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The neuromuscular blocking action of a series of bicyclic bis-onium esters

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1. Thirteen bicyclic dicholine esters have been tested on mammalian and avian skeletal muscle preparations.

2. One of the compounds exhibited depolarizing activity in all the preparations.

3. Three of the compounds exhibited depolarizing or dual-blocking activity in avian and denervated mammalian preparations, but exhibited non-depolarizing blocking activity in innervated mammalian preparations.

4. The remaining compounds exhibited non-depolarizing blocking activity with evidence of an additional facilitatory action.

5. The activity exhibited was dependent upon the onium substituents and the structure of the bicyclic ring.

6. All the compounds exhibited a choline-reversible block in the rapidly stimulated rat diaphragm preparation and six of them exhibited a secondary choline-reversible block of the rapidly stimulated cat tibialis anterior muscle.

Several dicholine esters of α, ω dicarboxylic acids containing cyclic structures have been synthesized and tested with the aim of producing a potent short-acting muscle relaxant without the depolarizing activity of suxamethonium (for reviews, see Bovet, 1951, 1959; Brücke, 1956). The experiments described in this paper were undertaken to investigate the neuromuscular blocking activity of a novel series of *bis*-β-trialkylammonium ethyl bicyclo [2.2.1] hept-2-ene, bicyclo [2.2.1] heptane and bicyclo [2.2.2] oct-2-ene, 5, 6 dicarboxylate diiodides and dibromides synthesized by Koch & Kotlan (1965). The formulae of the compounds are shown in Table 1.

Methods

The compounds were tested on the following preparations.

(*a*) The tibialis anterior muscles of cats anaesthetized with chloralose (80 mg/kg, injected intraperitoneally). Maximal twitches of a tibialis anterior muscle were elicited once every 10 sec by rectangular pulses (50 μsec duration) applied to the sciatic nerve. The strength of the shocks was about twice that required to evoke a maximal twitch. In some experiments maximal twitches of both tibialis anterior

muscles were elicited simultaneously; one was excited once every 10 sec, and the other once every sec through a 1:1 isolation transformer (Bowman & Rand, 1961: Bowman, Hemsworth & Rand, 1962). Drugs were injected intravenously or close-arterially by the technique of Brown (1938) as modified by Blaber (1960).

(*b*) Chronically denervated cat tibialis anterior and soleus muscles. The left sciatic nerves of cats were sectioned aseptically under pentobarbitone sodium anaesthesia and degeneration was allowed to proceed for 10–14 days. The cats were then anaesthetized with chloralose; muscle tension was recorded by means of an RCA 5734 mechano-electric transducer, and fibrillary potentials were recorded on an oscilloscope and counted as described by Bowman & Raper (1965). Drugs were injected close-arterially into the tibialis anterior or distant-arterially via the femoral artery.

(*c*) The tibialis anterior muscles of rabbits anaesthetized with urethane (6 ml/kg, 25% solution, injected intravenously). Maximal twitches of a tibialis anterior muscle were elicited once every 10 sec by rectangular pulses (100 μsec duration) applied to the peroneal nerve. Drugs were injected intravenously.

(*d*) The isolated phrenic nerve-hemidiaphragm of the rat (Bülbring, 1946). Both hemidiaphragms from the same rat were mounted together in Kreb-Henseleit solution at 32° C and of the following composition: (g/l.) NaCl 6.95, KCl 0.34, $CaCl_2$ 0.28, KH_2PO_4 0.162, $MgSO_4$ 0.294, $NaHCO_3$ 2.1, dextrose 2.0. The solution was continuously bubbled with 95% oxygen and 5% carbon dioxide. One muscle was excited once every sec and the other once every 10 sec (Bowman & Rand, 1961).

(*e*) Observations were made after intravenous injection of drugs into young conscious chicks (Buttle & Zaimis, 1949).

(*f*) The gastrocnemius muscles of adult domestic fowls anaesthetized with phenobarbitone sodium (200 mg/kg, injected intravenously). Maximal twitches of the lateral head of a gastrocnemius muscle were elicited once every 10 sec by rectangular pulses (50 μsec duration) applied to the sciatic nerve. Drugs were injected intravenously.

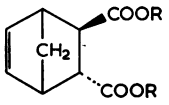
(*g*) The isolated chick semispinalis cervicis muscle preparation (Child & Zaimis, 1960). The semispinalis cervicis muscles of chicks aged 3 to 7 days were mounted in Tyrode solution at 40.5° C and of the following composition: (g/l.) NaCl 8.0, KCl 0.2, $CaCl_2$ 0.2, $MgCl_2$ 0.1, NaH_2PO_4 0.1, $NaHCO_3$ 1.0, dextrose 2.0. The solution was continuously bubbled with oxygen. The effects of the drugs under study were compared with responses produced by suxamethonium.

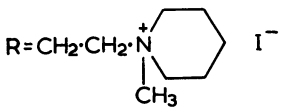
(*h*) The isolated chick biventer cervicis muscle preparation (Ginsborg & Warriner, 1960). The biventer cervicis muscles of chicks from 7 to 10 days old were dissected out and mounted in Krebs–Henseleit solution of the composition under (*d*) at 32° C; maximal twitches of the muscle were elicited by stimulation of the nerve within the tendon with rectangular pulses (0.5 msec duration).

The drugs used were: (+)-tubocurarine chloride (Duncan Flockhart), suxamethonium chloride (Allen & Hanburys), neostigmine methylsulphate and edrophonium chloride (Roche), phenobarbitone sodium (May & Baker), pentobarbitone sodium (Abbott), chloralose, urethane, atropine sulphate and choline chloride (British Drug Houses), and the compounds listed in Table 1. All doses refer to the salts which were dissolved in 0.9% w/v NaCl solution.

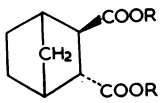
TABLE 1. *Structural formulae*

Series 1



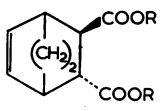
K1005	$R=CH_2CH_2\overset{+}{N}(CH_3)_3I^-$
K1009	$R=CH_2CH_2\overset{+}{N}(C_2H_5)_2CH_3\,I^-$
K1014	$R=\underset{\displaystyle CH_3}{\underset{\vert}{C}}HCH_2\overset{+}{N}(CH_3)_3\,I^-$
K1019	$R=CH_2CH_2\overset{+}{N}(CH_3)_2C_2H_5\,Br$
K1026	
K1030	$R=CH_2CH_2\overset{+}{N}(C_2H_5)_3\,Br^-$

Series 2



K1017	$R=CH_2CH_2\overset{+}{N}(CH_3)_3\,I^-$
K1033	$R=CH_2CH_2\overset{+}{N}(CH_3)_2C_2H_5\,Br^-$
K1034	$R=\underset{\displaystyle CH_3}{\underset{\vert}{C}}HCH_2\overset{+}{N}(CH_3)_3\,I^-$

Series 3



K1105	$R=CH_2CH_2\overset{+}{N}(CH_3)_3\,I^-$
K1112	$R=CH_2CH_2\overset{+}{N}(CH_3)_2C_2H_5Br^-$
K1106	$R=CH_2CH_2\overset{+}{N}(C_2H_5)_2CH_3Br^-$
K1115	$R=CH_2CH_2\overset{+}{N}(C_2H_5)_3\,I^-$

Results

The results obtained in conscious chicks showed that the compounds could be divided into three groups and their effects are described according to these groupings.

Compound K1005

In all the preparations used this compound produced effects entirely characteristic of a depolarizing drug. It produced contracture in the avian muscle preparations, and on the isolated chick semispinalis cervicis muscle preparation it acted additively with suxamethonium, its log dose/response line and that of suxamethonium not differing significantly from parallelism ($P=0.95$). In the cat tibialis anterior muscle preparation, K1005 increased the block produced by suxamethonium (Fig. 1), was

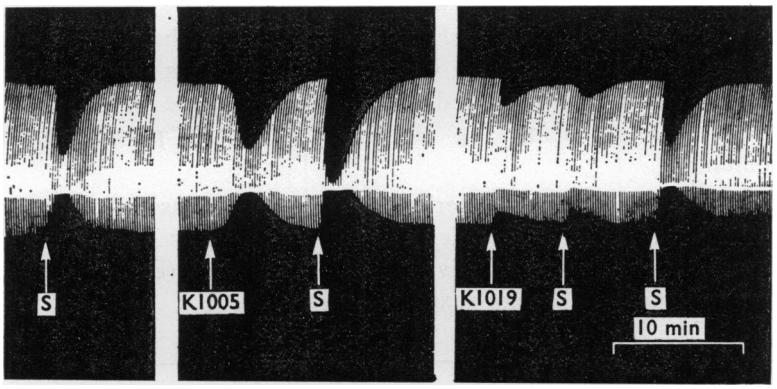


FIG. 1. Maximal twitches of the tibialis anterior muscle of a cat. Suxamethonium (40 μg/kg) was injected intravenously at S. The first panel shows one of a series of constant responses to this dose. K1005 (10 mg/kg) potentiated the response to the subsequent injection of suxamethonium, whereas K1019 (20 mg/kg) reduced the response to the subsequent injection of suxamethonium.

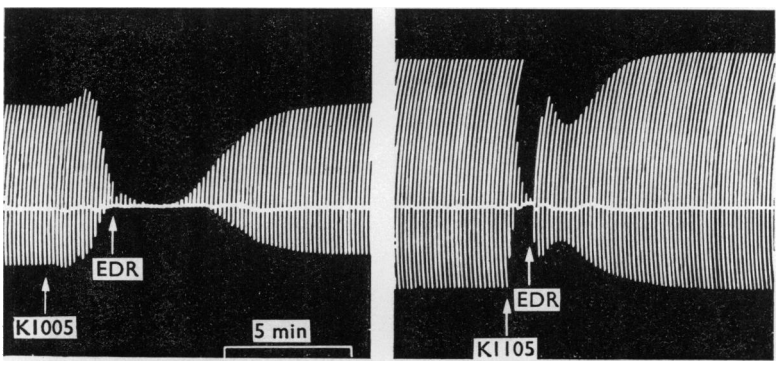


FIG. 2. Maximal twitches of the tibialis anterior muscle of a cat elicited once every 10 sec. K1005 (25 mg/kg) produced initial augmentation of twitch height followed by block which was not reversed by edrophonium (5 μg close-arterially). K1105 (50 mg/kg) produced immediate block without initial twitch potentiation and this block was reversed by edrophonium (5 μg close-arterially).

not antagonized by anticholinesterases (Fig. 2), and reversed block produced by tubocurarine (Fig. 3a).

The potency and duration of action of K1005 were assessed by direct comparison with suxamethonium at doses which caused 40–60% block of maximal twitch height (Table 2).

K1005 (200 μg i.a.) produced an increase in muscle tension and in the frequency of spontaneous potentials in denervated cat muscle.

Compounds K1017, K1019 and K1105

These compounds produced mixed effects characteristic of both depolarizing and non-depolarizing activity depending on the species and the tissue used. In the *in vivo* avian preparations used, the compounds produced an initial spastic paralysis or contracture, with a period of flaccid paralysis occurring before full recovery. In the adult anaesthetized fowl this was evidenced by a period of depression of maximal twitch height after the contracture, which was reversible by edrophonium. Subsequent equal doses produced diminishing contractures until the only effect produced was a decrease in twitch tension (Fig. 4). Surprisingly, results obtained in the isolated chick semispinalis muscle showed that on this tissue the compounds acted as agonists (Fig. 5), producing log dose/response lines parallel to that of suxamethonium.

On the cat and rabbit tibialis anterior muscle preparations the compounds produced effects characteristic of non-depolarizing drugs, being antagonized by anticholinesterases (Fig. 2), acting additively with tubocurarine (Fig. 3b) and reducing the response to a standard dose of suxamethonium (Fig. 1).

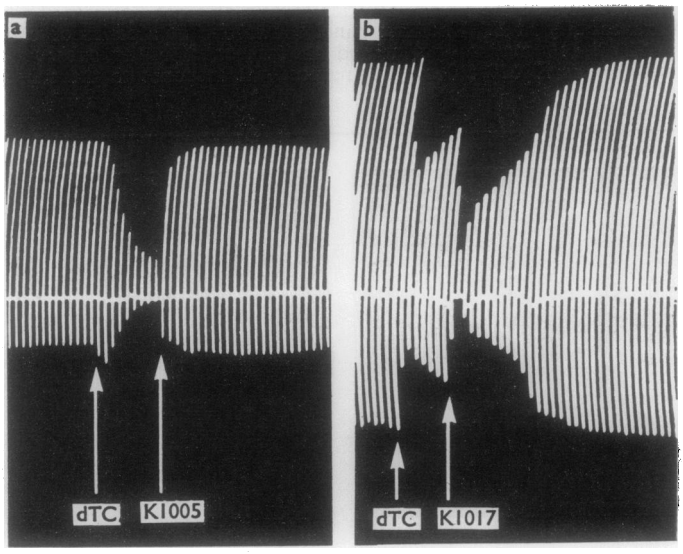


FIG. 3. Maximal twitches of the tibialis anterior muscles of cats. In (a), K1005 (0.4 mg close-arterially) reversed the block produced by tubocurarine 0.3 mg/kg (dTC, i.v.). In (b), the block produced by tubocurarine 50 μg (dTC) injected close-arterially was potentiated by K1017 (2 mg close-arterially).

The potencies and durations of action of the compounds were determined in the cat and rabbit by direct comparison with tubocurarine at doses which produced 40–60% block of maximal twitch height (Table 2).

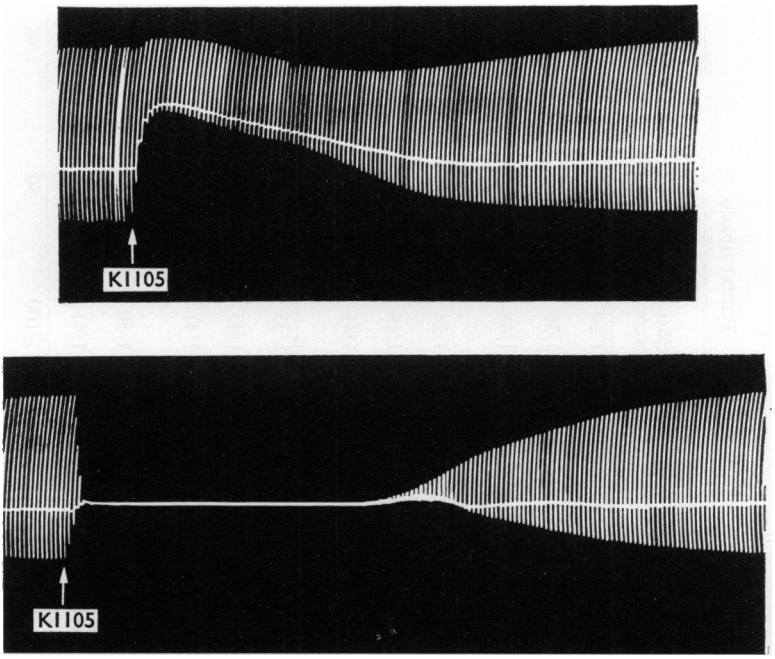


FIG. 4. Maximal twitches of the gastrocnemius muscle of a hen. Responses to the first and third of a series of doses of K1105 (20 mg/kg, i.v.). The first response is a large contracture with only a slight secondary depression of twitches, but by the third dose the contracture phase is absent, while the secondary depressant phase is dominant.

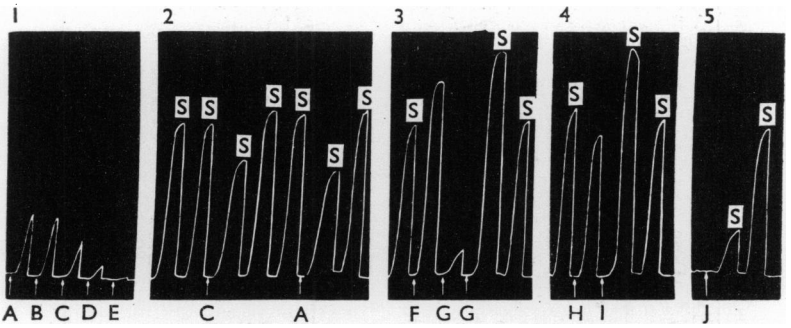


FIG. 5. Isolated chick semispinalis cervicis muscle. The contractions at S were produced by suxamethonium 0.15 μg/ml. added to the bath. At A, B, C, D and E, K1033 (200 μg/ml., 150 μg/ml., 100 μg/ml., 50 μg/ml. and 25 μg/ml. respectively) was added to the bath. In panel 2, C and A were added to the bath 15 sec before suxamethonium. Note the partial agonistic properties of K1033, shown by its production of submaximal contractures (panel 1) and inhibition of suxamethonium (panel 2). Panel 3 illustrates the agonistic action of K1017. At F and G, K1077 (10 μg/ml. and 2.5 μg/ml. respectively) was added to the bath. At the second G, K1077 (2.5 μg/ml.) was added to the bath 15 sec before suxamethonium. In panel 4 at H and I, K1005 (2.5 μg/ml. and 1 μg/ml., respectively) was added to the bath, I 15 sec before suxamethonium. Note the additive properties of K1017 and K1005 with suxamethonium. At J, K1112 (50 μg/ml.) was added to the bath 15 sec before suxamethonium. Note the reduction of the suxamethonium response, illustrating the antagonistic properties of K1112.

TABLE 2. *Potencies of compounds*

Compound	Conscious chick		Hen gastrocnemius			Rabbit tibialis		Cat tibialis	
	Effect (type of paralysis)	Dose (mg/kg)	Effect	Dose (mg/kg)	Standard for Comparison	Relative potency	Duration (% dTC or sux)	Relative potency	Duration (% dTC or sux)
K1005	Spastic	20	Contracture	0·5	Suxamethonium	1/20	120	1/330	150
K1009	Flaccid	20	—	—	(+)-Tubocurarine	1/36	52	1/40	17
K1014	Flaccid	20	—	—	(+)-Tubocurarine	1/48	67	1/36	57
K1019	Spastic/ flaccid	40	Contracture/ curare-like	5	(+)-Tubocurarine	1/24	35	1/65	20
K1026	Flaccid	20	—	—	(+)-Tubocurarine	1/19	53	1/15	20
K1030	Flaccid	20	—	—	(+)-Tubocurarine	1/41	37	1/40	29
K1017	Spastic/ flaccid	40	Contracture/ curare-like	5	(+)-Tubocurarine	1/35	35	1/150	15
K1033	Flaccid	20	—	—	(+)-Tubocurarine	1/43	29	1/60	10
K1034	Flaccid	20	—	—	(+)-Tubocurarine	1/41	50	1/40	58
K1105	Spastic/ flaccid	80	Contracture/ curare-like	20	(+)-Tubocurarine	1/50	30	1/130	15
K1112	Flaccid	20	—	—	(+)-Tubocurarine	1/41	44	1/40	11
K1106	Flaccid	20	—	—	(+)-Tubocurarine	1/100	50	1/96	30
K1115	Flaccid	20	—	—	(+)-Tubocurarine	1/100	55	1/48	33

In denervated cat muscles the compounds (1–2 mg. i.a.) produced an increase in the tension of the muscle and in the frequency of the spontaneous potentials.

Remaining compounds

The remaining compounds produced effects mainly characteristic of non-depolarizing blocking drugs, producing flaccid paralysis in conscious chicks.

With the exception of compound K1033, the results in conscious chicks were borne out on the isolated semispinalis cervicis muscle. Surprisingly, despite the absence of spastic paralysis in the chick, compound K1033 behaved as a partial agonist (Stephenson, 1956) on the isolated semispinalis and biventer cervicis muscles.

On the cat anterior tibialis muscle the responses to all the compounds were mainly characteristic of non-depolarization block, and in denervated cat muscle one of the

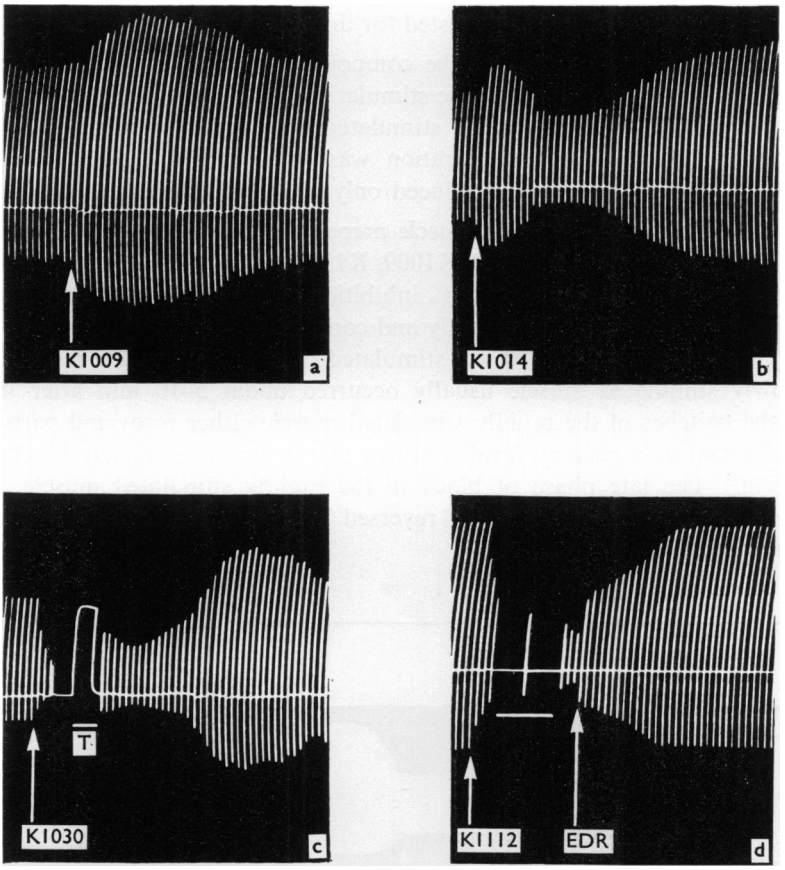


FIG. 6. Maximal twitches of the tibialis anterior muscles of cats. In (a) K1009 (10 mg/kg, i.v.) produced twitch potentiation only. In (b) K1014 (10 mg/kg, i.v.) produced twitch potentiation both before and after its blocking action. In (c) tetanus (50/sec for 5 sec) at T was maintained during the block produced by K1030 (10 mg/kg, i.v.). The block was nevertheless partially reversed by the tetanus, and post-blocking potentiation of twitch height was noted. In (d) edrophonium (5 μg close-arterially) at EDR weakly reversed the block produced by K1112 (10 mg/kg, i.v.). During the period marked by the horizontal bar the kymograph speed was increased to record a tetanus on the contralateral limb.

compounds (K1026, 4 mg i.a.) was without effect on the muscle tension or frequency of spontaneous potentials. Nevertheless, there was evidence of stimulant activity in the cat tibialis anterior muscle, as the twitch height was sometimes augmented by sub-blocking doses (Fig. 6a) and both before and after the block produced by larger doses (Fig. 6b). None of the compounds augmented twitch height in animals which had previously received tubocurarine. Although there was some relief of the block after a tetanus (50/sec for 5 sec), the tetanus did not wane during the period of repetitive stimulation.

The potencies and durations of action of these compounds were assessed by comparison with tubocurarine (Table 2).

Responses of rapidly stimulated preparations

A choline-reversible transmission failure produced selectively in a rapidly stimulated nerve-muscle preparation is an indication of a pre-junctional inhibitory action on acetylcholine synthesis (Reitzel & Long, 1959 ; Bowman, Hemsworth & Rand, 1967), and all the compounds were tested for this effect.

At doses of 100–150 μg/ml., all the compounds selectively blocked the isolated rat hemidiaphragm preparation (1/sec stimulation), while having no blocking action on the contralateral hemidiaphragm stimulated once every 10 sec. The block of the more rapidly stimulated preparation was reversed by choline (60 μg/ml.), whereas neostigmine (6 μg/ml.) produced only a transient reversal.

On the bilateral tibialis anterior muscle preparation of the cat, two distinct effects were noted. Large doses of K1030, K1009, K1014, K1034, K1106 and K1115 (4–10 times the dose required to produce 50% inhibition of maximal twitch height when the stimulation rate was 1/10 sec) abruptly and completely blocked the twitches of both rapidly (1/sec) and slowly (1/10 sec) stimulated muscles. Complete recovery of the more slowly stimulated muscle usually occurred about 5–10 min after injection, whereas the twitches of the rapidly stimulated muscle either recovered partially and became constant at a reduced level (Fig. 7a) or remained completely blocked for a long period. The late phase of block in the rapidly stimulated muscle, whether partial or complete, was permanently reversed by intravenous injection of 5 mg/kg of choline (Fig. 7a).

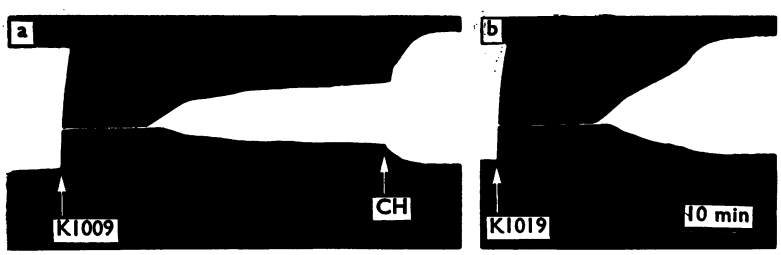
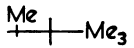
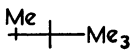
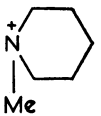


FIG. 7. Maximal twitches of the tibialis anterior muscles of cats stimulated once per second. In (a) K1009 (40 mg/kg) produced an immediate post-junctional block followed by a partial recovery. The second phase of block was reversed by choline (5 mg/kg, i.v.) at CH. In (b) K1019 (40 mg/kg) exhibited no secondary pre-junctional phase of block.

TABLE 3. *Qualitative effects of compounds in different test species*

Compound	Ring No.	N^+ subst.	Conscious chick	Hen gastrocnemius	Chick semispinalis	Rabbit tibialis	Cat tibialis	Smallest effective doses on rat diaphragm (μg/ml.)	Cat bilateral tibialis (l/sec)
K1005	1	Me_3	Depolarizer	Depolarizer	Depol.	Depolarizer	Depolarizer	Pre-junctional 150	Post-junctional
K1017	2	Me_3	Dual	Dual	Depol.	Curare-like	Curare-like	Pre-junctional 150	Post-junctional
K1105	3	Me_3	Dual	Dual	Depol.	Curare-like	Curare-like	Pre-junctional 100	Post-junctional
K1019	1	Me_2Et	Dual	Dual	Depol.	Curare-like	Curare-like	Pre-junctional 75	Post-junctional
K1033	2	Me_2Et	Curare-like	—	Dual	Curare-like	Curare-like	Pre-junctional 100	Post-junctional
K1112	3	Me_2Et	Curare-like	—	Non-depol.	Curare-like	Curare-like	Pre-junctional 100	Post-junctional
K1009	1	$MeEt_2$	Curare-like	—	Non-depol.	Curare-like	Curare-like	Pre-junctional 100	Pre-junctional
K1106	3	$MeEt_2$	Curare-like	—	Non-depol.	Curare-like	Curare-like	Pre-junctional 100	Pre-junctional
K1030	1	Et_3	Curare-like	—	Non-depol.	Curare-like	Curare-like	Pre-junctional 100	Pre-junctional
K1115	3	Et_3	Curare-like	—	Non-depol.	Curare-like	Curare-like	Pre-junctional 100	Pre-junctional
K1014	1		Curare-like	—	Non-depol.	Curare-like	Curare-like	Pre-junctional 150	Pre-junctional
K1034	2		Curare-like	—	Non-depol.	Curare-like	Curare-like	Pre-junctional 100	Pre-junctional
K1026	1		Curare-like	—	Non-depol.	Curare-like	Curare-like	Pre-junctional 100	Post-junctional

Similar biphasic blocks were produced by these compounds in animals which had been pretreated with neostigmine (0.1–0.4 mg/kg), indicating that the blocking effects were probably due to the parent compounds and not to products of hydrolysis by cholinesterase.

With the remaining compounds (K1005, K1017, K1105, K1019, K1033, K1112, K1026) recovery of both slowly and rapidly stimulated muscles of the cat occurred simultaneously and proceeded to complete recovery with no evidence of secondary pre-junctional activity (Fig. 7b).

The qualitative effects of all the compounds are summarized in Table 3.

Discussion

The results indicate that K1005 possesses depolarizing activity in all the preparations used, whereas compounds K1017, K1019 and K1105 act as depolarizing agents in the muscles of the domestic fowl which contain a large number of multiply-innervated fibres, and as non-depolarizing agents in mammalian muscle containing focally-innervated fibres. As far as the author is aware no other compounds have been reported to illustrate this pattern of species difference. The three compounds (K1017, K1019 and K1105) did produce evidence of depolarizing activity on denervated cat muscles, but tubocurarine and gallamine, both considered typical non-depolarizing blocking agents, also produce these effects in denervated muscles (McIntyre, King & Dunn, 1945; Jarcho, Berman, Eyzaguirre & Lilienthal, 1951; Luco & Sanchez, 1959; Bowman & Raper, 1964). Nevertheless, one of the non-depolarizing compounds from the series under test (K1026) exhibited no evidence of depolarizing activity even in denervated muscle, showing that not all non-depolarizing blocking drugs act in this way on denervated muscle.

The effects produced by the series of compounds studied, together with the known effects of other compounds active at the neuromuscular junction, indicate that a depolarizing component of drug action is more readily detectable in chronically denervated muscle than in multiply-innervated avian muscle, and least evident in innervated mammalian muscle. This is illustrated for a small group of drugs in Table 4.

The finding that some compounds produced evidence of depolarizing activity in avian but not in innervated mammalian muscle, however, may mean that different acetylcholine receptor configurations are present in the two types of muscle, and that the spread of acetylcholine receptors after denervation of mammalian muscle is accompanied by a change in the receptor configuration to one resembling that in avian muscle. Thus it is possible that some of the drugs under test (K1017, K1019

TABLE 4. *Responses of different types of muscle to neuromuscular blocking drugs*

	Chronically denervated	Avian	Innervated mammalian
K1005 or suxamethonium	+	+	+
K1017, K1019, K1105	+	+	−
Tubocurarine or gallamine	+	−	−
K1026	−	−	−

+, Stimulation of muscle. −, No stimulation of muscle.

and K1105) are able to approach the receptor sites more closely in avian and denervated muscle than in innervated muscle, and this may account for the depolarizing activity in the former.

The compounds described under the heading *Remaining compounds* possessed some stimulant action, although in the main they produced responses characteristic of non-depolarizing blocking drugs. It is possible that these compounds possess some anticholinesterase activity, which would account for the initial twitch augmentation, the weak antagonistic activity of edrophonium and the fact that during the block tetanic tension did not wane during the period of repetitive stimulation. The effect produced by any one compound possessing both neuromuscular blocking and facilitatory actions will depend on the size of the dose used (Blaber & Karczmar, 1967a, b) and the relative potency and duration of the two components of action. Thus, although some of the present series of compounds resembled the *bis*-quaternary oxamide ambenonium in producing twitch augmentation in low doses and neuromuscular block in higher doses (Karczmar, 1957; Blaber, 1960), they differed from this compound in being unable to reverse tubocurarine block. In this respect they resembled the blocking drug benzoquinonium, which also possesses anticholinesterase activity (Hoppe, 1951) but which lacks twitch augmenting properties (Bowman, 1958).

Table 3 shows that the changes in activity of the compounds correspond to changes in the size of the onium substituents; all the compounds with more than one ethyl substituent exhibited non-depolarizing blocking activity, whereas those with smaller substituents exhibited depolarizing, dual blocking or non-depolarizing activity, depending upon the type of bicyclic ring present. Thus ring 1, containing a double bond in the 2 position is preferential to ring 2, without the double bond, for depolarizing activity. Ring 3, containing an additional methylene group in the bridge, as well as a double bond in the 2 postion, exhibits the same activity as ring 2, indicating that the additional methylene group is nullifying the effect of the double bond. Both deviations from the structure of ring 1 increase the overall size of the ring system, which suggests that the lower depolarizing activity of the ring 2 and 3 derivatives may be due to stereochemical factors preventing simultaneous access of the onium and ester groups to the anionic and esteratic sites of the acetylcholine receptor surface (Ing, 1949; Lands, 1951).

Less specific drug-receptor interaction might also account for the production of non-depolarizing blocking activity on the introduction of β-methyl side chains in the N-trimethyl compounds, a property shared by succinyl-β-methylcholine (Vanderhaege, 1951; Beckett, 1961). The compounds of this type (K1014 and K1034) contain a centre of asymmetry in each of the choline moieties, with the possibility of four optically active isomers. These have not yet been optically resolved, but in view of Lesser's (1961, 1966) results with succinyl-β-methylcholine it seems unlikely that any of the isomers would exhibit qualitative differences in activity.

Bowman & Rand (1962) observed that pre-junctional blocking activity was more readily demonstrated on the rat diaphragm preparation than in the cat, and they suggested that this is because in general (but with the exception of tubocurarine) the rat muscle is less sensitive to post-junctional blockade which would mask any simultaneously occurring pre-junctional action. This difference between the two types of nerve-muscle preparation was also observed in the present experiments, all the compounds producing evidence of pre-junctional action in the rat muscle.

In the cat, however, the compounds produced such short-lasting post-junctional blockade that it is unlikely that the absence of evidence of a pre-junctional effect with some of the compounds was due to its being masked by the post-junctional action. More likely explanations of the greater susceptibility of the rat muscle to pre-junctional block may be first that, being an isolated preparation, it is deprived of plasma as a source of choline, and secondly that the inability of the isolated muscle to eliminate the compounds means that they may persist for a longer period in the region of the nerve endings.

The structural requirements of the onium substituents for producing pre-junctional block *in vivo* were similar to those in the series of choline analogues tested by Bowman & Rand (1962). That all the compounds tested exhibited some degree of pre-junctional activity, especially in the rat diaphragm, lends weight to the suggestion that most quaternary ammonium compounds, if tested under the correct conditions, may be shown to interfere with neuromuscular transmission by inhibition of acetylcholine synthesis in the nerve endings (Bowman & Hemsworth, 1965; Bowman, Hemsworth & Rand, 1967; Bhatnager & MacIntosh, 1967).

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