 1984, *22*, 311).

Introduction

Since its synthesis and testing in the mid 1970s,[1] butaclamol has been used in several attempts to design antipsychotic drugs[2-6] as well as for dopamine receptor mapping.[1] This interest derives from its high affinity in dopamine receptor binding assays, in which it is enantiospecific,[7] and because it is generally assumed to have

† Current address: Materials Research Laboratories, Department of Defence, Cordite Avenue, Maribyrnong, Victoria, Australia 3032.

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