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Action of some *bis*quaternary derivatives of phthalic acids and related substances on neuromuscular transmission

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Summary

1. All the *bis*quaternary derivatives of terephthalic acid with three methyl groups on each nitrogen atom (PK-107, PK-95, PK-97 and PK-126) were depolarizing neuromuscular blocking agents. The most active was the compound PK-97, in which the two quaternary groups are separated by sixteen atoms and are about 20 Å (2 nm) apart. Activity was reduced many fold either by decreasing the separation to twelve atoms or by increasing it to eighteen atoms. It was also reduced several hundred fold when one trimethylammonium group in PK-97 was replaced by a hydrogen atom (as in PK-119).

2. The presence and position of the ester groups in these compounds is important; depolarizing activity is in most cases greatest when the ester groups are the same distance from the quaternary nitrogen atoms as in acetylcholine, that is, in carbolonium, sebacoyldicholine and PK-154. The monoquaternary analogues of carbolonium and sebacoyldicholine are appreciably active, having between about one-tenth to one-fifth of the activity of their *bis*quaternary analogues.

3. The relationships between the structure and activity of these compounds are discussed, particular consideration being given to the structure of the chain separating the quaternary groups and the arrangement of acetylcholine receptors on cells and of esterbinding groups within these receptors.

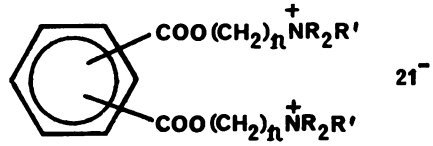
Introduction

The *bis*quaternary neuromuscular blocking drugs usually display their maximal potency when the internitrogen chain contains ten or sixteen atoms, and the two cationic heads of the compound are separated by a distance of about 14 Å (1·4 nm) or about 20 Å (2 nm) (see Barlow & Ing, 1948; Paton & Zaimis, 1949; Brücke, 1956; Bovet, 1959; Barlow & Zoller, 1964, Barlow, 1968).

A suggestion was recently made that individual acetylcholine receptors are unevenly distributed on the postsynaptic membrane of skeletal muscle and are collected together in oligomeric complexes, inside which they are arranged in two types of structure: (1) a 'C-10 structure' which can easily interact with *bis*quaternary compounds, like decamethonium or suxamethonium, which have ten atoms

between the cationic groups, and (2) a 'C-16 structure' which can easily interact with compounds like hexadecamethonium, sebacoyldicholine or carbolonium, which have sixteen atoms between the cationic groups (Rybolovlev, 1963; Michelson &

TABLE 1. *Analyses and (m.pts.) of compounds with the general formula:*



Number		*n*=	R=	R'=	Melting point (°C)	Solvent	N	I⁻ (%)
PK–107	*p*	2	Me	Me	276–7 (a)	A	4·47	42·7
140	*o*				228 (b)	C	4·83	42·6
					$C_{18}H_{30}N_2O_4I_2$	requires	4·72	42·9
91	*o*				168–170	C	No analysis	
					$C_{24}H_{42}N_2O_4I_2$	requires		37·6
109	*o*	2	*n*-BU	Me	>250	C+D	3·47	32·8
					$C_{30}H_{54}N_2O_4I_2$	requires	3·68	33·4
95	*p*	3	Me	Me	248–9 (a)	C+D		40·7
93	*o*				148–50	C+D		41·1
					$C_{20}H_{34}N_2O_4I_2$	requires		40·9
97	*p*	4	Me	Me	232–3	A		39·1
106	*o*				181–2	C+D		39·4
					$C_{22}H_{38}N_2O_4I_2$	requires		39·2
108	*p*	4	Et	Et	188–9	C+D		34·7
					$C_{28}H_{50}N_2O_4I_2$	requires		34·7
126	*p*	5	Me	Me*	145	B+D	4·63	27·3*
					$C_{24}H_{42}N_2O_4Br_2$	requires	4·81	27·4*
111	*p*	5	Et	Me	200–1	C	3·89	34·6
					$C_{28}H_{50}N_2O_4I_2$	requires	3·82	34·7

Compounds with the general formula

139	X=Y= $CH_2COO(CH_2)_2\overset{+}{N}Me_3$ I^-	201–2 (c)		
127	X=Y= $CH_2COO(CH_2)_3\overset{+}{N}M3_3$ I^-	226–7 (c)		39·0
		$C_{22}H_{38}N_2O_4I_2$	requires	39·2
154	X=Y= $CH_2CH_2COO(CH_2)_2\overset{+}{N}Me_3$ I^-	193	C 4·51	
		$C_{22}H_{38}N_2O_4I_2$	requires 4·32	
155	X=Y= $CH_2CH_2COO(CH_2)_3\overset{+}{N}Me_3$ I^-	224	C	37·5
		$C_{24}H_{42}N_2O_4I_2$	requires	37·4
158	X=Y= $CH_2CH_2COO(CH_2)_4\overset{+}{N}Me_3$ I^-	225	C 4·23	36·0
		$C_{26}H_{46}N_2O_4I_2$	requires 4·00	36·0
142	X=Y= $CH_2OOC(CH_2)_2\overset{+}{N}Me_3$ I^-	172	B+D 4·64	40·7
		$C_{20}H_{34}N_2O_4I_2$	requires 4·57	40·6
141	X= $COO(CH_2)_3\overset{+}{N}Me_3$ I^-	166–7	C 3·18	29·8
	Y=COOnPz	$C_{17}H_{26}NO_4I$	requires 3·22	29·2
119	X= $COO(CH_2)_4\overset{+}{N}Me_3$ I^-	208–9	B 3·14	27·3
	Y=COO*n*Bu	$C_{19}H_{30}NO_4I$	requires 3·02	27·4

All the compounds were di-iodides, except for PK–126, marked with an asterisk, which was a dibromide. The solvents used for recrystallization were: A, water; B, methanol; C, ethanol; D, ether. (a) Compounds PK–107 and PK–95 were described by Fusco, Palazzo *et al.* (1948, 1949) and by Fusco, Chiavarelli *et al.* (1948). (b) Compound PK–140 was described by Öskne (1959), who recorded m.pt. 230° C; (c) compounds PK–139 and 127 were described by Rosnati (1955) who recorded m.pts. 202° C and 225–227° C respectively.

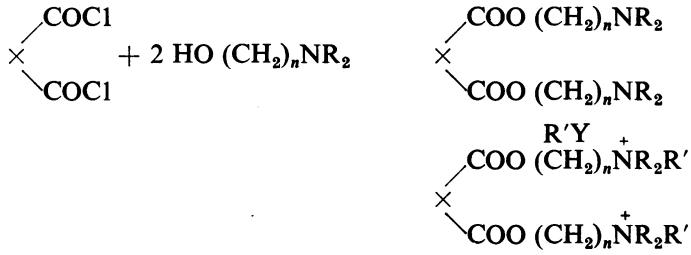
Khromov-Borisov, 1964; Khromov-Borisov & Michelson, 1966; Danilov, 1966, 1968; Michelson & Zeimal, 1970; see also Barlow, 1960).

To test these suggestions, new series of *bis*quaternary derivatives of terephthalic and phthalic acids and some related compounds were synthesized (Table 1) and their actions on the neuromuscular junction of different vertebrates were studied. Some members of these series were described previously by Bovet (1959) and some of our preliminary results have been published (Danilov, Eremina, Kvitko, Lavrentieva, Michelson, Porai-Koshits, Rozhkova & Shelkovnikov, 1968; Michelson, 1970).

Methods

Chemical

The quaternary salts listed in Table 1 were synthesized according to the following general scheme:



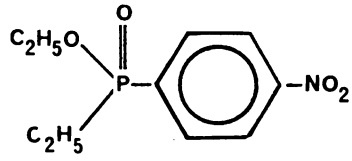
Phthalic acid and terephthalic acid were available. *p*-Phenylendiacetic, *p*-phenylendipropionic and *p*-phenylendiacrylic acids were obtained by methods described in the literature (Kipping, 1888; Ruggli & Staub, 1934; Ruggli & Thelheimer, 1941).

The acids were converted to their acid chlorides by treatment with thionyl chloride. The aminoesters were obtained by adding the dialkylaminoalcohol (0·04 mol), dissolved in benzene (20 ml), to a solution of the acid chloride (0·01 mol), dissolved in dry benzene (30 ml), at room temperature. The mixture was boiled under reflux for 5–10 hours. When it was cold, the benzene layer was separated from the residue and washed with water. The residue was dissolved in water, made alkaline to pH 10, and extracted with benzene. The combined benzene extracts were dried with anhydrous sodium sulphate and the benzene distilled off. When petroleum ether (b.p. 40–60° C) was added the aminoesters crystallized out and were recrystallized from a suitable solvent.

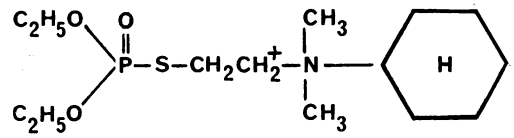
To obtain the quaternary salts, the aminoesters were dissolved in dry ether or in acetone and treated with a 5–6 fold excess of the alkylating agent. The mixture was left and the solid which crystallized out was filtered off, dried and recrystallized. Usually, ethanol was used as solvent but sometimes it was necessary to use methanol, water, or combinations of methanol or ethanol and ether. The overall yields were usually better than 60% but with the ethylated compound (PK-108), the yield was only about 25% and with the di-*n*-butylmethyl compound (PK-109) only 32%. Melting points and analyses are shown in Table 1.

Biological

Cats were anaesthetized with urethane (500 mg/kg) and chloralose (50 mg/kg) intraperitoneally; the sciatic nerve was stimulated with rectangular pulses of 0·5 ms duration at a frequency of 0·1 Hz, and with the size of the stimulus sufficient to produce maximal twitches of the anterior tibialis and the soleus muscles. The contractions of the two muscles were recorded isotonically with a Kymograph writing on a smoked drum. The blocking activity was estimated by finding the intravenous doses which reduced the contractions by 50%. For drugs which were readily hydrolysed by butyrylcholinesterase, the estimation of the blocking dose was carried out on cats given atropine (5 mg/kg, i.m.) followed by intravenous injection of an anticholinesterase: neostigmine (0·1 mg/kg), armine (0·5 mg/kg) or the compound GT-165, a selective inhibitor of butyrylcholinesterase (Brestkin, Brick, Volkova, Godovikov, Kabachnik & Teplov, 1965) (0·1–0·2 mg/kg).



Armine



GT-165

A method derived from Paton & Zaimis (1949, 1951) and Jewell & Zaimis (1954) was used to determine the type of blocking action. Experiments were made to see how the block was modified by decamethonium (0·03 mg/kg), (+)-tubocurarine chloride (0·1 mg/kg) and neostigmine (0·05–0·1 mg/kg). The effects on the block of reducing the interval impulses, of a tetanus, and the reaction after a tetanus, were also studied and comparisons were made between the blocking effects in the tibialis and in the soleus muscles. This information about the type of blocking action produced by the compounds was supplemented by comparisons of their effects on the frog rectus and chick biventer cervicis muscles, described below.

The neuromuscular blocking effects of the compounds *in rabbits* were assessed by measuring the head-drop dose during rapid intravenous injection (Hoppe, 1950).

The nerve-muscle preparation of the rat diaphragm (Bülbring, 1946) was mounted in 100 ml Liley solution (Liley, 1956) which was bubbled with air at room temperature. The phrenic nerve was stimulated with rectangular pulses of 0·1 ms duration at a frequency of 0·1 Hz. The contractions of the diaphragm were recorded isotonically on a smoked drum and the dose was found which reduced the height of contractions by 50% during 3 minutes. The drugs were added to the bath with a syringe in a volume not exceeding 1·0 ml. In order to inhibit butyrylcholinesterase the compound GT-165, or armine, was added to the bath in a concentration of $1{\cdot}0 \times 10^{-6}$M, left to act for about 40 min and then washed out.

Since the duration of exposure to drugs was relatively brief, experiments were performed on three compounds, PK-107, PK-95, and PK-97, to determine their potency ratios under conditions when time of action was unlimited. For these tests,

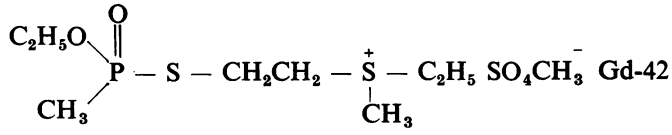
the minimal concentrations required just to produce full block were determined, and were about 3–4 times less than that required to produce 50% block in 3 minutes. The potency ratios, however, did not differ significantly between the two methods.

Frog rectus abdominis

Five or 10 muscles were set up simultaneously in one bath containing 200 ml frog-Ringer solution bubbled with air. Doses of depolarizing (acetylcholine-like) compounds were added and the heights of the contractures, recorded isotonically, were read on a millimetre scale. Cumulative log dose-response curves were plotted from the results and the values of EC50, were calculated, that is of the concentration producing a contracture which was half the maximum of which the tissue was capable. Some of the compounds were partial agonists and the maximum response obtainable with these was expressed as a fraction of the maximum response of which the tissue was capable. This fraction gives some idea of the intrinsic activity (Ariëns, 1964) or efficacy (Stephenson, 1956) of the compounds. All these experiments were performed in the presence of neostigmine, 2×10^{-6}M.

Since depolarizing drugs are liable to produce desensitization, and this could be particularly marked with cumulative dose-response experiments, estimates of the EC50 for PK-107, PK-95 and PK-97 were determined by a non-cumulative technique, in which the drug was washed out after each concentration tested, and an interval of 30 min left before testing the next concentration. The same values of EC50 were obtained as with the cumulative method. No signs of falling away of the contractions were seen when the drug was allowed to act on the muscle for 30 minutes.

The nerve-muscle preparation of the sartorius muscle of the frog (Ing & Wright, 1931) was suspended in a bath containing 25 ml Ringer solution bubbled with air. The sciatic nerve was stimulated with rectangular shocks of 0·1 ms duration at a frequency of 0·1 Hz, with the size of the stimulus sufficient to produce maximal twitches. The contractions of the muscle were recorded isotonically and the minimal concentrations of drugs which produced a complete block were estimated. The intervals between doses varied from 30 to 60 minutes. When it was designed to inhibit cholinesterases, an irreversible inhibitor, the compound Gd-42 (Volkova, Godovikov, Kabachnik, Magazanik, Mastrukova, Michelson, Rozhkova, Fruentov & Jakovlev, 1961) was added to the bath in concentration $2{\cdot}0 \times 10^{-6}$M, allowed to act for 30 min and then washed out.



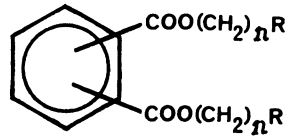
Gd-42

The biventer cervicis preparation from 7–12 day old chicks was set up as described by Ginsborg & Warriner (1960) in Locke's solution at 39–40° C bubbled with air, but the nerve was not stimulated and only contractures of the muscle were recorded. Neostigmine ($5{\cdot}0 \times 10^{-6}$M) was present in the bath which had a volume of 30 ml. Drugs were added by a pipette in a volume not exceeding 1·0 ml and allowed to act until the contracture had reached a maximum (usually for about 1–3 min).

Results

The main results are summarized in the Tables 2–4 and illustrated by Figs. 1–4.

TABLE 2. *Pharmacological properties of some phthalic acid derivatives*

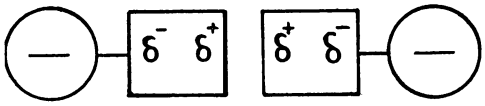
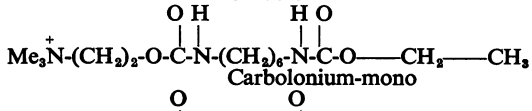
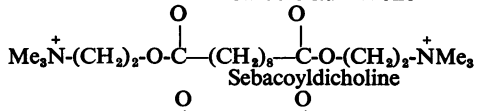
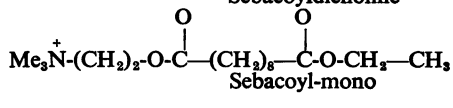
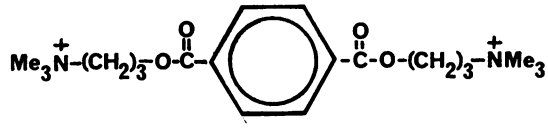
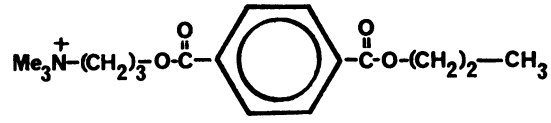
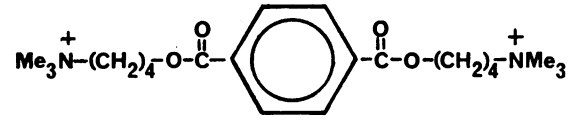
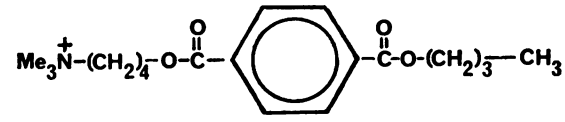


Compound PK–	R	*n*	$^{+}$ $^{+}$ N-N	Cat ED50 (μmol /kg)	Rabbit HDD (μmol /kg)	Rat diaphragm EC50 M	Sartorius EC50	Frog Rectus abdominis EC50 M	Max. resp.	Chick biventer EC50 M	Type
107 *p*	$^{+}NMe_3\ I^-$	2	12	0·4 ± 0·1 (6)	2·4 ± 0·4 (4)	$8·3\times10^{-5}$ ± $0·4\times10^{-5}$ (6)	$8·5\times10^{-4}$ ± $1·1\times10^{-4}$ (2)	$1·0\times10^{-5}$ ± $0·1\times10^{-5}$ (10)	0·7	$1·2\times10^{-5}$ ± $0·3\times10^{-5}$ (2)	D
95 *p*	$^{+}NMe_3\ I^-$	3	14	0·011 ± 0·006 (10)	0·05 ± 0·01 (5)	$7·3\times10^{-6}$ ± $0·8\times10^{-6}$ (7)	$1·1\times10^{-5}$ ± $0·3\times10^{-5}$ (4)	$1·0\times10^{-6}$ ± $0·2\times10^{-6}$ (10)	I	$2·7\times10^{-6}$ ± $0·4\times10^{-6}$ (3)	D
97 *p*	$^{+}NMe_3\ I^-$	4	16	0·005 ± 0·001 (10)	0·025 ± 0·008 (5)	$2·8\times10^{-6}$ ± $0·3\times10^{-6}$ (8)	$5·0\times10^{-6}$ ± $0·5\times10^{-6}$ (5)	$5·0\times10^{-7}$ ± $0·3\times10^{-7}$ (10)	0·9	$3·9\times10^{-7}$ ± $0·9\times10^{-7}$ (3)	D
126 *p*	$^{+}NMe_3\ I^-$	5	18	0·11 ± 0·02 (5)	0·35 ± 0·06 (3)	$2·6\times10^{-5}$ ± $0·2\times10^{-5}$ (4)		$4·0\times10^{-7}$ ± $0·2\times10^{-7}$ (10)	0·4	$1·5\times10^{-7}$ ± $0·8\times10^{-7}$ (3)	D
108 *p*	$^{+}NEt_3\ I^-$	4	16	0·5 ± 0·04		$1·0\times10^{-4}$ ± 0×10^{-4}		Cholinolytic			ND
111 *p*	$^{+}NEt_2Me\ I^-$	5	18	4·0 ± 0·5 (5)		$1·5\times10^{-4}$ ± $0·2\times10^{-4}$ (7)		Cholinolytic			ND
140 *o*	$^{+}NMe_3\ I^-$	2	10	100·0 (3)	15·0 ± 2·0 (3)	$1·0\times10^{-4}$ ± 0×10^{-4} (2)		Cholinolytic			ND
93 *o*	$^{+}NMe_3\ I^-$	3	12	3·0 ± 0·3 (5)	5·0 ± 1·5 (4)	$1·3\times10^{-4}$ ± $0·3\times10^{-4}$ (5)		$5·0\times10^{-5}$ ± $0·5\times10^{-5}$ (10)	0·3		
106 *o*	$^{+}NMe_3\ I^-$	4	14	0·5 ± 0·1 (5)	1·0 ± 0·2 (4)	$5·0\times10^{-6}$ ± $0·9\times10^{-6}$ (7)		$2·0\times10^{-5}$ ± $0·2\times10^{-5}$ (10)	0·4		
91 *o*	$^{+}NEt_3\ I^-$	2	10	15·0 ± 2·0 (5)		$6·1\times10^{-4}$ ± $1·5\times10^{-4}$ (6)	$1·0\times10^{-4}$ ± $0·4\times10^{-4}$ (7) $4·2\times10^{-5}$* ± $0·3\times10^{-5}$ (4)	Cholinolytic			ND
109 *o*	$^{+}NMe\ nBu_2\ I^-$	2	10	2·0 ± 0·2 (3)		$1·0\times10^{-4}$ (1)		Cholinolytic			ND
Acetylcholine								$3·0\times10^{-7}$		$1·6\times10^{-6}$	

* After the action of an anticholinesterase drug Gd-42. Numbers indicate mean values ± standard errors, with the number of estimations shown in parentheses. The column $^{+}$ $^{+}$ N-N indicates the number of atoms separating the charged nitrogen atoms; D indicates a depolarizing blocking action; ND indicates a non-depolarizing type of action. The column headed maximum response indicates the maximal contracture of the rectus muscle obtainable with the drug, expressed as a fraction of the maximum of which the tissue was capable. Compounds 107, 97, 126, 93, 106 were all partial agonists; the last three in particular had only low efficacy.

Tests with compounds liable to enzymatic hydrolysis by cholinesterases (PK-154, for instance) were made in the presence of anticholinesterases. Although different inhibitors were used in different tests, the choice of inhibitor did not appear to affect the results to any great extent. Blocking doses on the cat tibialis preparation, for instance, were much the same whether the anticholinesterase was neostigmine, armine, Gd-42 or GT-165.

TABLE 3. *The blocking potency of some* bis *quaternary compounds and their* mono *quaternary derivatives*

	N-N	Cat ED50 (μmol/kg)	Rabbit HDD (μmol/kg)	Rat diaphragm EC50 M
	16	0·008 ±0·001 (5) ×10	0·06 ±0·012 (4) ×6·6	$1·3\times10^{-5}$† $\pm0·2\times10^{-5}$ (6) ×3
		0·08 ±0·005 (5)	0·4 ±0·04 (4)	$3·9\times10^{-5}$† $\pm2·0\times10^{-5}$ (5)
	16	0·02*		$5·0\times10^{-7}$*
		×5 0·1*		×4 $2·0\times10^{-6}$*
	14	0·011 ±0·006 (10) ×30	0·05 ±0·01 (5) ×40	$7·4\times10^{-6}$ $\pm0·9\times10^{-6}$ (5) ×7
		0·3 ±0·06 (5)	2·0 ±0·3 (5)	$5·0\times10^{-5}$ $\pm0·0\times10^{-5}$ (2)
	16	0·005 ±0·001 (10) ×700	0·025 ±0·008 (5) ×400	$2·9\times10^{-6}$ $\pm0·1\times10^{-6}$ (5) ×35
		3·5 ±0·4 (5)	10·0 ±0·75 (5)	$1·0\times10^{-4}$ $\pm0·0\times10^{-4}$ (4)
Me_4N^+ TMA		3·0 ±0·08 (5)	12·0 ±1·7 (5)	$5·4\times10^{-4}$ $\pm2·0\times10^{-4}$ (3)

* After anticholinesterase armine; †, the results of Rybolovlev (1963).

Discussion

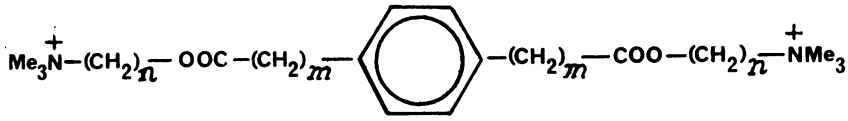
Type of blocking action

All *bis*quaternary derivatives of terephthalic acid with three methyl groups on each nitrogen atom (PK-107, PK-95, PK-97, PK-126) were neuromuscular blocking agents of a depolarizing type (Table 2). The action of PK-97 in the cat is illustrated by Fig. 1.

In depolarizing compounds the replacement of methyl groups on the nitrogen atoms by ethyl or more bulky groups converted them to neuromuscular blocking agents of a non-depolarizing type and of low potency as described by Bovet (1959).

In non-depolarizing compounds the replacement of methyl groups on quaternary nitrogen atoms by more bulky groups usually enhanced the potency without changing the type of action. (Compare PK-140 with PK-91 and PK-109, Table 2).

TABLE 4. *Pharmacological properties of some* bis *quaternary tere-phthalic acid derivatives and their analogues with different structure of internitrogen chain*



Compound PK–	*n*	m	N-N	Cat ED50 (μmol/kg)	Rabbit HDD (μmol/kg)	Frog Rat diaphragm EC50 M	Frog Sartorius EC50 M	Frog Rectus EC50 M	Max. resp.	Chick biventer EC50 M
97	4	0	16	0·005 ± 0·001 (10)	0·025 ± 0·008 (5)	$2·8\times10^{-6}$ ± $0·3\times10^{-6}$ (8)	$5·0\times10^{-6}$ ± $0·5\times10^{-6}$ (5)	$5·0\times10^{-7}$ ± $0·3\times10^{-7}$ (10)	0·9	$3·9\times10^{-7}$ ± $0·9\times10^{-7}$ (3)
127	3	1	16	1·3 ± 0·2 (5)	9·0 ± 1·6 (5)	$5·6\times10^{-4}$ ± $0·2\times10^{-4}$ (4)				
154	2	2	16	0·05 ± 0·01 (8)		$6·3\times10^{-7}$ ± $0·5\times10^{-7}$ (3)	$2·6\times10^{-6}$ ± $0·3\times10^{-6}$ (8)	$2·6\times10^{-7}$ ± $0·3\times10^{-7}$ (5)	I	$2·0\times10^{-7}$ (1)
95	3	0	14	0·011 ± 0·006 (10)	0·05 ± 0·01 (5)	$7·3\times10^{-6}$ ± $0·8\times10^{-6}$ (7)	$1·1\times10^{-5}$ ± $0·3\times10^{-5}$ (4)	$1·0\times10^{-6}$ ± $0·2\times10^{-6}$ (10)	I	$2·7\times10^{-6}$ ± $0·4\times10^{-6}$ (3)
139	2	1	14	7·5 ± 0·8 (3)	7·5 ± 0·5 (3)	$2·7\times10^{-4}$ ± $0·6\times10^{-4}$ (5)	$4·0\times10^{-4}$ ± $1·0\times10^{-4}$ (3)	very weak contraction		$3·0\times10^{-5}$ (1)
142	2	1	14	0·02 ± 0·007 (4)	0·04 ± 0·01 (4)	$3·7\times10^{-6}$ ± $0·6\times10^{-6}$ (5)	$1·2\times10^{-6}$ ± $0·3\times10^{-6}$ (3)	$5·9\times10^{-7}$ ± $0·4\times10^{-7}$ (5)	I	$8·0\times10^{-7}$ (1)
126	5	0	18	0·11 ± 0·02 (5)	0·35 ± 0·025 (5)	$2·6\times10^{-5}$ ± $0·2\times10^{-5}$ (5)				$1·5\times10^{-7}$ ± $0·8\times10^{-7}$ (3)
155	3	2	18	0·4 ± 0·01 (4)	6·0 ± 0·9 (4)	$6·0\times10^{-6}$ ± $0·4\times10^{-6}$ (4)	$6·5\times10^{-5}$ ± $3·0\times10^{-5}$ (4)	$1·6\times10^{-5}$ ± $0·2\times10^{-5}$ (5)	0·4	
158	4	2	20	0·5 ± 0·0014 (4)	8·0 ± 1·2 (3)			$6·0\times10^{-6}$ ± $1·0\times10^{-6}$ (3)	0·3	

All the columns (except *n* and m) are as in Table 2. Figures for the compound PK–154 were obtained after the action of anticholinesterases.

* In the compound PK-142 the COO groups are reversed (see the structure in Fig. 4 or in Table 1).

Distance between the quaternary nitrogen atoms

Among the *bis*quaternary derivatives of terephthalic acid having two trimethylammonium cationic heads the compound PK-97, with an internitrogen chain containing sixteen atoms, and a distance between the nitrogens of about 20 Å, proved to be the most potent neuromuscular blocking agent (see Table 2 and Fig. 2). Shortening of the inter-nitrogen chain to fourteen and, especially, to twelve atoms

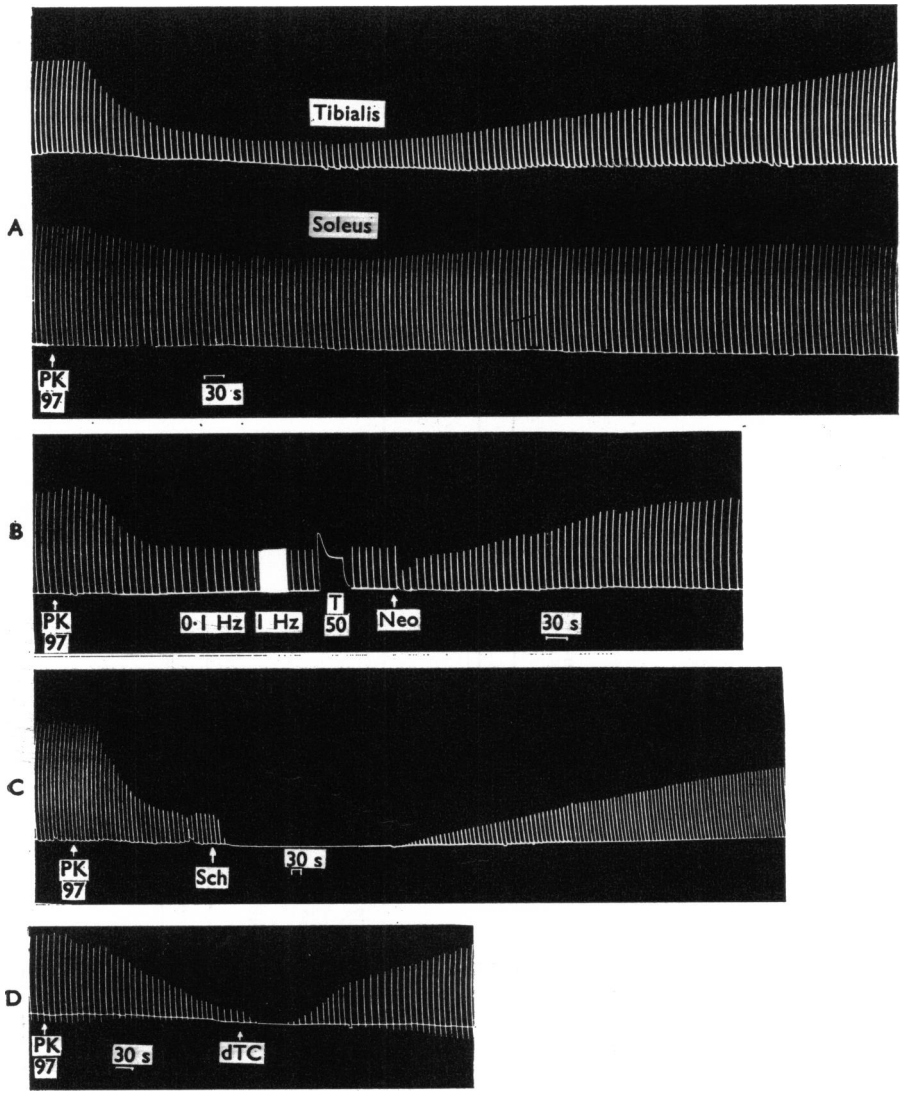
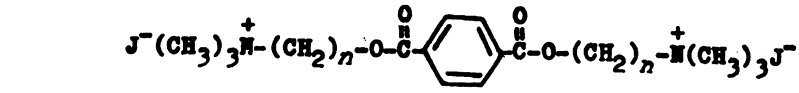


FIG. 1. The type of blocking action of the compound PK-97. Cat. A, Contractions of tibialis muscle (upper record) and soleus muscle (lower record). PK-97 (0·005 μmol/kg i.v.) produces a more pronounced block in the tibialis muscle. B, Tibialis muscle after prior injection of atropine, 5 mg/kg, intramuscularly. The blocking effect of PK-97 is not changed by the increase in the frequency of stimulation from 0·1 Hz to 1·0 Hz; tetanization at 50 Hz results in a well sustained contraction and in the post-tetanic period the level of the block is not changed; neostigmine (NEO) 0·1 mg/kg, intravenously does not antagonize the blocking action of PK-97. C, Succinylcholine (Sch) 0·05 μmol/kg increases the block induced by PK-97 in tibialis. D, (+)-Tubocurarine (TC) 0·3 μmol/kg produces a short increase of the block induced by PK-97 and then antagonizes this block in tibialis muscle.

(as in PK-95 and PK-107 respectively) or lengthening it to eighteen atoms (as in PK-126) resulted in a many fold decrease in potency. When one trimethylammonium group of the most potent compound PK-97 was replaced by a hydrogen atom (as in PK-119) the potency decreased 700 fold (Table 3). These results are consistent with the suggestion that a ' C-16 structure ' exists on the cholinoceptive membrane of the muscles studied and the interaction of the two cationic heads of PK-97 with the two anionic sites of neighbouring cholinoceptive units forming this structure seems indispensable for its action. The results in cats, rabbits, on the rat diaphragm and on the frog sartorius nerve-muscle preparation are all similar (Table 2) and the blocking potency on these preparations parallels their stimulant activity on the frog rectus abdominis muscle. On the chick biventer cervicis



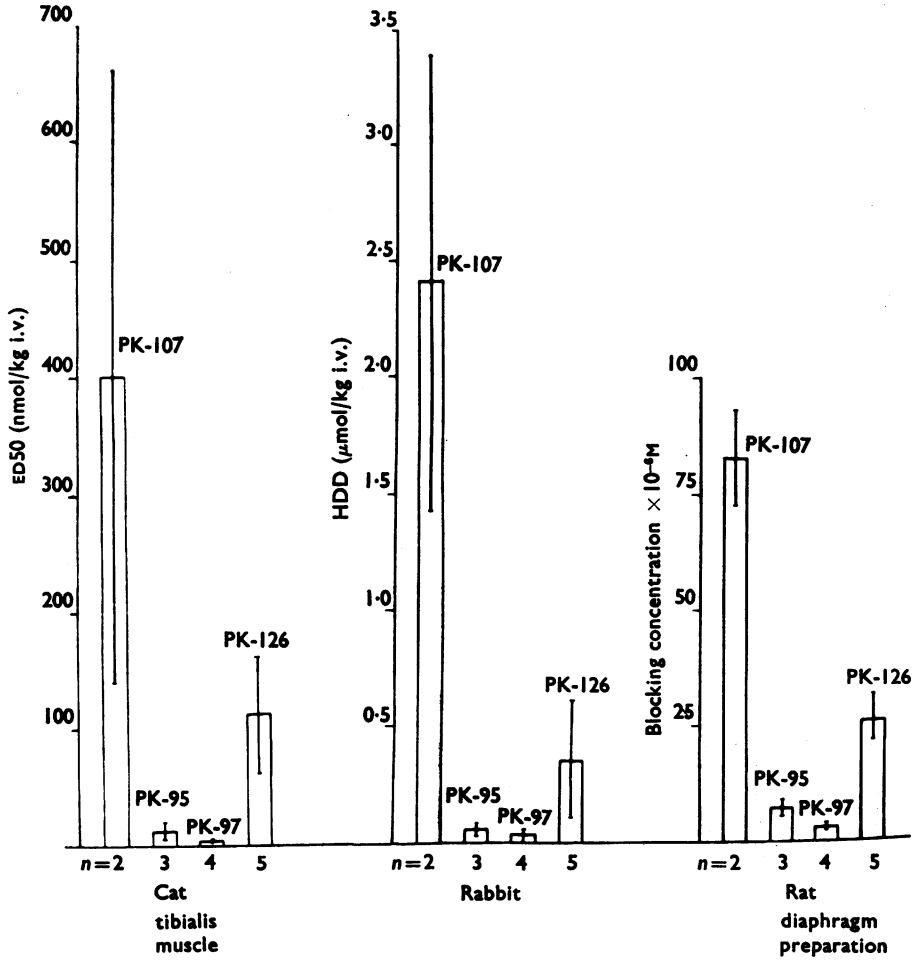


FIG. 2. Neuromuscular blocking action of the derivatives of terephthalic acid as a function of the internitrogen chain length. The height of the columns represents the blocking dose in cats (nmol/kg, i.v.) and rabbits (μmol/kg, i.v.) and the blocking concentrations ($\times 10^{-6}$M) in the rat diaphragm preparation. *n* is the number of methylene groups. Note that the compound PK-97, containing sixteen atoms in its internitrogen chain, is the most potent.

muscle, however, stimulant activity increases with chain length right up to the compound PK-126, with eighteen atoms between the trimethylammonium groups. Such variation in the optimal internitrogen distance is already known (Barlow & Zoller, 1964).

Derivatives of phthalic acid are generally far less potent than derivatives of terephthalic acid even when they have the same number of atoms between the cationic groups (Table 2). A possible explanation is that in phthalic acid derivatives the internitrogen chain cannot reach an extended conformation and the real distance between the quaternary nitrogens is less than in the analogous terephthalic derivatives.

Importance of the position of ester links in the internitrogen chain

When one trimethylammonium group in the compound PK-97 is replaced by a hydrogen atom (as in PK-119) the blocking potency is dramatically decreased: 700 fold in the cat and 400 fold in the rabbit (Table 3). With PK-141, the mononitrogen analogue of the compound PK-95, the decrease in potency is less pronounced (30–40 times). In monoquaternary analogues of carbolonium and of sebacoyldicholine however, activity is only reduced to between one-tenth and one-quarter of that of the analogous bisquaternary compounds (Rybolovlev, 1963; Danilov, 1966).

An important difference between the structures of these compounds is that in carbolonium and sebacoyldicholine the ester links are separated from the quaternary nitrogen atoms by two methylene groups (as in acetylcholine itself), whereas in PK-95 they are separated by three methylene groups and in PK-97 by four methylene groups. It seems possible, therefore, that carbolonium and sebacoyldicholine can

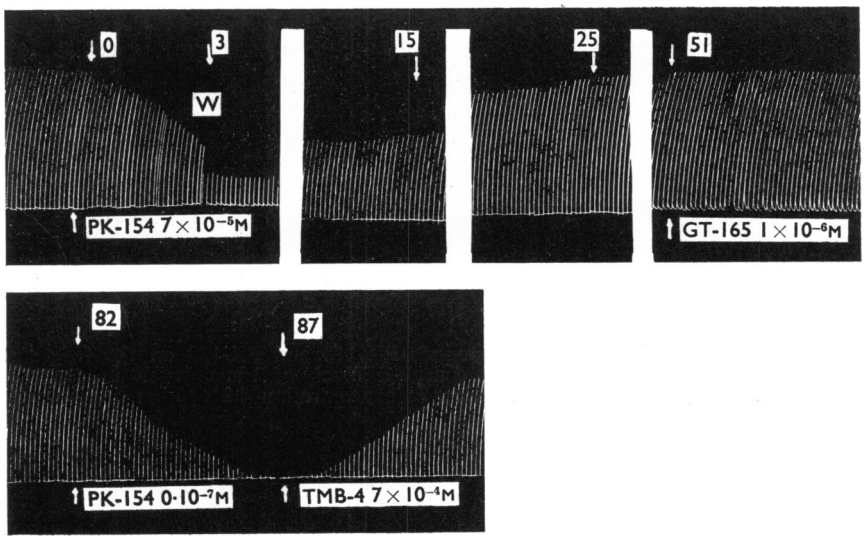


FIG. 3. The blocking action of the compound PK-154 on the rat diaphragm preparation before and after the inhibition of cholinesterase. After the action of the selective inhibitor of butyrylcholinesterase (compound GT-165) the blocking potency of the compound PK-154 increases 100 fold. Reactivation of cholinesterase by the compound TMB-4 quickly restores the transmission blocked by PK-154 (results obtained by L. L. Protas). The numbers above the trace show time in minutes. W—Wash.

interact with the 'C-16 structure' (see the scheme in the top of the Table 3) at four places: the two quaternary nitrogens react with the two anionic sites and the two ester groups react with the two esterophilic sites. In the compounds PK-95 and, especially in PK-97, the position of the ester links is 'wrong' and their interaction with the esterophilic sites of the C-16 structure is hindered (PK-95) or impossible (PK-97).

The monoquaternary analogues of carbolonium and sebacoyldicholine could still interact simultaneously with both cholinoceptive units which form the C-16 structure: with the two sites (anionic and esterophilic) of one receptor and with the esterophilic site of the other one (see the scheme in Table 3). On the other hand PK-119, the monoquaternary analogue of PK-97 can probably react only with one site of the C-16 structure (one anionic site) and is not more potent than tetramethylammonium (Table 3).

These results are understandable if we suppose that the C-16 structure is actually formed of two cholinoceptive units with their esterophilic sites facing each other (Rybolovlev, 1963; Khromov-Borisov & Michelson, 1966).

The results obtained with PK-154 are compatible with this idea. The internitrogen chain of PK-154 has the same length and the same components as the chain in PK-97, but in PK-154 the esteric links are in the 'right' position: the ether oxygens are separated from quaternary nitrogens by two methylene groups, just as in the acetylcholine molecule itself. Being an ester of choline with a dicarboxylic acid (see Brücke, 1956) the compound is readily hydrolysed by butyrylcholinesterase (Volkova, 1972) but after the inhibition of the enzyme it proved to be a potent neuromuscular blocking drug (Fig. 3). PK-154 is more active than PK-97 on the

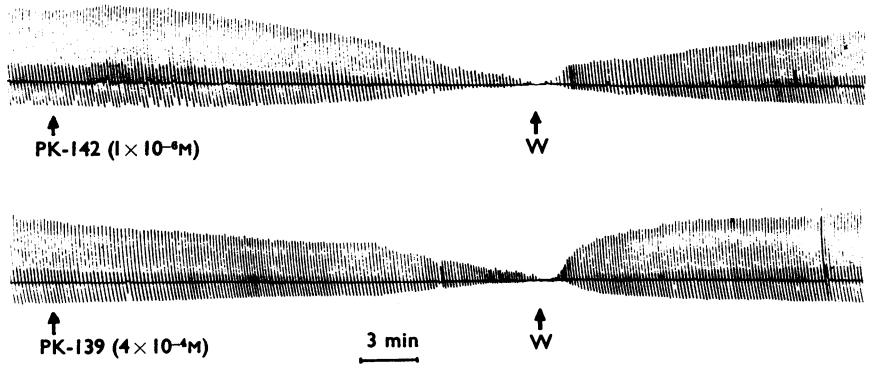
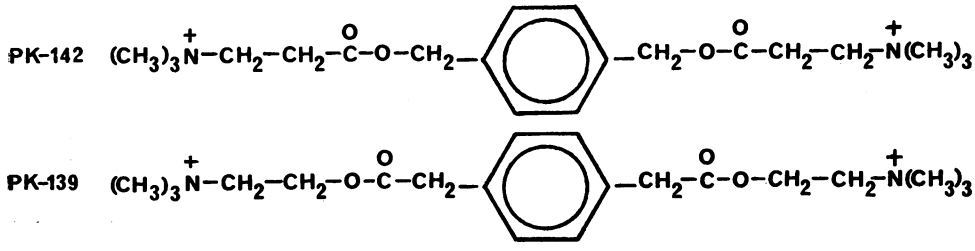


FIG. 4. The blocking action of the compounds PK-142 and PK-139 on the frog sartorius muscle preparation. PK-142 produces a complete block of transmission in concentration $1{\cdot}0\times10^{-6}$M; PK-139 produces the same effect in concentration $4{\cdot}0\times10^{-4}$M. W—wash.



rat diaphragm, on the frog sartorius muscle preparation, on the rectus abdominis muscle of the frog and on the chick biventer muscle (Table 4). Only in the cat is PK-154 less potent than PK-97. On the neurones of a mollusc *Limnaea stagnalis*, after the inhibition of cholinesterases, PK-154 is an order of magnitude more potent as a depolarizing agent than PK-97 (Ger, Zeimal & Kvitko, 1971).

General structure of the internitrogen chain

The changes in the potency of the compound studied can be ascribed not only to the changes in the distance between the cationic heads and in the position of the esteric links but also to the alterations in the flexibility of the molecule and its optimal conformations, in the density and distribution of charge, in the balance of hydrophilic and hydrophobic properties, in the degree of hydration and so forth (compare with Paton & Waud, 1962). For example one of the possible explanations of the extremely weak potency of the compounds PK-127 and PK-139, in which one methylene group is inserted between the carbonyl carbons and the ring (Table 4), is that it is due to severe steric hindrance preventing free rotation in the region of the ester links, which can be seen from atomic models (Ger *et al.*, 1971). The reversal of the ester groups in PK-139 makes free rotation possible (PK-142) and produced more than a 100 fold increase in potency (Table 4, Fig. 4). If the ester groups are attached directly to the ring (PK-97 and PK-95) or two methylene groups are inserted between the carbonyl groups and the ring (PK-154) there is no steric hindrance and all these compounds are very potent (Table 4).

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