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A STUDY *IN VITRO* OF NEW SHORT-ACTING, NON-DEPOLARIZING NEUROMUSCULAR BLOCKING AGENTS*

RICHARD J. KITZ, JOANNES H. KARIS and SARA GINSBURG

Departments of Anesthesiology and Neurology, Columbia University, College of Physicians and Surgeons, New York, N.Y., U.S.A.

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Abstract—Presently available short-acting, depolarizing myoneural blocking agents have significant side effects such as increased intraocular tension, cardiac arrhythmias desensitization of the postjunctional membrane, skeletal muscle injury and muscle pains. Non-depolarizing blocking drugs do not produce profound blocks of short duration. A program was evolved to synthesize compounds producing a non-depolarizing block at low concentrations with a brief action span requiring no antagonists.

Several series of mono- and diquaternary esters of alkyl aminoethanols were prepared and studied *in vitro*. The rates of spontaneous hydrolysis and the hydrolysis catalyzed by acetylcholinesterase and plasmacholinesterase were measured. In addition, the anticholinesterase effects of these agents were evaluated. The myoneural blocking properties were studied with intracellular recordings in frog sciatic nerve–sartorius muscle preparations. Activity-structure relationships are reported. Several compounds warrant further study including investigations *in vivo*.

NEUROMUSCULAR blocking agents are widely used in the practice of anesthesia to provide the skeletal muscle relaxation required during surgery and electroshock treatment.[1] Normally the propagation of an impulse from nerve to muscle occurs by a sequence of events that starts with the release of the transmitter acetylcholine and the activation of the cholinergic receptor at the postjunctional membrane. The neuromuscular blocking agents can interrupt this transmission process in two major ways: (1) By activating the receptors and thus depolarizing the postjunctional membrane to a level at which the membrane becomes refractory to released acetylcholine. Succinyldicholine and decamethonium are examples. These so-called depolarizing muscle relaxants can, therefore, not be reversed by anticholinesterase agents. (2) By "occupation" of the receptors so that the released acetylcholine cannot activate these sites. Of the non-depolarizing muscle relaxants, *d*-tubocurarine and gallamine (Flaxedil) are the most commonly used. Since both these drugs possess a relatively long duration of action they cannot be used when a brief period of profound relaxation is required. Although this "curare type" block can be antagonized, the drugs (atropine and anticholinesterase agents) used for this purpose are potentially dangerous by virtue of their action on the autonomic nervous system and cardiac muscle.

Succinyldicholine and decamethonium are the depolarizing muscle relaxants most commonly used in clinical practice. The former is especially valuable since it is able to

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produce a profound block of only minutes duration when administered as a single dose, or for longer periods when given by continuous slow infusion. However, depolarizing neuromuscular blocking agents may have certain side effects in man, such as increased intraocular pressure,[2,3] cardiac arrhythmias,[4,5] desensitization of the postjunctional membrane,[6,7] skeletal muscle injury,[8,9] and muscle pain.[10,11] Occasionally a succinylcholine-induced neuromuscular block may be markedly prolonged because of a genetic lack of plasmacholinesterase, which normally is responsible for the hydrolytic decomposition of the drug.[12,13] Other less well documented untoward effects have been reported.[14–17]

Non-depolarizing muscle relaxants such as *d*-tubocurarine or gallamine do not seem to share the untoward effects detailed above. However, the duration of action of these agents does not depend upon hydrolysis (as with succinyldicholine), but mainly on their redistribution and excretion which are relatively slower processes.[13] A profound neuromuscular block with those agents can, therefore, only be obtained when a long duration of action is acceptable.

An overview of the situation described above leads to the conclusion that a most useful drug would produce a non-depolarizing block at the skeletal muscle motor end plate, at sufficiently low concentrations, with a short duration of action and, therefore, require no antagonists.

In this paper we report the synthesis of compounds designed with these characteristics in mind. The results of a structure–activity analysis *in vitro* of the new agents are also reported and compared with data from appropriate reference compounds. Several of these agents appear to warrant further study and investigation *in vivo*.

METHODS

Synthesis. All compounds are carboxylic acid esters of alkyl aminoethanols. The various aminoethanols and carboxylic acids are commercially available (Aldrich Chemical Co., Milwaukee, Wisc.). The corresponding acid chlorides are either commercially available or were prepared in the conventional manner by refluxing the acid with an excess of thionylchloride. The excess thionylchloride was eliminated in vacuum and the residue was purified through crystallization from chloroform or benzene and petrol ether. 1-Naphthylacetylchloride,[18] the phenylenediacelylchlorides[19] and nitrocinnamoylchlorides[20,21] had been previously synthesized.

Tertiary amino esters were prepared by the method of Rosnati and Bovet-Nitti:[22] the acid chloride dissolved in benzene was treated with small portions of dialkylaminoethanol at room temperature. In order to complete the reaction, the mixture was refluxed for approx. 20 min, cooled and treated with dilute HCl. The benzene layer was discarded and the aqueous acidic layer alkalinized with potassium carbonate and extracted with ether. The etherial solution was decolorized with charcoal and dried over Na_2SO_4. The crude residue was used without purification for the quaternization step.

When the acid chloride was not commercially available, an alternative method of Horenstein and Pählicke[23] was sometimes used for the preparation of tertiary esters. This method gave a more pure product, but yields were not always satisfactory, particularly when the carboxylic acid was poorly soluble in methanol, as was the case for all nitrocinnamic acids. The general procedure was as follows: diethylaminoethylbromide hydrobromide was treated with an equimolar amount of 2 N KOH in

methanol; the precipitated KBr was filtered off and the corresponding carboxylic acid was added to the methanolic solution of aminobromide. The solvent was flashed off and the residue heated on a steam bath for about 4 hr. The syrupy mass was dissolved in water and treated with potassium carbonate, extracted with ether, decolorized and dried in the manner described above. The yield averaged 30–50 per cent.

Quaternary meth- and eth-iodides were prepared by conventional methods, generally using dimethylformamide as the solvent and heating 30–60 min on a steam bath. The crude product was precipitated with ether and purified through crystallization from isopropanol or isopropanol and ether. Better results were obtained for the synthesis of compounds 4 and 5, (Table 1) by using acetone as the solvent and longer heating (3–4 hr).

Compounds quaternized with 9-bromofluorene could not be prepared by conventional methods. However, excellent yields were obtained as follows: The tertiary amino esters were allowed to react with 9-bromofluorene in nitrobenzene at room temperature for 3–6 days. The bromide salt was precipitated with ether. The crude product was extracted with acetone to eliminate acetone-soluble impurities and finally recrystallized in the usual manner. Cinnamates were obtained in yields of 70 per cent crude and up to 50 per cent of pure compound. For the acetates and phenylacetates the yields ranged between 20–40 per cent.

Bromides of *m*- and *p*-nitrocinnamates are poorly soluble in water. They were transformed into tosylates by treating with silver *p*-toluenesulfonate in acetonitrile.

Fluorenyl homologs of choline and ethocholine (compounds 28 and 29, Table 1) were synthesized by reacting the tertiary amino alcohols with 9-bromofluorene in nitrobenzene on a steam bath for 30 min. The acetyl esters of these fluorenyl homologs were prepared by acetylation of the quaternary alcohol with acetic anhydride.

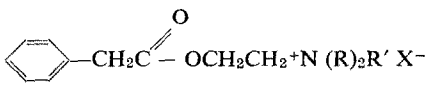
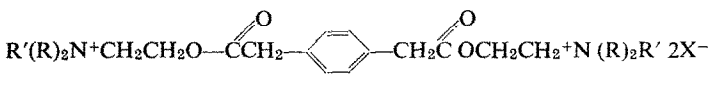
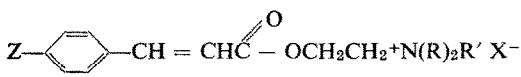
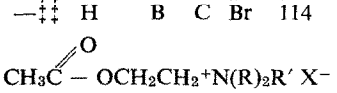
Hydrolysis. The duration of action of these esters *in vivo* is presumably related to their hydrolysis rate, as in the case with the diester succinylcholine.[13] Therefore, the rates of spontaneous hydrolysis and the hydrolysis catalyzed by acetylcholinesterase (EC 3.1.1.7) and plasmacholinesterase (EC 3.1.1.8) were measured in a pH stat titrator. CO_2 free techniques were employed. The spontaneous rate of hydrolysis of 1×10^{-3} M solution of the compound was measured in 75 ml of a medium consisting of 0·1 M NaCl, 0·02 M $MgCl_2$, 0·005 % gelatin, 1×10^{-5} M EDTA, at pH 7 and 25°. The rate is equal to the μmoles of standardized 0·01 N NaOH automatically added to maintain a constant pH.

After the rate of spontaneous hydrolysis was measured, 0·01 ml of a solution of acetylcholinesterase was added and the rate of enzymatically catalyzed hydrolysis measured. Acetylcholinesterase was prepared from Electrophorus electricus and has an activity of 90 m-moles acetylcholine hydrolyzed per ml per hr at 25°, pH 7, and 1×10^{-3} acetylcholine bromide.[24]

In another series of experiments the spontaneous hydrolysis was again determined but now 0·1 ml of human plasmacholinesterase solution was added and the rate of enzymatically catalyzed hydrolysis was measured. Plasmacholinesterase was partially purified from the commercial preparation (Cutter Laboratories, Berkeley, Calif.). The activity measured as above was 0·022 m-mole acetylcholine hydrolyzed ml/hr.

Neuromuscular blocking activity. The neuromuscular blocking properties of the compounds were tested *in vitro*. This approach provides evidence of the mode of action at the neuromuscular junction and eliminates many complicating factors which

TABLE 1. MONO- AND DIQUATERNARY ESTERS OF ALKYLAMINOETHANOLS

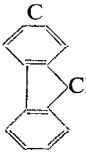
No.	*	Z†	R′	R′	X	M.p.(°C)§	Formula	Calcd. (%) C	Calcd. (%) H	Calcd. (%) N	Found (%) C	Found (%) H	Found (%) N
													
1	—	—	A	A	I	158‖(a)	—	—	—	—	—	—	—
2	—	—	B	B	I	72‖(b)	—	—	—	—	—	—	—
3	—	—	B	C	Br	117	$C_{27}H_{30}NBrO_2$	67·50	6.29	2·92	67·17	6·35	2·87
4	—	—	B	D	Br	152	$C_{21}H_{27}N_2BrO_4$	55·88	6·03	6·21	55·62	6·06	6·35
5	—¶	—	B	D	Br	160	$C_{21}H_{26}N_3BrO_6$	50·81	5·28	8·47	51·20	5·39	8·26
6	—**	—	B	B	I	126	$C_{16}H_{32}NIO_2$	48·36	8·12	3·53	48·52	8·09	3·64
7	—††	—	B	B	I	107	$C_{20}H_{28}NIO_2$	54·43	6·39	3·17	54·15	6·39	3·37
													
8	1,2	—	A	A	I	227	$C_{20}H_{34}N_2I_2O_4$	38·72	5·53	4·52	38·96	5·55	4·81
9	1,2	—	B	B	I	177	$C_{26}H_{46}N_2I_2O_4$	44·33	6·58	3·98	44·2	6·58	4·21
10	1,3	—	A	A	I	142	$C_{20}H_{34}N_2I_2O_4$	38·72	5·53	4·52	39·03	5·61	4·68
11	1,3	—	B	B	I	131	$C_{26}H_{46}N_2I_2O_4$	44·33	6·58	3·98	44·03	6·60	4·07
12	1,4	—	A	A	I	201‖(c)	—	—	—	—	—	—	—
13	1,4	—	B	B	I	182‖(d)	—	—	—	—	—	—	—
													
14	—	H	B	B	I	153	$C_{17}H_{26}NIO_2$	50·62	6·50	3·47	51·34	6·56	3·49
15	—	H	B	C	Br	145	$C_{28}H_{30}NBrO_2$	68·29	6·14	2·85	67·85	6·23	2·81
16	4	Cl	B	B	I	193	$C_{17}H_{25}NClIO_2$	46·64	5·76	3·20	47·06	5·97	3·64
17	4	Cl	B	C	Br	138	$C_{28}H_{29}NClBrO_2$	63·82	5·55	2·66	63·79	5·26	2·87
18	2	NO_2	B	B	I	166	$C_{17}H_{25}N_2IO_4$	45·54	5·62	6·25	45·60	5·78	6·12
19	2	NO_2	B	C	Br	130	$C_{28}H_{29}N_2BrO_4$	62·57	5·44	5·21	62·64	5·39	4·97
20	3	NO_2	B	B	I	167	$C_{17}H_{52}N_2IO_4$	45·54	5·62	6·25	45·26	5·67	6·21
21	3	NO_2	B	C	Tos	141	$C_{35}H_{36}N_2SO_7$	66·86	5·77	4·46	66·95	5·92	4·78
22	4	NO_2	B	B	I	168	$C_{17}H_{25}N_2IO_4$	45·54	5·62	6·25	45·86	5·73	6·18
23	4	NO_2	B	C	Tos	192	$C_{35}H_{36}N_2SO_7$	66·86	5·77	4·46	66·77	5·80	4·38
24	—‡‡	H	B	B	I	123	$C_{17}H_{28}NIO_2$	50·37	6·96	3·46	50·74	7·18	3·45
25	—‡‡	H	B	C	Br	114	$C_{28}H_{32}NBrO_2$	68·01	6·52	2·83	68·65	6·71	2·85
													
26	—	—	A	C	Br	167	$C_{19}H_{22}NBrO_2$	60·64	5·89	3·72	60·75	6·09	3·62
27	—	—	B	C	Br	122	$C_{21}H_{26}NBrO_2$	62·37	6·48	3·47	62·19	6·49	3·30
			$HOCH_2CH_2{}^+N(R)_2R'\ X^-$										
28	—	—	A	C	Br	142	$C_{17}H_{20}NBrO$	61·08	6·03	4·19	61·28	6·11	4·13
29	—	—	B	C	Br	155	$C_{19}H_{24}NBrO$	62·98	6·68	3·87	62·76	6·78	3·76

* Refers to the position of substitution on the ring.
† Z = Ring substituent.

R and R′

A 

B 

C 

D 

‡ Melting point taken on a Uni-Melt apparatus.
§ Compare reference 21: (a) 150°–151°; (b) oil; (c) 201°–202°; (d) 182°–184°.
‖ *p*-Nitrophenylacetyl instead of phenylacetyl.
¶ Cyclohexylacetyl instead of phenylacetyl.
** 1-Naphthylacetyl instead of phenylacetyl.
†† Hydrocinnamoyl (phenylpropionyl) instead of cinnamoyl.

operate *in vivo*. The methods were basically those described by Nastuk[25] and Nastuk and Alexander.[26] The frog sciatic nerve–sartorius muscle was isolated and mounted in a dish containing Ringers solution. Control measurements of the resting membrane potential were taken by impaling the cell at an area distant from the junction. A glass microelectrode containing 3 M KCl was used to measure the potential difference between the inside of the cell and the surrounding bathing solution. The drug under investigation was added at a relatively low concentration and changes in the resting potential were measured. The sciatic nerve was then stimulated with a supramaximal stimulus and the action or endplate potential recorded for each impaled cell. The drug concentration was increased until a level was reached at which most muscle fibers did not produce an action potential after nerve stimulation. The type of block (depolarizing or non-depolarizing) was noted and the lowest concentration necessary to block transmission was measured.

Anticholinesterase properties. All quaternary ammonium compounds have anticholinesterase activity at appropriate concentrations. This is an undesirable quality in any neuromuscular blocking agent. A simple test system *in vitro* was used to study anticholinesterase activity of these compounds and comparisons were made with some reference compounds. Measurements were made in a pH stat titrator under the same conditions described above. A dual syringe technique was used in which one syringe contained 0·012 N NaOH and the other 0·012 M acetylcholine bromide, allowing measurements at low but constant substrate concentrations.[27] An appropriate amount of the test compound was added to 75 ml of the reaction medium described which also contained 5×10^{-4} M acetylcholine and eel acetylcholinesterase. The decrease in the rate of acetylcholine hydrolysis was measured and compared to the rate recorded in the absence of the test compound. The measurements were repeated at three to four concentrations of the test agent varied over a 5-fold range. It was necessary to correct some values for spontaneous hydrolysis of the compound.

None of the compounds produced progressive enzyme inhibition even at relatively high concentrations (1×10^{-4} M) for long periods (1 hr). Dilution experiments demonstrated that the inhibition is immediately reversible indicating that the mechanism is probably one of simple reversible inhibition where

$$I + E \underset{}{\overset{K_I}{\rightleftarrows}} E \cdot I$$

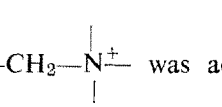
An approximate value of K_I was calculated by substituting the data into the equation

$$\frac{v^\circ}{v} = 1 + \frac{I}{K_I\left(1 + \frac{S}{K_m}\right)}$$

where v° is the rate of acetylcholine hydrolysis in the absence of the test compound and v is the rate in the presence of the drug. The other symbols have their usual meanings. The measured value of K_m was $1{\cdot}05 \times 10^{-4}$ M. The apparent value of K_I was used to calculate an approximate I_{50} value for our conditions of measurement. A more accurate method of measuring binding constant values was not used because precise data are not essential for screening procedures.

RESULTS AND DISCUSSION

An inspection of the structural formulae of *d*-tubocurarine, gallamine and other non-depolarizing blocking drugs reveals several common traits: all are rather large, bulky molecules containing quaternary ammonium groups, characteristics recognized by Bovet.[28] Their duration of action is limited principally by distribution and excretion mechanisms,[13] but evidently few ester compounds containing appropriate bulky substituents have been prepared and tested for their myoneural blocking properties. While esters have varying degrees of lability and choline esters in particular are generally susceptible to esterase hydrolysis, the degree of ester bond lability is influenced by such factors as adjacent electronegative atoms, electron withdrawing groups, benzene ring substituents, double bond systems and other functions. Thus, ester compounds are a group offering the possibility of "chemical tailoring" to meet the requirement of short action spans. An agent producing a non-depolarizing block with the potency and duration of action approaching succinyldicholine is desirable. If compounds could be synthesized which were hydrolyzed by acetylcholinesterase (red cell cholinesterase), the inherent problem of the genetic variants of the plasma enzyme would be avoided.

Because of its general complementarity with the esterases, the basic body structure of  was adopted. Other studies had indicated that quaternization of the nitrogen with ethyl groups produced compounds having non-depolarizing blocking properties and methyl quaternization yielded depolarizing agents.[28] Previous work in this laboratory revealed that hexafluorenium (Mylaxen) produces a non-depolarizing block.[29] Reasoning from this observation, the fluorenyl group was used to quaternize the tertiary amine functions in many of the new compounds.

Several benzoic acid and phenylacetic acid ester compounds previously synthesized by Rosnati and Bovet-Nitti[22] were reprepared. These compounds and the classic non-depolarizing and depolarizing drugs were tested as reference agents. New agents were then prepared in which the nitrogen was quaternized with combinations of methyl, ethyl, fluorenyl and nitrobenzyl groups. Diquaternary compounds and derivatives of cinnamic acid, phenylproprionic acid, cyclohexylacetic and naphthylacetic acid were prepared and studied. The simple test systems *in vitro* used as screening procedures have the advantages of allowing evaluation of specific parameters (hydrolysis, anti-cholinesterase properties, etc.) which may be altered by appropriate chemical manipulation.

Table 1 contains structural formulae and the values of melting points and elemental analysis. The format of Table 1 is used in Table 2 to facilitate comparisons. Hydrolysis and neuromuscular blocking data are recorded in Table 2. Anticholinesterase data are reported in Table 3.

An analysis of the data (Table 2) concerning the monoquaternary esters of phenylacetic acid (compounds 1,2,3,4) verifies previous work in that quaternization of the nitrogen atom with methyl groups produces depolarizing agents. Ethyl group quaternization yields non-depolarizing compounds but of decreased potency. When one ethyl group is replaced with a *p*-nitrobenzyl substituent, the potency is further

decreased (compound 4). A fluorenyl group was used to quaternize diethylaminoethylphenyl acetate (compound 3). This agent is the most potent non-depolarizing compound of the series, paralleling succinyldicholine (SDC) in this regard. The hydrolysis data of these compounds indicates that compound 3 is hydrolyzed by plasmacholinesterase twice as fast as succinyldicholine. The spontaneous hydrolysis rate is five times in excess of SDC. The hydrolysis rate by acetylcholinesterase is 0·7 per cent/hr in our system. This may be of importance when the total amount of

TABLE 2. RATES OF HYDROLYSIS AND NEUROMUSCULAR BLOCKING CONCENTRATIONS

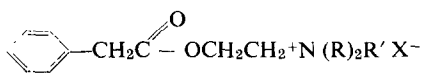
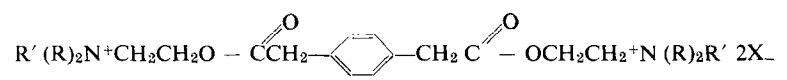
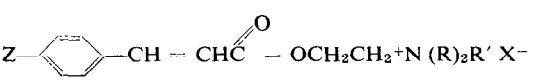
No.	*	Z†	R	R′	Hydrolysis						Neuromuscular block	
					Spont.		AChE		PChE			
					(%/hr)	(±SDC)	(%/hr)	(±SDC)	(%/hr)	(±SDC)	concn (M)	Type§
	Acetylcholine				0·1	−3	260	+	2·3	+1·5	5×10^{-6}	D
	Benzoylcholine				0	0	0	0	3·7	+2·5	5×10^{-4}	D
	Succinylcholine				0·3		0		1·5		5×10^{-5}	D
	Decamethonium				0	0	0	0	0	0	5×10^{-5}	D
	d-Tubocurarine				0	0	0	0	0	0	5×10^{-6}	N
	Gallamine				0	0	0	0	0	0	5×10^{-4}	N
	Hexafluorenium				0	0	0	0	0	0	2×10^{-5}	N
												
1	—	—	A	A	0·9	+3·0	0	0	2·7	+1·8	3×10^{-5}	D
2	—	—	B	B	1·5	+5·0	0	0	30	+20·0	1×10^{-4}	N
3	—	—	B	C	1·5	+5·0	0·7	+	3·9	+2·6	3×10^{-5}	N
4	—	—	B	D	0·3	+1·0	0·7	+	18	+12·0	2×10^{-4}	N
5	—‖	—	B	D	2·3	+7·7	2·0	+	7·2	+4·8	1×10^{-4}	N
6	—¶	—	B	B	0	0	1·8	+	90	+60	2×10^{-4}	N
7	—**	—	B	B	0·4	+1·3	1·1	+	5	+3·3	8×10^{-5}	N
												
8	1,2	—	A	A	0·1	−3	0	0	0·7	−2	6×10^{-4}	D
9	1,2	—	B	B	0·6	+2	0	0	18	+12	5×10^{-4}	N
10	1,3	—	A	A	0·3	1	0·3	+	6·3	+4·2	1×10^{-6}	D
11	1,3	—	B	B	3·7	+12	0	0	5	+3·3	$1{\cdot}5 \times 10^{-4}$	N
12	1,4	—	A	A	0·2	−0·5	2·3	+	4·5	+8·4	5×10^{-7}	D
13	1,4	—	B	B	4·6	+15	0·6	+	7·8	+5·2	1×10^{-4}	N
												
14	—	H	B	B	0	0	0	0	1·0	−0·7	2×10^{-4}	N
15	—	H	B	C	0	0	0	0	0	0	5×10^{-6}	N
16	4	Cl	B	B	0	0	0	0	0	0	5×10^{-5}	N
17	4	Cl	B	C	0	0	0	0	0	0	$2{\cdot}5 \times 10^{-6}$	N
18	2	NO_2	B	B	0	0	0	0	1·8	+1·2	2×10^{-4}	N
19	2	NO_2	B	C	0	0	0	0	0·7	−2	2×10^{-5}	N
20	3	NO_2	B	B	0	0	0	0	2·0	+1·6	4×10^{-4}	N
21	3	NO_2	B	C	0·1	−3	0	0	0	0	$1{\cdot}5 \times 10^{-5}$	N
22	4	NO_2	B	B	0	0	0	0	3·7	+2·5	1×10^{-4}	N
23	4	NO_2	B	C	0	0	0	0	25	+17	1×10^{-5}	N
24	—††	H	B	B	0	0	0	0	7·5	+5	1×10^{-4}	N
25	—††	H	B	C	0·5	−0·2	0	0	0	0	1×10^{-5}	N

TABLE 2.—*continued*

No.	*	Z†	R	R′	Hydrolysis						Neuromuscular block	
					Spont.		AChE		PChE			
					(%/hr)	(±SDC)	(%/hr)	(±SDC)	(%/hr)	(±SDC)	concn (M)	Type§
					$CH_3C(=O)-OCH_2CH_2{}^+N(R)_2R'\ X^-$							
26	—	—	A	C	0·4	+1·3	0	0	0	0	3×10^{-4}	N
27	—	—	B	C	0	0	0	0	0	0	5×10^{-4}	N
					$HOCH_2CH_2{}^+N(R)_2R'\ X^-$							
28	—	—	A	C	—	—	—	—	—	—	8×10^{-5}	N
29	—	—	B	C	—	—	—	—	—	—	2×10^{-4}	N

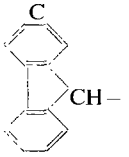
* Refers to the position of substitution on the ring.

† Z = Ring substituent.

R and R′

A: CH_3-

B: C_2H_5-

C: 

D: $O_2N-C_6H_4-CH_2-$

‡ D = Depolarizing block; N = Nondepolarizing block.

§ *p*-Nitrophenylacetyl instead of phenylacetyl.

‖ Cyclohexylacetyl instead of phenylacetyl.

¶ 1-Naphthylacetyl instead of phenylacetyl.

** Hydrocinnamoyl (phenylpropionyl) instead of cinnamoyl.

The first five columns are taken from Table 1 and are included here to facilitate comparisons. The named compounds are reference agents. The hydrolysis data are recorded absolutely in per cent per hour and relatively to the standard succinyldicholine (column ± SDC) measured similarly at 25°, pH 7, and 1×10^{-3}M. The neuromuscular blocking data are recorded in terms of the concentrations necessary to completely interrupt myoneural transmission in the frog sciatic nerve–sartorius muscle preparation. The techniques are described in the text.

red-cell cholinesterase present in an adult is considered and may be expected to contribute to the possible short action span *in vivo*.

If the phenyl head is replaced successively with a *p*-nitrophenyl (compound 5), cyclohexyl (compound 6), or naphthyl (compound 7) moiety, the potency does not change significantly over compound 3. The rate of hydrolysis by plasmacholinesterase is considerably higher in compound 6; 90 per cent is hydrolyzed in 1 hr compared to 3·7 per cent of the reference compound benzoylcholine.

Most potent neuromuscular blocking compounds have two or three quaternary ammonium functions. Therefore, symmetrical diquaternary analogues of the phenylacetate series were prepared. Although several methyl quaternized derivatives were potent depolarizing agents (compound 12 is ten times more potent than acetylcholine) the non-depolarizing ethyl substituted derivatives were surprisingly not more potent than the monoquaternary analogues. The hydrolysis data revealed no significant trends. Unfortunately, we were unable to prepare the diquaternary fluorenyl substituted derivatives of this series.

Choline esters of cinnamic acid have been reported to be reversible inhibitors of fly brain cholinesterase.[30] Some derivatives are hydrolyzed by chymotrypsin.[31] The fluorenyl and ethyl quaternized derivatives of diethylaminoethyl-cinnamate were prepared and studied. All were non-depolarizing myoneural blocking agents. Half of the ten derivatives prepared were more potent than SDC and two were as potent as

TABLE 3. INHIBITION OF ACETYLCHOLINESTERASE

No.	Apparent K_I	Apparent I_{50}
Succinyldicholine	1×10^{-5}	$6{\cdot}0 \times 10^{-5}$
Decamethonium	$1{\cdot}5 \times 10^{-6}$	$9{\cdot}0 \times 10^{-6}$
d-Tubocurarine	$2{\cdot}6 \times 10^{-5}$	$1{\cdot}5 \times 10^{-4}$
Gallamine	$6{\cdot}6 \times 10^{-5}$	$4{\cdot}0 \times 10^{-4}$
Hexafluorenium	$1{\cdot}1 \times 10^{-7}$	$6{\cdot}6 \times 10^{-7}$
Edrophonium (Tensilon)	$1{\cdot}3 \times 10^{-7}$	$7{\cdot}5 \times 10^{-7}$
Choline	$2{\cdot}2 \times 10^{-4}$	$1{\cdot}3 \times 10^{-3}$
Ethocholine	$1{\cdot}7 \times 10^{-4}$	$1{\cdot}0 \times 10^{-3}$
3	$8{\cdot}5 \times 10^{-6}$	$5{\cdot}1 \times 10^{-5}$
7	$7{\cdot}2 \times 10^{-5}$	$4{\cdot}3 \times 10^{-4}$
15	$1{\cdot}9 \times 10^{-6}$	$1{\cdot}1 \times 10^{-5}$
16	$3{\cdot}8 \times 10^{-6}$	$2{\cdot}3 \times 10^{-5}$
17	$1{\cdot}0 \times 10^{-7}$	$6{\cdot}0 \times 10^{-7}$
19	$2{\cdot}2 \times 10^{-7}$	$1{\cdot}3 \times 10^{-6}$
21	$5{\cdot}6 \times 10^{-8}$	$3{\cdot}4 \times 10^{-7}$
23	$5{\cdot}6 \times 10^{-8}$	$3{\cdot}4 \times 10^{-7}$
25	$8{\cdot}5 \times 10^{-6}$	$5{\cdot}1 \times 10^{-5}$
28	$8{\cdot}6 \times 10^{-6}$	$5{\cdot}2 \times 10^{-5}$
29	$1{\cdot}2 \times 10^{-5}$	$7{\cdot}2 \times 10^{-5}$

The named compounds are reference agents. The numbers in the first column refer to compounds numbered similarly in Table 1. The dimensions for K_I and I_{50} are moles per liter. The values of the constants are the averages of 3–4 measurements varied over a 5-fold range. The term 'apparent' is used to indicate the relatively less precise method selected as the screening test for anticholinesterase activity. All measurements were made at 25°, pH 7, in a medium of 0·1 M NaCl, 0·02 M $MgCl_2$, 0·005 % gelatin, 1×10^{-5} M EDTA and 5×10^{-4} M acetylcholine bromide with electric eel acetylcholinesterase as described in text.

d-tubocurarine (compounds 15 and 17). The potency was consistently increased by a factor of ten or more when a fluorenyl group was substituted for an ethyl moiety on the nitrogen. This is in agreement with the "bulky group" concept. All the compounds are stable in solution and are not susceptible to hydrolysis by acetylcholinesterase measured under our conditions. The introduction of a NO_2 group into the ortho, meta or para positions yields derivatives which are subject to plasmacholinesterase catalyzed hydrolysis (except compound 21). Compound 19 (ortho NO_2 substituted) combines stability in solution (no spontaneous hydrolysis) with an appropriate rate of plasmacholinesterase hydrolysis (50 per cent less than SDC) and proper potency (twice as potent as SDC). The meta NO_2 substituted analogue (compound 21) is also potent but resistant to enzymatic hydrolysis. Compound 23 (para NO_2 substituted) is five times more potent than SDC but is hydrolyzed by the plasma enzyme seventeen times faster than SDC. The para chloro substituted analogue (compound 17) has twice the potency of *d*-tubocurarine but is not spontaneously or enzymatically hydrolyzed.

The influence of the cinnamic acid double bond was assessed by preparing two phenylproprionic (hydrocinnamic) acid esters (compounds 24 and 25). The potency was half their respective cinnamic acid ester analogues.

The acetate esters of quaternary aminoethanol (dimethylfluorenyl, compound 26 and diethylfluorenyl, compound 27) were synthesized. They were stable to enzymatic hydrolysis and were not especially potent as non-depolarizing compounds.

The quaternary hydrolysis products of the most potentially useful compounds so far prepared are expected to be fluorenyldiethylaminoethanol (compound 30) and fluorenyldimethylaminoethanol (compound 29). This reasoning follows from the fact that the alcohol moiety is split out as the first product of hydrolysis.[32] Therefore, these two amino alcohols were synthesized and tested for their neuromuscular blocking properties. Their potency is about 10-fold less than their (respective) ester analogues.

The data in Table 3 allow comparison of the anticholinesterase activities of the reference agents with those of the new compounds. Edrophonium, a potent anticholinesterase agent, and choline derivatives were also tested. Decamethonium has the lowest value for K_I among the clinical useful neuromuscular blocking agents tested. Hexafluorenium and edrophonium also have the low values anticipated. Phenyl ring substitution in the cinnamic acid derivatives (compounds 17, 19, 21, 23) increases anticholinesterase activity 10- to 20-fold when compared to the unsubstituted derivative (compound 15) and the reference agent, decamethonium. Compound 29, the hydrolysis product of some of the new agents has a K_I value of $1{\cdot}2 \times 10^{-5}$ M, and is approximately five times as active as choline (K_I, $2{\cdot}2 \times 10^{-4}$ M). Compounds 3, 7, 15 and 16 are all less potent than decamethonium as inhibitors of acetylcholinesterase.

This study has produced several potentially useful compounds which warrant pilot investigations *in vivo*. These have begun. Other structural modifications are being made and studied for increased potency and reduced anticholinesterase activity.

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