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Inhibition of High-Affinity Choline Transport in Rat Striatal Synaptosomes by Alkyl Bisquaternary Ammonium Compounds

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SUMMARY

HOLDEN, J. T., ROSSIER, J., BEAUJOUAN, J. C., GUYENET, P. & GLOWINSKI, J. (1975) Inhibition of high-affinity choline transport in rat striatal synaptosomes by alkyl bisquaternary ammonium compounds. *Mol. Pharmacol.*, 11, 19-27.

The high-affinity choline transport system in rat striatal synaptosomes was inhibited competitively by a series of long-chain alkyl bisquaternary ammonium compounds. BTE18 [octadecamethylenebis(triethylammonium bromide)] had a K_i value of 28 nM, and BHDM18 [octadecamethylenebis((2-hydroxyethyl)dimethylammonium bromide)] had a K_i value of 75 nM. BTE18 and hemicholinium-3 (HC-3) were equipotent competitive inhibitors. The transport of neither tryptophan nor dopamine was inhibited significantly by these compounds. Inhibitory activity increased as the alkyl chain length was increased, but reached a maximum at 17 or 18 methylene groups for bistriethyl- and bistrimethylammonium compounds. Triethylammonium compounds were more active than trimethylammonium compounds. Monoquaternary alkyltrimethylammonium compounds with 5-12 methylene groups were at least as active as the corresponding bis compounds. Various other choline-related analogues yielded the following findings. Hemicholinium-15 was much less active than HC-3. Troxonium and troxypyrrolium tosylate were moderately effective inhibitors, as was the cholinesterase inhibitor BW 284 C51. Long-chain alkyl bisquaternary ammonium compounds may be useful high-affinity analogues in studying the properties of this synaptosomal choline transport system.

INTRODUCTION

The physiological importance of choline as a precursor of the neurotransmitter acetylcholine is reflected in the large number of studies devoted to its transport and metabolism in nervous tissue. The existence of a so-called high-affinity choline transport system ($K_m \sim 1$-4 μM) has been detected in several recent investigations (1-5) in addition to a previously reported system of lower affinity (6-9). Since this system may play a special role in the functioning of cholinergic nerve endings, a more complete description of its properties would be desirable. Of special interest is the identification of high-affinity competi-

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tive inhibitors, since such substances, beyond their potential pharmacological usefulness, might facilitate the isolation and chemical characterization of choline transport catalysts. A previous report from this laboratory (4) has demonstrated the competitive inhibition of the high-affinity choline transport system in rat striatal synaptosomes by very low concentrations of hemicholinium-3 ($K_i \sim 25$ nM). This report will describe the comparably high affinity for this system of several long-chain alkyl bisquaternary ammonium compounds. These substances inhibit choline transport competitively, and they do not significantly affect tryptophan or dopamine transport.

MATERIALS AND METHODS

Chemicals. Except for hexamethonium and decamethonium, the alkyl bisquaternary ammonium compounds used in this study were obtained from Dr. R. B. Barlow, University of Edinburgh. They have the general structure $(R)_3—N^+—(CH_2)_n—N^+—(R)_3$. Abbreviations described by Barlow and Zoller (10) will be used.[2] These are summarized in Table 1 together with the structures of other compounds used in this study. A series of *n*-alkyl quaternary ammonium compounds, referred to as ATM (Table 1), also were studied. These compounds were obtained from Dr. N. J. M. Birdsall, National Institute for Medical Research, London, and were recrystallized from ethanol–ethyl acetate mixtures prior to use. Some of the inhibition data will be discussed in terms of the estimated total chain length of a compound. This refers to all the atoms in the longest chain, including both nitrogen atoms and one of the other three substituents on each nitrogen atom. Thus BTE16 has a chain length of 22 and BTM16 a chain length of 20 (Table 1). The chain length of heterogeneously substituted compounds (e.g., BHDM18) was calculated using an average value for the three shorter substituents.

Compound CT 5263, containing two triethyl substituted quaternary ammonium groups (11), was obtained from Dr. J. C. Meunier, Institut Pasteur, Paris. The bisnormethyl analogue of HC-3 was obtained from Drs. A. Marquet and B. Moreau, Collège de France, Paris. HC-3 and HC-15 were obtained from Aldrich Europe, Beerse, Belgium. The acetylcholinesterase inhibitor BW 284 C51 [1,5-bis(4-alkyldimethylammonium phenyl)pentan-3-one dibromide] was obtained from Burroughs Wellcome.

Troxonium tosylate (triethyl[2-(3,4,5-trimethoxybenzoyloxy)ethyl]ammonium *p*-toluenesulfonate) and troxypyrrolium tosylate (1-ethyl-1-[2-(3,4,5-trimethoxybenzoyloxy)ethyl]pyrrolidinium *p*-toluenesulfonate) were gifts from Dr. F. L. Chubb, Frank W. Horner, Ltd., Montreal.

[*methyl*-^{3}H]Choline chloride (16.5 Ci/mmole), [^{14}C]choline chloride (61 mCi/mmole), and [^{3}H]tryptophan (4.7 Ci/mmole) were obtained from the Radiochemical Centre, Amersham. [^{3}H]Dopamine (9.3 Ci/mmole) was obtained from New England Nuclear Corporation. All other substances were the highest grade commercially available.

Striatal synaptosomes. All experiments were performed using synaptosomes prepared as described previously (4), following a modification (12) of the method of Gray and Whittaker (13).

Uptake experiments. Uptake experiments were performed by a previously described procedure (4) with minor modifications. Striatal synaptosomes were first incubated at 37° for 3 min with gentle shaking under a 95% O_2–5% CO_2 atmosphere in 1 ml of a standard physiological medium (NaCl, 136 mM; KCl, 5.6 mM; $NaHCO_3$, 16.2 mM; NaH_2PO_4, 1.2 mM; $MgCl_2$, 1.2 mM; $CaCl_2$, 2.2 mM) containing glucose (2 mM) and eserine (20 μM) as described previously (4). Uptake was initiated by adding 50 μl of the glucose- and eserine-supplemented buffered medium containing [*methyl*-^{3}H]choline (1.0 μCi; final concentration at 1.05 ml, usually 2

[2] The abbreviations used are: HC-3, hemicholinium-3; HC-15, hemicholinium-15. Additional abbreviations for the quaternary compounds studied are included with a description of these substances in the text and in Table 1.

TABLE 1

Description of compounds

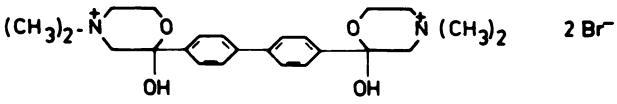
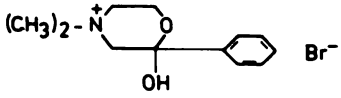
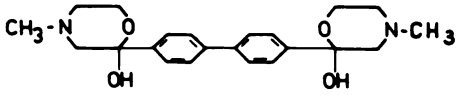
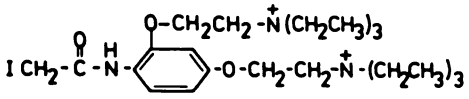
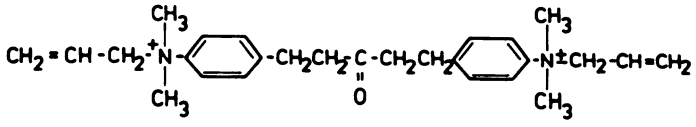
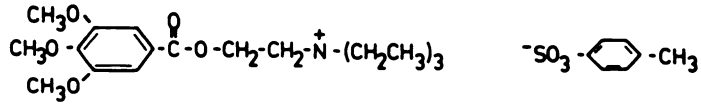
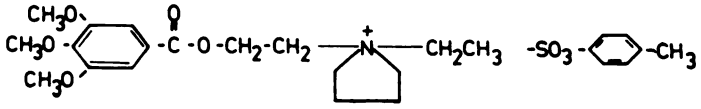
ALKYL - BIS QUATERNARY AMMONIUM SALTS

abbreviation	structure	chain length
BTM (n)	$(CH_3)_3-\overset{+}{N}-(CH_2)_n-\overset{+}{N}-(CH_3)_3\ 2X^-$	n + 4
BTM 16	$(CH_3)_3-\overset{+}{N}-(CH_2)_{16}-\overset{+}{N}-(CH_3)_3\ 2Br^-$	20
BTE (n)	$(CH_3CH_2)_3\overset{+}{N}-(CH_2)_n-\overset{+}{N}-(CH_2CH_3)_3\ 2X^-$	n + 6
BTE 16	$(CH_3CH_2)_3-\overset{+}{N}-(CH_2)_{16}-\overset{+}{N}-(CH_2CH_3)_3\ 2Br^-$	22
BHDM 18	$(HOCH_2CH_2)(CH_3)_2-\overset{+}{N}-(CH_2)_{18}-\overset{+}{N}-(CH_3)_2(CH_2CH_2OH)\ 2Br^-$	23.4
BHDE 18	$(HOCH_2CH_2)(CH_3CH_2)_2-\overset{+}{N}-(CH_2)_{18}-\overset{+}{N}-(CH_2CH_3)_2(CH_2CH_2OH)\ 2Br^-$	24.6

ALKYL QUATERNARY AMMONIUM SALTS

ATM (n)	$(CH_3)_3\overset{+}{N}-(CH_2)_{n-1}CH_3\ X^-$	n + 2
ATM 8	$(CH_3)_3-\overset{+}{N}-(CH_2)_7CH_3\ I^-$	10

ADDITIONAL COMPOUNDS

name	structure
HEMICHOLINIUM-3	
HEMICHOLINIUM-15	
BIS NOR METHYL HEMICHOLINIUM-3	
CT 5263	
BW 284 C 51	
TROXONIUM TOSYLATE	
TROXYPYRROLIUM TOSYLATE	

μM) and various amounts of the inhibitors to be tested. Incubations were terminated after 1 min by the rapid addition of 10 ml of ice-cold buffered medium lacking glucose. The cooled tubes were centrifuged at 19,200 $\times$ g for 15 min. The supernatant solution was removed, and the tube and pellet were rinsed (without disturbing the pellet) with a second 10-ml volume of cold buffer. Large buffer droplets were carefully removed from the inside of the tube by suction, and the rinsed pellet was resuspended in 0.5 ml of 1% Triton X-100. After standing overnight, 0.3-ml aliquots were taken for isotope determination and 50-μl aliquots for protein determination by the method of Lowry *et al.* (14) with bovine serum albumin as standard. Isotope content was converted to amounts of choline, using appropriate standards, and all uptake values were normalized to a standard synaptosomal protein level (usually 1 g). All experiments included blank tubes containing a large excess of nonradioactive choline (final concentration, 10 mM) to determine and allow a correction for noncatalyzed choline uptake. Uptake during 1 min provided an estimate of the initial transport rate from which kinetic parameters could be calculated.

In some experiments a double labeling protocol was used to measure simultaneously [^{14}C]choline and either [^{3}H]tryptophan or [^{3}H]dopamine uptake. In these experiments choline was used at a concentration of 2 μM, tryptophan at 5 μM, and dopamine at 0.1 μM, and initial 1-min uptake rates were determined as described above. Nonspecific uptake was estimated in blanks which contained a large excess of nonradioactive choline (10 mM) and either tryptophan (1 mM) or dopamine (0.1 mM). The findings with dopamine were not significantly altered when blanks containing benztropine, an inhibitor of dopamine uptake (15), was used to estimate nonspecific dopamine uptake.

RESULTS

Preliminary experiments revealed that very low concentrations of several long-chain alkyl bisquaternary ammonium compounds inhibited the high-affinity choline transport system. Accordingly, experiments were conducted to determine the nature of this inhibition, the susceptibility of some other transport systems, and the relation between chain length, nitrogen substituents, and affinity for the transport catalysts.

Competitive inhibition by BTE18 and BHDM18. The triethyl-substituted C_{18} compound BTE18 and the corresponding dimethylhydroxyethyl compound BHDM18 both inhibited the high-affinity choline transport system in a competitive manner (Fig. 1A and B). The data provide the following estimates of K_i values: for BTE18, 28 nM, and for BHDM18, 75 nM. These values fall in the activity range previously reported (4) and repeatedly confirmed during this study for HC-3 (approximately 25 nM). There was no indication that inhibition was dependent on the presence of a hydroxyl group.

Specificity of inhibition by alkyl bisquaternary ammonium compounds. Although the competitive nature of choline transport inhibition was clearly established, these experiments did not preclude an effect of these compounds on other transport systems, especially since these substances might have detergent properties (10). However, as shown in Fig. 2 and Table 2, transport systems for tryptophan and dopamine were not significantly affected by relatively high concentrations of several bisquaternary compounds. Since dopamine uptake, like high-affinity choline transport, is stimulated by sodium ion, these experiments also indicated that the inhibition of choline transport is not likely to be related to antagonistic effects on an ion-dependent, energy-coupling mechanism.

Effect of chain length on inhibition of choline transport. Figure 3 illustrates the effect of chain length on the relative inhibitory activity of compounds in the bis-*N*-triethyl and bis-*N*-trimethyl series. Comparisons were made in terms of the concentration of each substance required to reduce the initial transport rate to 60% of the control value (40% inhibition). Using the

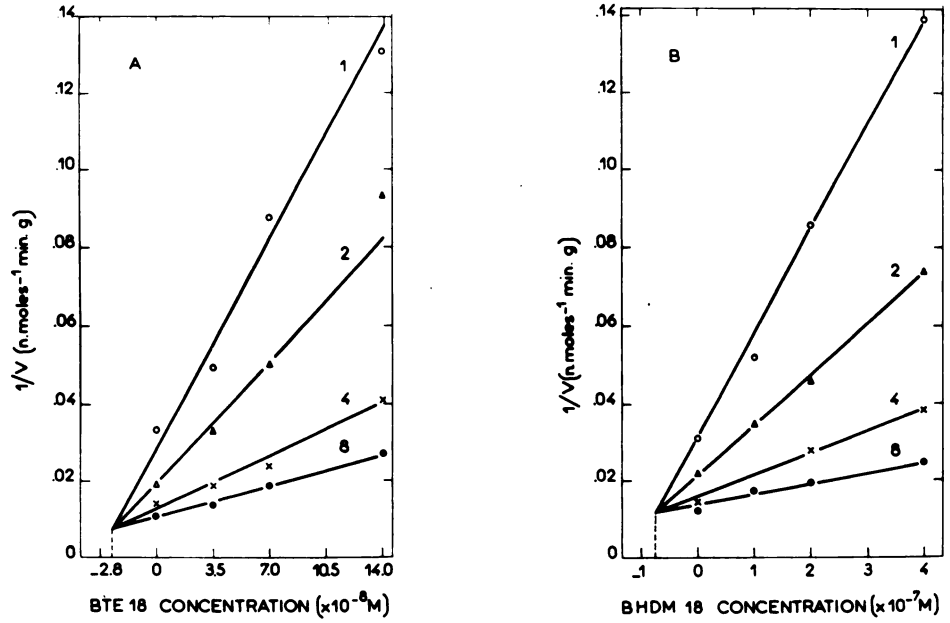


FIG. 1. *Competitive inhibition of choline uptake by BTE18 (A) and BHDM18 (B)*

Initial rates of [^{3}H]choline uptake were measured using choline at 1 μM (O——O), 2 μM (Δ——Δ), 4 μM (×——×), and 8 μM (●——●), in the absence and presence of the indicated concentrations of inhibitor. The intersection of the reciprocal rate curves for the four choline concentrations indicates K_i values of 28 nM for BTE18 and 75 nM for BHDM18.

substrate choline at 50% (2 μM) of its K_m value (4 μM), a competitive inhibitor would reduce the transport rate to 60% of the control level at a concentration which corresponds to its K_i value. HC-3 was used in each experiment as a control to verify the degree of sensitivity of each synaptosomal preparation to inhibition by bis-onium salts. In a large series of experiments 40% inhibition of choline transport was observed with HC-3 concentrations between 20 and 29 nM, with an average value of 24 nM, in good agreement with the previously reported K_i value of 25 nM (4). While the comparative data in Fig. 3 represent approximate estimates, it should be noted that the concentration of BTE18 producing 40% inhibition in these experiments (41 nM) also agreed fairly well with the K_i value (28 nM) obtained in the experiments summarized in Fig. 1A.

There was a regular increase in the apparent affinity in both series of compounds as the chain length was increased. Compounds in the BTE series were more effective than these in the BTM series when compared on the basis of either equal total chain length (e.g., chain length 18; BTE12 vs. BTM14) or the length of the alkyl constituent (BTE12 vs. BTM12). Both series of compounds displayed a peak affinity which occurred at chain length 22 for BTM and between 23 and 24 for BTE compounds, corresponding to alkyl chains of 18 methylene groups in the BTM series and either 17 or 18 methylene groups in the BTE series. In the BTM series there was a sharp reduction in affinity somewhere between BTM10 (decamethonium) and BTM6 (hexamethonium). BTE compounds of comparably short chain length were not available for testing.

Several *N*-trimethyl monoquaternary ammonium compounds (designated ATM) also were tested (Table 3). ATM12 (chain length 14) had an apparent affinity approximately equal to the comparable BTM compound (BTM10). At shorter chain lengths the monoquaternary compound was distinctly more active than the compa-

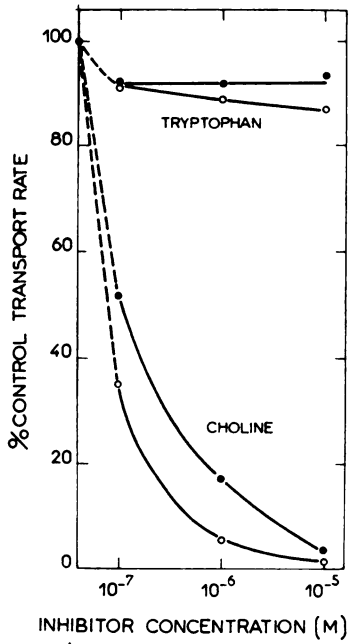


FIG. 2. *Specificity of choline transport inhibition by BTE18* (O——O) *and BHDM18* (●——●).

Striatal synaptosomes were incubated for 1 min with [^{14}C]choline (2 μM) and [^{3}H]tryptophan (5 μM) to measure the transport rates of these substances simultaneously in the absence and presence of BTE18 and BHDM18 at the indicated concentrations.

TABLE 2

Comparative sensitivity of choline and dopamine transport to inhibition by bisquaternary ammonium compounds

Striatal synaptosomes were incubated at 37° for 1 min with [^{14}C]choline at 2 μM and [^{3}H]dopamine at 0.1 μM as described under MATERIALS AND METHODS. Uptake of both isotopes was corrected using the appropriate blank controls. The values shown are percentages of the uninhibited control rates. Closely similar values were observed in another experiment, in which dopamine and choline uptake were measured separately.

Compound	Concentration	Rate	
		Choline	Dopamine
	M	*% control*	
BTE18	10^{-6}	10	73
	10^{-5}	5.2	106
BHDM18	10^{-6}	16	91
	10^{-5}	5.3	73
BHDM10	10^{-5}	15	121
HC-3	10^{-6}	23	91
	10^{-5}	17	92

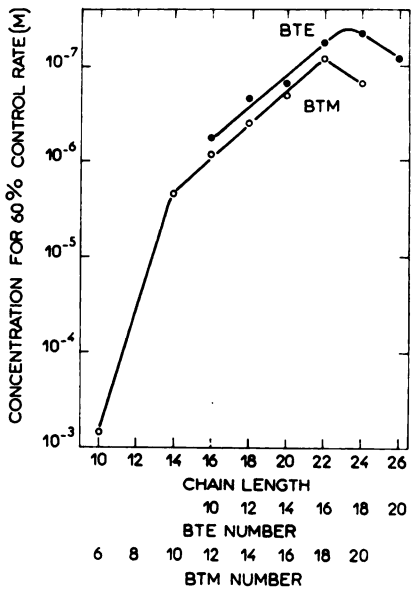


FIG. 3. *Effect of chain length on inhibition of choline transport in striatal synaptosomes by BTM and BTE compounds.*

[^{3}H]-Choline uptake was determined in the absence and presence of the inhibitors at several concentrations that would progressively reduce the initial transport rate by 20–80%. Graphical analysis of these data yielded the indicated values for the inhibitor concentration that reduced the transport rate to 60% of the control value (40% inhibition). In all cases the choline concentration was 2 μM. The values shown represent averages of at least two experiments. HC-3 was used in all experiments as a control to verify the sensitivity of each synaptosomal preparation to inhibition by a bis-onium compound.

rable bis compound. ATM10 and ATM12 were essentially equally active, indicating either a leveling off or a peak in the chain length–activity relationship.

Inhibitory activity of additional compounds. Table 4 summarizes the results of choline transport inhibition experiments using various additional substances. The *N*-hydroxyethyldiethyl compound BHDE10 was slightly more active than the corresponding dimethyl compound, BHDM10. Both compounds were slightly less active than the comparable BTE or BTM compound of equal chain length. HC-15, which corresponds to one half the HC-3 molecule, was several orders of magnitude less active than HC-3, and the bisnormethyl analogue

of HC-3, which lacks quaternary nitrogen groups, was essentially inactive even at 10 μM.

Compound CT 5263, which has been used in affinity columns for the purification of an acetylcholine receptor (11), was only as active as HC-15. On the other hand, the acetylcholinesterase inhibitor BW 284 C51 was a relatively effective inhibitor of choline transport. The trimethoxybenzoic acid esters troxonium tosylate and troxypyrrolium tosylate were active in the 0.1 μM range. Papaverine, which has recently been reported to inhibit choline transport competitively in hepatoma cells (16), was without significant effect in this striatal synaptosome system. Iodoacetamide up to 1 mM was not inhibitory, and *N*-ethylmaleimide was only slightly inhibitory at 1 mM.

TABLE 3

Effect of alkyltrimethylammonium compounds on choline transport

Initial rates of [^{3}H]choline transport were measured from a 2 μM solution as described under MATERIALS AND METHODS. Rates in the absence and presence of several concentrations of the indicated quaternary ammonium compounds were used to estimate the percentage of the control rate at these inhibitor concentrations. A plot of these data was used to estimate the inhibitor concentration that reduced transport to 60% of the control rate. The values shown represent averages of two experiments. BTM data are from Fig. 4.

Chain length	ATM		BTM	
	No.	Concentration for 60% of control rate	No.	Concentration for 60% of control rate
		M		*M*
7	5	1.3×10^{-4}		
9	7	2.8×10^{-5}		
10	8	2.5×10^{-5}	6	6.9×10^{-4}
12	10	2.4×10^{-6}		
14	12	2.7×10^{-6}	10	2.2×10^{-6}

DISCUSSION

The experiments reported here have demonstrated that long-chain alkyl bisquaternary ammonium salts are very effective competitive antagonists of high-affinity choline transport in rat striatal synaptosomes. In some cases the K_i values observed were 50–100 times lower than the apparent K_m of choline for this system, and in the range previously found for the highly effective inhibitor HC-3. These findings suggest that such compounds might be

TABLE 4

Inhibition of choline transport by various drugs and metabolic inhibitors

Initial rates of [^{3}H]choline transport were measured from a 2 μM solution as described under MATERIALS AND METHODS in the absence and presence of the indicated concentrations of various inhibitors. The rates in the presence of inhibitor are presented as a percentage of the control, uninhibited rate. The data shown are single estimates from several experiments in which the control rate varied from 45.0 to 53.3 nmoles/min/g of protein.

Compound	Rate at					Concentration for 60% of control rate
	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M	10^{-3} M	
			% control			*M*
BHDM10		76	33	9.6		2.5×10^{-6}
BHDE10		54	25	5.0		8.3×10^{-7}
CT 5263		91	48	27		5.2×10^{-6}
BW 284 C51	80	50	16	6.8		4.1×10^{-7}
HC-15		91	56			7.7×10^{-6}
Bisnormethyl HC-3	94	96	89			$>10^{-5}$
Troxonium tosylate	104	49	31	18		6.3×10^{-7}
Troxypyrrolium tosylate	66	38	23	12		1.7×10^{-7}
Triethylcholine		83	64			1.5×10^{-5}
Papaverine	92	80	86			$>10^{-5}$
Iodoacetamide				99	102	
N-Ethylmaleimide				88	77	$>10^{-3}$

usefully employed in the isolation and characterization of the substrate specificity-conferring component(s) of the choline transport system.

The pharmacological properties of these compounds have been investigated previously, using several peripheral nervous system preparations. For example, Barlow and Zoller (10) demonstrated that compounds in the BTE series had neuromuscular and ganglion-blocking activities which progressively increased and reached a maximum as the polymethylene chain was lengthened up to 15–17 units. In the BTM series ganglion-blocking activity reached a maximum at a slightly longer (n = 16–18) chain length. These findings resemble the observations made in the present study, which indicate that choline transport was maximally inhibited by BTE17 or 18 and BTM18. While the ganglion- and neuromuscular blocking activities of these compounds may include effects on pre- and postsynaptic cholinergic receptors, the coincidence in the two sets of data suggests that the physiological response to these substances may also involve inhibition of presynaptic choline uptake, resulting in decreased acetylcholine release.

Several previous investigations also considered the effect of some of these compounds on choline transport. Martin (17) observed a progressive increase in the affinity of alkyl mono- and bistrimethylammonium salts for the choline transport system of human erythrocytes as chain length was increased. A maximum in the inhibitory activity–chain length curve was not observed, but the experiments did not include compounds with polymethylene chain lengths longer than 22 (BTM18). The reduction in affinity of these compounds was much less pronounced for the striatal choline transport system than in the human erythrocyte when the polymethylene chain was shortened from 18 to 10 units. As a result, BTM10 had an affinity 50–100 times greater for the rat striatal than for the erythrocyte transport system. Another consequence of this more gradual decline in affinity was that decamethonium (BTM10) and ATM12 (chain length 14) had essentially equal inhibitory activity, whereas in the red cell the monoquaternary salt had an affinity almost two orders of magnitude greater than the corresponding bis compound. It is not known to what extent these differences in response observed with human erythrocytes and rat striatal synaptosomes originated in differences in the choline transport catalysts or in neighboring membrane components.

The ability of decamethonium and hexamethonium to inhibit choline uptake in guinea pig cortical synaptosomes also has been reported by Hemsworth *et al.* (18). However, the choline transport system studied had an apparent K_m of 152 μM and the bis-onium compounds had a much lower affinity than was observed in the present study.

Yamamura and Snyder (5) recently described the sensitivity of the high-affinity choline transport system in rat striatal synaptosomes to a variety of inhibitors, including iodoacetamide and *N*-ethylmaleimide, both of which were observed to be more effective than we found them to be. This difference may be due to procedural differences in the two studies. Yamamura and Snyder incubated synaptosomes with prospective antagonists for 10 min prior to choline addition, whereas in our study antagonists and choline were added simultaneously to the synaptosome suspension.

The trimethoxybenzoic acid esters troxonium tosylate and troxypyrrolium tosylate have prejunctional blocking effects in peripheral nervous system preparations that are antagonized by choline, implying an effect on choline transport and/or acetylcholine synthesis (19, 20). In keeping with these findings, both substances inhibited synaptosomal choline transport at relatively low concentrations (0.63 and 0.17 μM, respectively). The substantially lower efficacy of triethylcholine as a competitor indicates that troxonium tosylate acted as a competitor without prior hydrolysis to yield triethylcholine.

BW 284 C51, a relatively specific inhibitor of the so-called true cholinesterase, clearly inhibited the high-affinity choline transport system in rat striatal synap-

tosomes.[3] It appeared to have a similar affinity for this transport system and the acetylcholinesterase in rat brain and human red cells. The inhibition of choline reuptake by BW 284 C51 would tend to counteract its effectiveness in prolonging synaptic acetylcholine activity.

Finally, it should be noted that while a few of the alkyl bisquaternary ammonium compounds approached the activity of HC-3 as antagonists of the synaptosomal high-affinity choline transport system, none of them was distinctly superior to HC-3 in this respect. They do, however, provide an alternative high-affinity molecular probe for some components of this system.

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REFERENCES

1. Yamamura, H. I. & Snyder, S. H. (1972) *Science*, **178,** 626–628.
2. Haga, T. & Noda, H. (1973) *Biochim. Biophys. Acta*, **291,** 564–575.
3. Dowdall, M. J. & Simon, E. J. (1973) *J. Neurochem.*, **21,** 969–982.
4. Guyenet, P., Lefresne, P., Rossier, J., Beaujouan, J. C. & Glowinski, J. (1973) *Mol. Pharmacol.*, **9,** 630–639.
5. Yamamura, H. I. & Snyder, S. H. (1973) *J. Neurochem.*, **21,** 1355–1374.
6. Marchbanks, R. M. (1968) *Biochem. J.*, **110,** 533–541.
7. Potter, L. T. (1968) in *The Interaction of Drugs and Subcellular Components of Animal Cells* (Campbell, P. N., ed.), pp 293–304, Churchill, London.
8. Diamond, I. & Kennedy, E. P. (1969) *J. Biol. Chem.*, **244,** 3258–3263.
9. Diamond, I. & Milfay, D. (1972) *J. Neurochem.*, **19,** 1899–1909.
10. Barlow, R. B. & Zoller, A. (1964) *Br. J. Pharmacol. Chemother.*, **23,** 131–150.
11. Olsen, R. W., Meunier, J. C. & Changeux, J.-P. (1972) *FEBS Lett.*, **28,** 96–100.
12. Israël, M. & Frachon-Mastour, P. (1970) *Arch. Anat. Microsc. Morphol. Exp.*, **59,** 383–391.
13. Gray, E. G. & Whittaker, V. P. (1962) *J. Anat.*, **96,** 79–88.
14. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.*, **193,** 265–275.
15. Coyle, J. T. & Snyder, S. H. (1969) *Science*, **166,** 899–901.
16. Plagemann, P. G. W. & Sheppard, J. R. (1974) *Biochem. Biophys. Res. Commun.*, **56,** 869–875.
17. Martin, K. (1969) *Br. J. Pharmacol. Chemother.*, **36,** 458–469.
18. Hemsworth, B. A., Darmer, K. I., Jr. & Bosman, B. H. (1971) *Neuropharmacology*, **10,** 109–120.
19. Bhatnagar, S. P., Lam, A. & McColl, J. D. (1965) *Biochem. Pharmacol.*, **14,** 421–434.
20. Marshall, I. G. (1969) *Eur. J. Pharmacol.*, **8,** 204–214.

[3] Choline transport inhibition by BW 284 C51 also has been observed by G. B. Ansell (personal communication).