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INHIBITION OF CHOLINE ACETYLTRANSFERASE BY TERTIARY ALKYLAMINOETHYL ESTERS

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Abstract—Several dimethylaminoethyl esters were synthesized in order to develop a compound which would be better able to cross cell membranes and to inhibit choline acetyltransferase (ChAc) within the nerve terminal. A structure–activity relationship study demonstrated three requirements for ChAc inhibition by the alkylaminoethyl esters: (1) a terminal cationic head on the amine end of the molecule, (2) the ability to stabilize a partial negative charge on the acyl end, and (3) a leaving group on the α-carbon on the acyl end. Three tertiary amine esters which fulfilled these requirements were studied: *N,N*-dimethylaminoethyl chloroacetate (Cl-DMA); *N,N*-dimethylaminoethyl bromoacetate (Br-DMA); and *N,N*-dimethylaminoethyl acrylate (acryl-DMA). The I_{50}'s of these compounds to inhibit ChAc were 1.29–5.75 × 10^{-4} M in both extracted cell-free ChAc system and cellular minced brain ChAc system. These compounds showed reversible inhibition and were uncompetitive in nature with respect to both substrates, choline and acetyl CoA. The interaction of Cl-DMA and acryl-DMA with acetylcholinesterase and butyrylcholinesterase was also investigated. Cl-DMA and acryl-DMA were 20–150-fold and 3–5-fold less potent, respectively, to inhibit cholinesterases than to inhibit ChAc, demonstrating fair specificity toward ChAc inhibition, particularly with Cl-DMA. The hydrolysis of the ester linkage in either compound was not affected by these cholinesterases. Both compounds produced blockade of indirectly stimulated nerve-muscle preparations both *in vitro* and *in vivo*, and the blockade in the isolated preparation was reversible with washing. Therefore, Cl-DMA, in particular, would be a compound of interest to inhibit ChAc in the whole animal.

In recent years, a number of compounds have been described which are able to inhibit choline acetyltransferase (Choline acetylase, ChAc, I.U.B.2.3.1.6), the enzyme responsible for the synthesis of acetylcholine (ACh) from choline and acetyl-CoA. All of these inhibitors have several disadvantages which have prevented their general use *in vivo*. These disadvantages include rapid metabolism, isomerization, non-specific action, and inability to cross cell membranes. Styrylpyridines, which are the most widely studied of these inhibitors, possess most if not all of these disadvantages [1–5]. Since several analogs of the choline esters have been shown to be inhibitors of ChAc *in vitro* [6–8], but also possessing similar disadvantages as styrylpyridines, it was felt that a further study of the tertiary nitrogen analogs of some of these compounds might produce a compound which would not have these drawbacks and could be used as a ChAc inhibitor *in vitro*. Two compounds emerged as inhibitors deserving further study: *N,N*-dimethylaminoethyl acrylate (acryl-DMA) and *N,N*-dimethylaminoethyl chloroacetate (Cl-DMA). In this report, the structure–activity relationships of the esters studied are described, and the enzymatic inhibitory action and kinetics of inhibition are presented. The effects of Cl-DMA and acryl-DMA on *in vivo* and *in vitro* preparations are also investigated in this research.

MATERIALS AND METHODS

Materials. Drugs used in this study are listed in Table 1: vinylcholine ether bromide (VCE, compound No. 1); carbamylcholine chloride (carbachol, compound No. 2); 3,3-dimethyl-1-butanol choloacetate (Cl-DBA, compound No. 3); *N,N*-dimethylaminoethyl chloroacetate (Cl-DMA, compound No. 4); *N,N*-dimethylaminoethyl bromoacetate (Br-DMA, compound No. 5); *N,N*-dimethylaminoethyl acrylate (acryl-DMA, compound No. 6); *N,N*-dimethylaminoethyl methacrylate (methacryl-DMA, compound No. 7); acrylic acid (compound No. 8) and *N,N*-dimethylaminoethanol (DMA, compound No. 9). The drugs used to test ChAc inhibitory activity in these studies were either obtained commercially (carbachol, acrylic acid, DMA, methacryl-DMA) or synthesized in this laboratory (VCE, Cl-DBA, Cl-DMA, Br-DMA, acryl-DMA). Acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) was obtained from Nutritional Biochem. Corp. The radioisotopic [1-^{14}C]acetyl-CoA was obtained from International Chem. and Nuclear Corp. and had a specific activity of 50 mCi/m-mole. Acetylthiocholine and 5,5′-dithiobis-2-nitrobenzoate (DTNB) were obtained from Aldrich Chem. Co., Inc.

Inhibition of ChAc. The ChAc for all studies except minced brain preparation was extracted from the acetone powder of Holtzman rat brain [9] with ice-cold extraction medium (NaCl, 100 mM; EDTA, 1 mM; cysteine, 4 mM; bovine serum albumin, 0.05%; and NaH_2PO_4–Na_2HPO_4, 10 mM at pH 7.0) modified from Burger *et al.* [10]. The acetone powder was extracted with the extraction medium [25 mg/ml] for 20 min with constant stirring. The extract was centrifuged at 9000 *g* at 4° for 10 min and the supernatant fraction containing ChAc was retained. Acetylcholine synthesis was determined by radioassay using a modified method of Schrier and Shuster [11]. The enzyme extract [0.05 ml] was pre-incubated with the inhibitor

Table 1. Structure activity relationship of ChAc inhibitors

Compound No.	Name*	Structure	Per cent Inhibition
1	VCE	$CH_2{=}CH{-}O{-}CH_2{-}CH_2{-}\overset{\oplus}{N}(CH_3)_3$ (O)	0.1 ± 2.6
2	Carbachol	$NH_2{-}C{-}O{-}CH_2{-}CH_2{-}\overset{\oplus}{N}(CH_3)_3$ (O)	0.1 ± 0.9
3	Cl-DBA	$Cl{-}CH_2{-}C{-}O{-}CH_2{-}CH_2{-}C(CH_3)_3$ (O)	11.0 ± 1.6
4	Cl-DMA	$Cl{-}CH_2{-}C{-}O{-}CH_2{-}CH_2{-}N(CH_3)_2$ (O)	98.0 ± 0.3
5	Br-DMA	$Br{-}CH_2{-}C{-}O{-}CH_2{-}CH_2{-}N(CH_3)_2$ (O)	75.8 ± 1.1
6	Acryl-DMA	$CH_2{=}CH{-}C{-}O{-}CH_2{-}CH_2{-}N(CH_3)_2$ (CH_3, O)	72.1 ± 6.5
7	Methacryl-DMA	$CH_2{=}C{-}C{-}O{-}CH_2{-}CH_2{-}N(CH_3)_2$ (CH_3, O)	1.4 ± 1.1
8	Acrylic Acid	$CH_2{=}CH{-}C{-}OH$ (O)	1.1 ± 0.5
9	DMA	$HO{-}CH_2{-}CH_2{-}N(CH_3)_2$	4.0 ± 9.8

* Chemical names of these compounds may be found in the section of Materials and Methods.

† All compounds present in the incubation system at 1×10^{-3} M concentrations. Means ± S.E.M. ($N = 4$ for all studies except carbachol where $N = 3$).

(or water in the case of control) in 0.2 ml of incubation medium (NaCl, 150 mM; neostigmine, 0.05 mM; choline, 25 mM; and sodium phosphate buffer pH 7.0, 25 mM). After 20 min pre-incubation at 37°, 0.01 ml of [1-^{14}C]acetyl-CoA was added to give a final concentration of 0.065 mM containing 0.15 μCi radioactivity. The reaction was continued at 37° for 30 more min. The reaction was then stopped by adding 4.3 ml of ice-cold water and immediately thereafter 500 mg of Bio-Rad AG 1 × 1 anion exchange resin to remove unreacted acetyl-CoA. After vigorous stirring for 30 sec, each incubation tube was centrifuged for 5 min at 1500 g. Then, 0.5 ml of the supernatant fraction was transferred to 10 ml of scintillation solution (2,5-diphenyloxazole, 7 g; naphthalene, 100 g; dioxane, 1000 ml) and counted in a liquid scintillation counter (Beckman Model 1650). The remaining supernatant was transferred to a clean centrifuge tube and 250 mg of Amberlite CG 120 cationic exchange resin was added to trap choline and acetylcholine. After stirring and centrifuging as above, 0.5 ml of the supernatant was again added to 10 ml of scintillation solution for counting. The amount of acetylated product was calculated by subtracting the cpm obtained after both anionic and cationic exchange from the cpm obtained after anionic exchange alone. 10,000 cpm is equivalent to 1.04 nmoles of acetylated product synthesized/min/mg acetone powder.

For studies on the reversibility of ChAc inhibition by Cl-DMA, Br-DMA and acryl-DMA, the concentration of ChAc varied from 10 to 40 mg acetone powder/ml. For studies on inhibition kinetics of Cl-DMA and acryl-DMA, either the acetyl-CoA concentration varied from 0.016 to 0.065 mM while the concentration of choline remained constant at 25 mM; or the choline concentration varied from 0.4 to 4.0 mM while the acetyl-CoA concentration remained constant at 0.65 mM.

ChAc inhibition in minced brain. Holtzman rats were decapitated and their brains removed and immediately placed in 0.9% ice-cold saline. The brains were then minced gently with a mortar and pestle in cold saline for several min. The brain tissue was transferred to a centrifuge tube and centrifuged at 9000 g for 2 min. The supernatant was discarded and the separated cellular fraction was transferred to another centrifuge tube and resuspended in saline. These cells were then centrifuged, the supernatant discarded, and the cells again resuspended in saline. This was repeated twelve times to remove all soluble ChAc. At the final time, a sample of the supernatant was collected for a test as a blank to insure that all the soluble ChAc had been removed. This procedure for the assay of ChAc was the same as described above, except that the extracted ChAc was replaced with 50 μl of the minced brain tissue with 10 mg wet wt in each incubation sample, and the brain tissue was homogenized prior to the ACh determination.

Cholinesterase inhibition. The inhibition of cholinesterases was studied according to the method of Ellman *et al.* [12] in which the cholinesterase hydrolyzes acetylthiocholine yielding thiocholine which in turn reacts with DTNB to yield the yellow 5-thio-2-nitrobenzoic anion. The colorimetric determination was made with a Gilford Automatic Recording Spectrophotometer (Model 2400) at 412 mμ. The reaction system consisted of 3 ml of sodium phosphate buffer (pH 8.0), 10 mM; DTNB, 0.32 mM; and AChE, 0.083 U/ml or BuChE, 0.017 U/ml. This was preincubated with the inhibitor in concentrations ranging from 1.0 to 10.0 mM for 15 min at 25° in the spectrophotometric cuvette. Ten μl of acetylthiocholine was

then added for a final concentration of 6.0×10^{-4} M and the rate of hydrolysis was measured with time as a function of the change in absorbance at 412 mμ.

Hydrolysis of esters. To determine the stability of the ester linkage of Cl-DMA and acryl-DMA, the rate of autohydrolysis and hydrolysis in the presence of AChE and BuChE were measured by the acid production with a modified method of Chiou [13]. The reaction was run at 25° for 20 min with 10 ml of an aqueous barbital buffer solution (sodium barbital, 1.5 mM; KH_2PO_4, 0.3 mM; NaCl, 150 mM) containing either no enzyme or 0.83 units of AChE or 0.17 units of BuChE. Since acryl-DMA was used as the free base, the pH was adjusted with 1 N HCl (0.05 ml) so that the addition of the acryl-DMA gave an initial pH of 7.3–7.6. Acryl-DMA or Cl-DMA was added to give a final concentration of 5.0 mM and the change in pH with time was measured and compared with a standard curve obtained with identical solutions titrated with acrylic or chloroacetic acid. The pH determinations were made with a Beckman SS-3 pH meter with a Beckman combination electrode No. 39030.

Frog sciatic nerve gastrocnemius muscle preparation in vitro. *Rana pipiens* weighing 20–25 g were stunned by a blow to the head, decapitated, and pithed. The skin was removed from the leg and the sciatic nerve was isolated, tied, and cut at its upper end. A loop of suture was tied around the tendon at the lower end of the gastrocnemius muscle and the tendon was cut distal to the tie. The femur was cut, leaving the joint still attached to the muscle. A long thread was attached to the joint bones. The gastrocnemius muscle with the sciatic nerve attached was then removed from the animal and placed in 40 ml of frog Ringer solution (NaCl, 110 mM; KCl, 2.01 mM; $CaCl_2$, 0.77 mM; $NaHCO_3$, 4.25 mM; and dextrose, 11.1 mM) in an organ bath oxygenated with 95% O_2 5% CO_2 at pH 7.4 and 25°. The loop at the distal end of the muscle was attached to a stationary support and the tie at the other end on the joint bone was attached to a Narco Bio-Systems Myograph-B isometric force transducer (E & M Instrument Co. Inc., Houston, Texas) with 1 g initial tension. The nerve was passed through a platinum two-ring electrode and stimulated with supramaximal voltage (10 V), 0.5 msec duration and 250 Hz frequency administered in monophasic square wave pulses for 0.1 sec every 10 sec. Muscle contractions measured by the Myograph were recorded on a Model Four-A Narco Bio-Systems Physiograph (E & M Instrument Co., Inc.). Drugs were added to the bath in volumes not exceeding 0.8 ml.

Rat sciatic nerve gastrocnemius muscle preparation in situ. Holtzman rats weighing 300–500 g were anesthetized with an i.p. injection of ethyl carbamate, 1.6 g/kg. An incision was made on the neck and the carotid artery was cannulated and the tubing from the artery containing heparinized saline (10 U/ml) was attached to a pressure transducer (Model P-1000 A, Narco Bio-Systems Inc.) for recording blood pressure and heart rate. The jugular vein was also cannulated for injection of drugs. The leg was opened to expose the sciatic nerve. This was tied and cut distal to the gastrocnemius muscle. The tendon of the gastrocnemius muscle at the back of the leg was tied with suture, and as much of the muscle as possible was isolated while still maintaining blood supply. The rat was then positioned so that the leg could be immobilized by securing it with nails to the mounting board. The tendon tie was attached to a Myograph-C force transducer. The sciatic nerve was passed through a platinum two-ring electrode and stimulated at supramaximal voltage (3 V) of 0.5 msec duration and 250 Hz frequency administered for 0.1 sec every 10 sec. Muscle contractions were recorded as described previously. Drugs dissolved in saline were administered intravenously in doses not exceeding 0.5 ml.

Statistical analysis. Standard errors of the means (S.E.M.) are given for mean figures. The 95% confidence limits for the ED_{50}'s were computed according to Litchfield and Wilcoxon [14].

RESULTS

ChAc inhibitory activities of alkylaminoethyl esters. In order to determine the relationship of ChAc inhibition with different alkylaminoethyl esters and their derivatives, a number of compounds were tested for inhibition of cell-free ChAc *in vitro*. The results of this study are presented in Table 1. It can be seen that at a concentration of 1×10^{-3} M, only three of these compounds, Cl-DMA (compound No. 4), Br-DMA (compound No. 5), and acryl-DMA (compound No. 6) produced an effective inhibition of the enzyme. The hydrolysis products of these compounds, however, did not produce any inhibition of ChAc. To determine the potency of these three compounds, the I_{50}'s were determined and the results are presented in Table 2. They are approximately 10-fold less potent than their quaternary analogs, reported by others [6, 8, 15]. This might be expected mainly because of the difference in conformation due to the removal of a methyl group, but also because these compounds are not permanently charged.

Table 2. I_{50}'s of ChAc inhibition

	I_{50} ($M \times 10^4$)			
Compound	N	Cell-free ChAc extracted from brain acetone powder	N	Cellular ChAc in minced brain tissue
Cl-DMA	4	1.29 ± 0.05*	4	2.25 ± 0.23
Br-DMA	4	5.80 ± 0.09		--
Acryl-DMA	4	5.02 ± 0.95	3	5.75 ± 0.91

* Mean ± S.E.M.

Since these compounds are tertiary amines which are able to exist in the uncharged form, they should be able to penetrate biological membranes to inhibit ChAc in the intracellular site. To investigate this, a minced brain preparation was used in which the ChAc was still contained within the intact nerve terminal. The I_{50}'s for Cl-DMA and acryl-DMA were determined and compared with the potency in the cell-free extracted ChAc assay (Table 2). It is seen that both Cl-DMA and acryl-DMA are almost as potent in the minced brain, demonstrating their ability to gain entry into the nerve terminal and inhibit ChAc in the inside.

The reversibility of Cl-DMA, Br-DMA, and acryl-DMA was tested on cell-free ChAc *in vitro* and the results in Fig. 1 show that all three produced a reversible inhibition of ChAc. Originally, it was thought that Cl-DMA and Br-DMA might show differences in their reversibility on ChAc. That this might be the case was suggested by the studies of Chiou and Sastry [16] showing that the quaternary derivatives of Cl-DMA and Br-DMA, chloroacetylcholine and bromoacetylcholine respectively, had different binding characteristics on the cholinergic receptor. The former produced reversible binding to the cholinergic receptor whereas the latter produced irreversible binding. Although chloroacetylcholine has been shown to produce reversible inhibition of ChAc [6], the reversibility of bromoacetylcholine has not been reported. Since Br-DMA was not irreversible (Fig. 1), it apparently has no advantages over Cl-DMA; and since it is more hygroscopic and more difficult to synthesize, it was decided that of the two, only the chloro-compound would be investigated further.

The kinetics of the inhibition seen with Cl-DMA and acryl-DMA was investigated and the results are presented in Fig. 2. Both Cl-DMA and acryl-DMA showed an uncompetitive type of inhibition with respect to both substrates of ChAc, choline and acetyl-CoA.

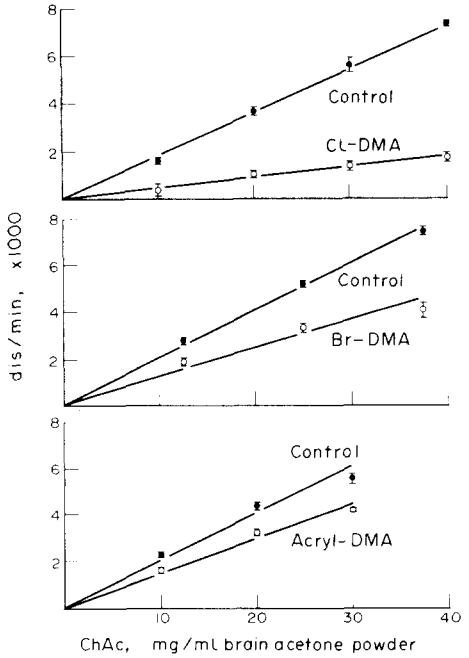

Fig. 1. Reversibility of ChAc inhibitors. All three inhibitors in incubation medium were at 5×10^{-4} M concentration. Means ± S.E.M. were presented with $N = 3$. Note the inhibited line changed the slope and originated from the orgin, indicating a reversible inhibition.

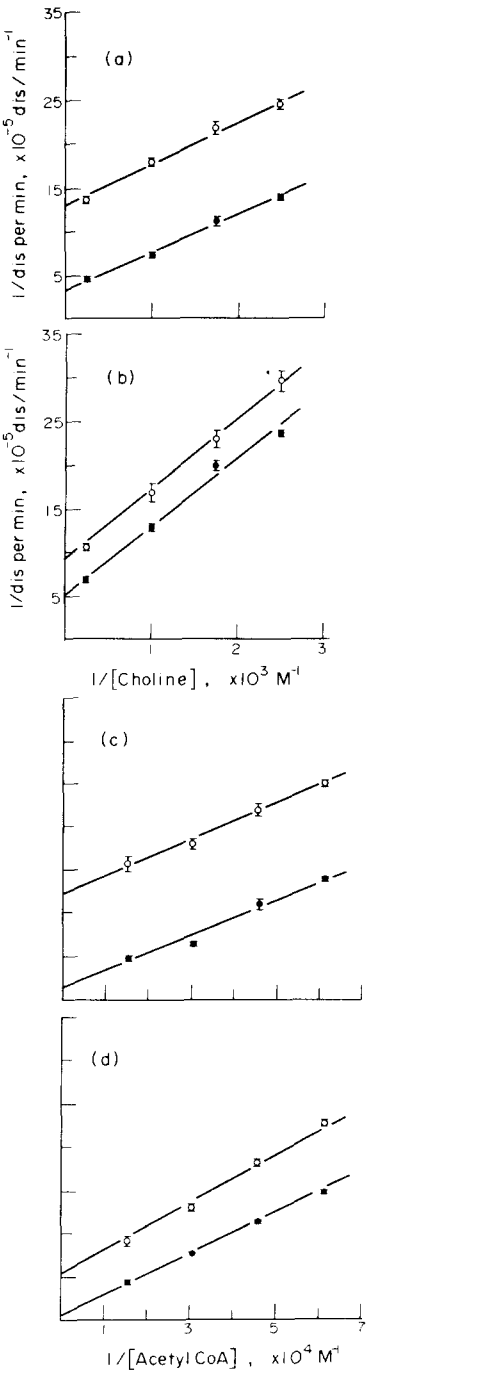

Fig. 2. Kinetics of ChAc inhibition. Assay was done on cell-free ChAc extracted from rat brain acetone powder. Acetyl-CoA concentrations varied from 0.0163 mM to 0.065 mM. Choline concentrations varied from 0.4 mM to 4 mM. Means ± S.E.M. were presented with $N = 3$. Where, (●), control; (○), with Cl-DMA or Acryl-DMA; (A), with 1×10^{-4} M of Cl-DMA; (B), with 2×10^{-4} M of acryl-DMA; (C), with 4×10^{-4} M of Cl-DMA; (D), with 1×10^{-3} M of acryl-DMA. Note the parallel shift of control lines by inhibitors indicating an uncompetitive type of inhibition.

Interaction with cholinesterases. To determine the specificity of Cl-DMA and acryl-DMA to inhibit ChAc, the I_{50}'s for inhibition of both AChE and BuChE were determined. Table 3 shows that both compounds are more potent inhibitors of ChAc than either of these two cholinesterases, 20–150 times more potent in the case of Cl-DMA and 3–5 times more potent for acryl-DMA.

Table 3. Inhibition of cholinesterases

Compound	I_{50} (M × 10^3) AChE	BuChE
Cl-DMA	2.64 ± 0.04*	19.60 ± 3.08
Acryl-DMA	2.24 ± 0.11	1.60 ± 0.04

* Mean ± S.E.M., $N = 3$.

The stability of the ester linkage of these two compounds was investigated, and the rates of autohydrolysis and the rates of enzymatic hydrolysis in the presence of AChE and BuChE are shown in Table 4. The rates of hydrolysis of acetylcholine in this system are also presented for comparison. It can be seen that neither Cl-DMA nor acryl-DMA are significantly hydrolyzed by either enzyme. The autohydrolysis rates, while much faster, are not unmanageable. The $t_{1/2}$'s for hydrolysis at pH 7.4 are 24 hr for acryl-DMA and 5 hr for Cl-DMA.

Effects on biological preparations. Finally the ability of these compounds to block muscle contractions in indirectly stimulated nerve-muscle preparations was investigated both *in vitro* and *in vivo*. A record of the blockade by both Cl-DMA and acryl-DMA in the isolated frog sciatic nerve gastrocnemius muscle preparation is presented in Fig. 3; and a record for acryl-DMA in the rat sciatic nerve gastrocnemius muscle preparation *in situ* is presented in Fig. 4. Both compounds produced a slow blockade of twitch contractions in both preparations. The blockade was reversible upon washing in the isolated preparation with ED_{50}'s, measured after 1 hr of drug administration of 42.0 (20.0–88.2) × 10^{-4} M for Cl-DMA and 5.5 (2.02–12.63) × 10^{-4} M for acryl-DMA. Control preparations were stable for over 3 hr. In *in vivo* experiments, both drugs produced an immediate but transient hypotension when injected (Figure 4). This is probably due to the direct action of these compounds on the vascular smooth muscle.

DISCUSSION

This study on the structure–activity relationship of alkylaminoethyl esters has revealed three requirements for ChAc inhibition: (1) a cationic terminal charge on the amine end of the molecule, (2) the ability to stabilize a partial negative charge on the acyl end, and (3) a leaving-group on the α-carbon of the acyl end (Fig. 5). These requirements were demonstrated by the following results.

To determine the importance of the terminal cationic head on ChAc inhibition, Cl-DBA (compound No. 3) was tested but did not show ChAc inhibition. Since Cl-DBA is the exact molecule as chloroacetylcholine, a potent ChAc inhibitor [6], except that the nitrogen is replaced with a carbon to eliminate the positive charge, this shows that the cationic head is essential for the ChAc inhibitory activity.

Therefore, in order to retain the positive charge but still allow cell membrane penetration, a number of tertiary amine esters were tested. All of these compounds had a significant inhibitory action on ChAc except methacryl-DMA (compound No. 7). The reason for this exception will be discussed later. Since the compound must have a cationic head, or possess the potential for a positive charge formation, this requirement can be satisfied either by quaternary or tertiary amines. The latter are over 90% ionized at physiological pH. Compounds such as Cl-DBA (compound No. 3), which cannot be charged, are not ChAc inhibitors.

Secondly, there is a requirement for stabilizing a partial negative charge on the acyl end of the molecule. Electron withdrawal groups such as halogen substitution or a combination of carbonyl and vinyl group satisfy this requirement. For example, α,β-unsaturated esters satisfy this requirement by resonance-stabilization of the negative charge. If the carbonyl group of acryloylcholine is removed it becomes VCE (compound No. 1) which does not allow resonance-stability, and consequently, does not inhibit ChAc. An adjacent halogen also causes electron withdrawal. For this reason, chloroacetylcholine is a potent inhibitor of ChAc [6] but acetylcholine is not [17].

Finally, there is a requirement for a leaving group on the acyl end of the molecule (Fig. 5). This is evidenced by the fact that as the acidity of the α-hydrogen increases, as with halogen substitution or with a vinyl group, the potency of ChAc inhibition increases. Substitution of the hydrogen with a methyl group, as in the case of methacryl-DMA (compound No. 7), or replacement of the acetyl group with a

Table 4. Hydrolysis of esters

Compound*	Rate of hydrolysis (moles/min × 10^8) Autohydrolysis	AChE+	BuChE†
Cl-DMA	8.74 ± 0.14§	0.22 ± 0.05	0.32 ± 0.21
Acryl-DMA	1.75 ± 0.04	0.07 ± 0.01	0.06 ± 0.01
ACh	1.19 ± 0.03	1.46 ± 0.03	6.57 ± 0.17

* All compounds present at 5 × 10^{-3} M.
+ AChE, 0.083 U/ml.
† BuChE, 0.017 U/ml.
§ Mean ± S.E.M., $N = 3$.

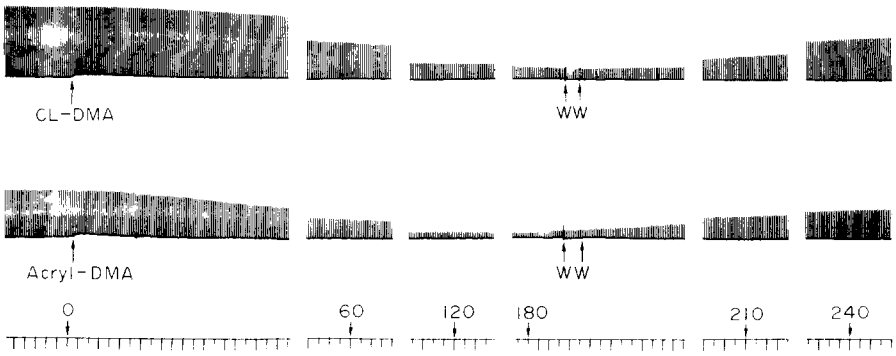

Fig. 3. Effect of Cl-DMA and acryl-DMA on muscle contractions in the frog sciatic gastrocnemius preparation *in vitro*. Cl-DMA, 3×10^{-3} M; acryl-DMA, 1×10^{-3} M; W, washing. Each time interval represents 1 min.

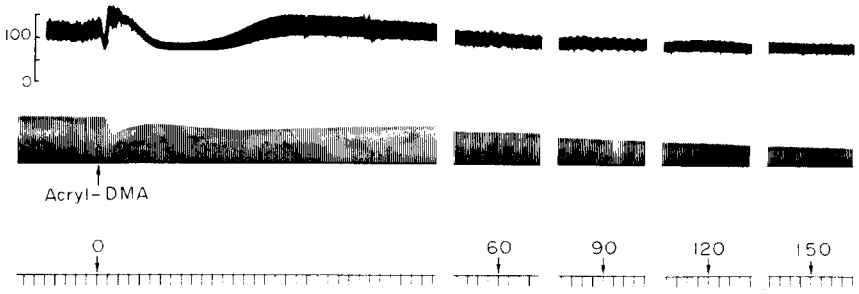

Fig. 4. Effect of acryl-DMA on blood pressure and muscle contractions in the rat sciatic gastrocnemius preparation *in situ*. Acryl-DMA, 100 mg/kg. Each time interval represents 1 min. Blood pressure calibrated in mm Hg.

carbamyl group, as with carbachol, (compound No. 2) completely eliminates inhibition. Since haloacetylcholines and acryloylcholine have been shown to inhibit ChAc [6, 8], VCE and carbachol (compounds Nos 1 and 2 in Table 1) were tested as compounds which would be more stable to hydrolysis. Neither of these, however, inhibited ChAc because of the aforementioned reasons.

The results of the kinetic studies show that all of the three inhibitors studied are reversible inhibitors of ChAc. Cl-DMA and acryl-DMA (compounds Nos 4 and 6) also produce uncompetitive inhibition with respect to both substrates, choline and acetyl-CoA. It appears that all of the alkylaminoethyl esters produce inhibition of ChAc via the same mechanism since all have been shown to produce uncompetitive inhibition with respect to both substrates [6, 8, 15].

Neither Cl-DMA nor acryl-DMA are suitable substrates for hydrolysis by AChE or BuChE. Although the rate of autohydrolysis is fairly rapid with Cl-DMA and may present a problem in animal studies, this could be overcome by a more frequent administration. Because Cl-DMA and acryl-DMA are more potent inhibitors of ChAc than cholinesterases, they appear to be sufficiently specific to inhibit ChAc and to allow further study in animal preparations.

Both Cl-DMA and acryl-DMA are able to produce neuromuscular blockade *in vitro* and *in vivo*. Since the effects are reversible with washing after 3 hr *in vitro*, it indicates that the compounds are still active over this period of time.

In conclusion, the results of these studies show an interesting structure activity relationship for ChAc inhibitors. Two compounds, Cl-DMA and acryl-DMA, have demonstrated sufficient potency and specificity for use in biological studies, and each is able to inhibit neuromuscular contractions both in isolated preparations and *in vivo*.

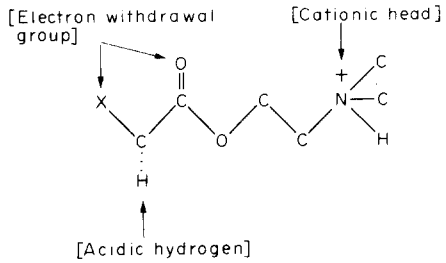

Fig. 5. Molecular structure of dimethylaminoethyl esters that inhibit ChAc.

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