Br. J. Pharmac. Chemother. (1968), **32**, 624–634.

EFFECTS OF QUINURONIUM METHYLSULPHATE ON VOLUNTARY MUSCLE

BY

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(*Received November* 14, 1967)

Quinuronium methylsulphate is regarded as one of the most toxic chemotherapeutic substances in veterinary medicine. A dose of 1 mg/kg used in the treatment of babesiasis (piroplasmosis), induces profuse salivation, defaecation, micturition and dyspnoea. As long ago as 1935 these profound symptoms were said to be caused by " parasympathetic stimulation " (Cernaianu, Schuldner & Magureanu, 1935). The drug has been shown to inhibit cholinesterases *in vitro* and *in vivo* in many species (Rümmler & Laue, 1961 ; Eyre, 1966a), and most of the pharmacodynamic actions of quinuronium may be ascribed in whole or part to this activity (Eyre, 1967).

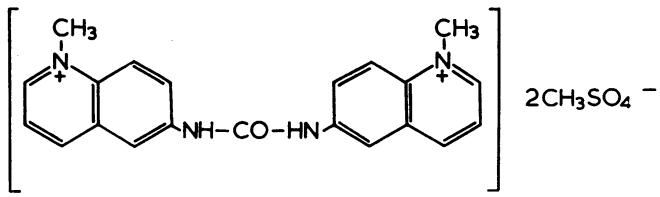
Kikuth (1935) compared some of these symptoms with " shock " but did not investigate the problem further. Quinuronium was shown later to release histamine in several species (Eyre, 1966b).

A number of antagonists has been investigated both pharmacologically and clinically: adrenaline (Cernaianu *et al.*, 1935 ; Kikuth, 1935 ; Kronfeld, 1959 ; Eyre, 1966c) ; mepyramine (Eyre, 1966c, 1967) ; pryidine aldoxime methiodide (Rümmler & Laue, 1961 ; Eyre, 1966a, 1966c), and atropine (Rümmler & Laue, 1961 ; Eyre, 1966a, 1966c ; Callow & Mellors, 1966). While no antagonist may be regarded as ideal, atropine appears to be the antidote of choice (Eyre, 1966c).

Higher doses of quinuronium (>1–2 mg/kg) cause, in addition to the signs described, severe dyspnoea or apnoea, muscular tremors and incoordination, cyanosis and collapse, sometimes followed by death. Kronfeld (1959) reported a " respiratory " death and showed that quinuronium depressed cellular oxygen metabolism. No antidote was of any value in preventing or treating these profound symptoms (Kronfeld, 1959 ; Eyre, 1967).

The methyl substituted bis-quarternary nature of the drug, coupled with the symptoms of apnoea, and effects on skeletal muscles suggested that the drug might affect neuromuscular transmission (Paton & Zaimis, 1949 ; Barlow, 1964). This possibility has now been investigated.

The results of preliminary investigations were communicated to the British Pharmacological Society at Dundee, July 6, 1966.



Quinuronium methylsulphate

METHODS

Isolated rat phrenic nerve-diaphragm

The preparation was as described by Bülbring (1946), using the modified electrode of Cooper & Marshall (1962) to record the responses to direct and indirect stimulation. A bath of 50 ml. capacity at 35° C was used, containing Krebs–Henseleit solution of the following composition (moles/l.): Na 144, K 5.9, Ca 2.54, Mg 1.2, Cl 128.3, H_2PO_4 1.2, HCO_3 24.9, SO_4 1.2, glucose 10 (Krebs & Henseleit, 1932). The solution was gassed with 5% carbon dioxide in oxygen and the muscle stimulated supramaximally with 8 rectangular impulses/min either directly or through the nerve. Isometric contractions were recorded kymographically. In order to observe the effects of adding calcium and potassium, the concentrations of the two ions were doubled (K 11.8 and Ca 5.08 moles/l.).

Quantitative comparison of quinuronium and tubocurarine

By adding a drug in dose increments to the organ bath in which the preparation was stimulated indirectly, three or four levels of steady partial neuromuscular block were obtained between 20% and 80% (Blackman & Ray, 1964). The log molar concentration response curves were constructed for tubocurarine and quinuronium and were parallel straight lines. The molar concentration of each drug which produced 50% neuromuscular block (EC50) could be estimated graphically.

Isolated chick biventer cervicis nerve-muscle preparation

A preparation of the biventer muscle was made as described by Ginsborg & Warriner (1960) in the Krebs–Henseleit solution gassed with 5% carbon dioxide in oxygen. The nerve-tendon was stimulated with 8 rectangular pulses/min and semi-isometric contractions were recorded kymographically.

Isolated chick semispinalis muscle

This isolated muscle (obtained from chicks less than 7 days old) was set up in Krebs–Henseleit solution in the manner of Child & Zaimis (1960) in a 5 ml. organ bath modified from that described by Bennett (1964). Isotonic contractions were recorded kymographically.

In vivo, peroneal nerve—digital extensor muscle group of the rabbit

Experiments were performed on eight rabbits weighing between 1.5 and 3 kg, of mixed breed and sex, anaesthetized with urethane.

The muscles used in this preparation were the dorso-lateral group of the pelvic limb comprising the long digital extensor and the anterior tibial muscle, whose combined function is to extend the digits and flex the hock joint so causing a forward kick of the foot. The motor nerve supply is the peroneal nerve which lies in a subcutaneous position at the lateral aspect of the tibio-tarsal joint. The apparatus was described by Eyre & Goff (1967).

The nerve was ligated centrally and was stimulated with 8 rectangular pulses/min at approximately 10 V. Muscle twitches were recorded with a flat steel spring lever by means of a thread attached to the foot.

Carotid blood pressure was recorded kymographically with a Condon manometer, and respiratory movements were taken from a tracheal cannula with a simple bellows recorder. Drugs were administered intravenously.

The drugs used were tubocurarine chloride; acetylcholine chloride; decamethonium iodide; physostigmine (eserine) salicylate and quinuronium methylsulphate. The doses quoted in the text refer to the salts, which were dissolved in 0.15 M sodium chloride for intravenous injection and otherwise in distilled water.

RESULTS

Isolated rat phrenic nerve—diaphragm

Quinuronium (10^{-6}–10^{-5} M) caused an increase in the twitch response of the indirectly stimulated rat diaphragm. Concentrations greater than 10^{-5} M caused neuromuscular block, which was sometimes preceded by augmentation of the twitch (Fig. 1a).

Figure 1b shows the actions of quinuronium on a rat diaphragm–phrenic nerve stimulated with four shocks alternately through the nerve and directly. Quinuronium 3×10^{-4} M caused partial neuromuscular block. The responses to direct stimulation were only slightly diminished.

The preparation returned to normal after washing, but it was difficult to repeat the augmentation of the indirect twitch response in the same tissue. After approximately 1 hr of repeated washing the twitch potentiation could be demonstrated again, but less markedly.

In a fresh preparation, the addition of quinuronium 10^{-5} M during a partial neuromuscular block by tubocurarine 10^{-6} M partially relieved the block (Fig. 1c). Raising the concentration of quinuronium to 5×10^{-5} M and 1×10^{-4} M in the presence of tubocurarine produced further neuromuscular block. The concentration of quinuronium required to produce an "anti-curare" action was a threshold or sub-threshold blocking concentration of between 10^{-6} and 10^{-5} M.

Stimulation of the nerve with 60 shocks/sec for 10 sec during steady partial neuromuscular block by quinuronium produced a contraction of the muscle followed by a brief period of slightly enhanced indirect twitch response; this effect was similar to but smaller than the effect of a tetanus during block produced by tubocurarine.

The addition of eserine 7.5×10^{-6} M during partial block by quinuronium did not reverse the block, whereas doubling the concentration of potassium (Wilson & Wright, 1937) to 11.8×10^{-3} M or calcium to 5.08×10^{-3} M in the Krebs-Henseleit solution produced reversal of the neuromuscular block elicited by both tubocurarine and quinuronium (Fig. 1d).

Quantitative comparison of quinuronium and tubocurarine

Quinuronium and tubocurarine were compared by estimating the EC50 of each graphically. In the case of quinuronium the initial twitch height before drug application was taken as the control, the increased twitch response at sub-threshold blocking concentrations being disregarded. This may have had the effect of underestimating slightly the potency of quinuronium in blocking the preparation. The respective molar EC50s were tubocurarine 1.80×10^{-6} M and quinuronium 1.45×10^{-4} M.

Isolated chick biventer cervicis nerve-muscle preparation

Quinuronium (approximately 10^{-6} M) occasionally produced a very small increase in twitch height of the biventer muscle. Quinuronium concentrations higher than 10^{-6} M

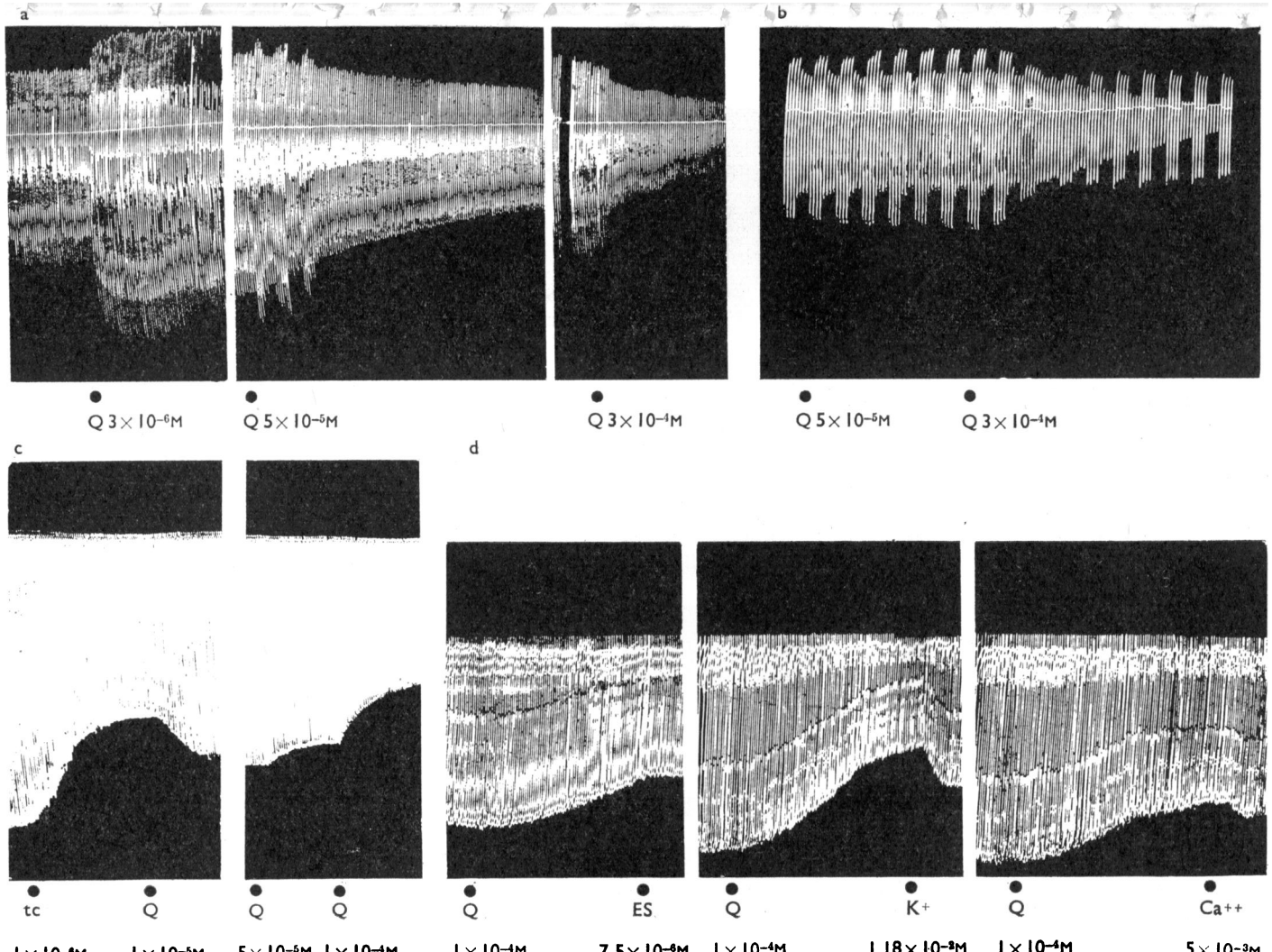


Fig. 1. Rat phrenic nerve-diaphragm in Krebs–Henseleit solution at 35° C. (a) Indirect stimulation with 8 shocks/min at 4 V intensity. Effect of quinuronium (Q) 3×10^{-6} M, 5×10^{-5} M and 3×10^{-4} M. (b) Diaphragm stimulated (8/min at 4 V) with 4 shocks alternately through the nerve (5 V stimulus, greater twitch height) and directly (50 V stimulus, lesser twitch height). Effect of quinuronium (Q) 5×10^{-5} M and 3×10^{-4} M. (c) Indirect stimulation (8/min at 4 V). Effect of increasing concentration of quinuronium (Q) 1×10^{-5} M, 5×10^{-5} M and 1×10^{-4} M on the blocking action of *d*-tubocurarine (tc) 1×10^{-6} M. (d) Indirect stimulation (8/min at 4 V). Effect of eserine (ES) 7.5×10^{-6} M, potassium (K^+) 1.18×10^{-2} M and calcium (Ca^{++}) 5×10^{-3} M on the blocking action of quinuronium (Q) 1×10^{-4} M.

produced neuromuscular block which was indistinguishable from that produced by a comparable concentration of tubocurarine (Fig. 2a and b). Quinuronium 10^{-5} M antagonized the contraction of the biventer muscle produced by decamethonium 5×10^{-7} M (Fig. 2c and d), but in contrast to the effect on the rat diaphragm, showed no tendency to relieve the steady partial neuromuscular block produced by tubocurarine (Fig. 2b). Again in contrast to the effect in the rat diaphragm, quinuronium block was partially antagonized by eserine 10^{-5} M (Fig. 2e).

A comparison of the EC50s of tubocurarine and quinuronium revealed that in this preparation the relative values were tubocurarine 2.1×10^{-6} M and quinuronium 6.6×10^{-6} M ; this shows that quinuronium is a much more potent neuromuscular blocking agent in the chicken than in the rat.

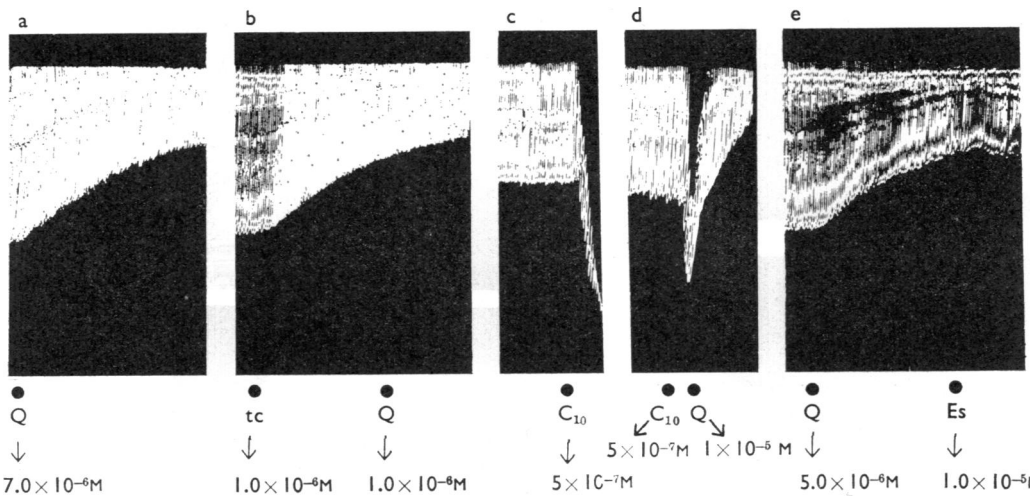


Fig. 2. Chicken biventer cervicis nerve muscle preparation in Krebs-Henseleit solution at 35° C. Indirect stimulation with 8 shocks/min at 4 V intensity. (a) Effect of quinuronium (Q) 7.0×10^{-6} M. (b) Effect of quinuronium 1×10^{-6} M on the blocking action of tubocurarine (tc) 1.0×10^{-6} M. (c) Effect of decamethonium (C10) 5×10^{-7} M. (d) Effect of quinuronium 1.0×10^{-5} M on the blocking action of decamethonium 5×10^{-7} M. (e) Effect of eserine (Es) 1.0×10^{-5} M on the blocking action of quinuronium 5×10^{-6} M.

Isolated chick semispinalis muscle

Quinuronium did not produce contracture of this muscle at any concentration, but inhibited the contractures induced by nicotine, decamethonium, suxamethonium, or acetylcholine in the presence of eserine. Figure 3 shows the inhibition by quinuronium (2, 4 and 6 μg) of contractures of the semispinalis muscle induced by decamethonium 0.2 μg.

In vivo *peroneal nerve–digital extensor muscle group of the rabbit*

Intravenous injections of quinuronium sulphate produced changes in neuromuscular transmission, blood pressure and respiratory volume. Quinuronium (250 μg/kg) caused

a marked augmentation of the extensor muscle twitch, and a fall in carotid blood pressure, which persisted for several minutes. Quinuronium (750 μg/kg) caused a similar change in blood pressure, a smaller increase in the muscle twitch and a transient reduction in respiratory volume (Fig. 4a).

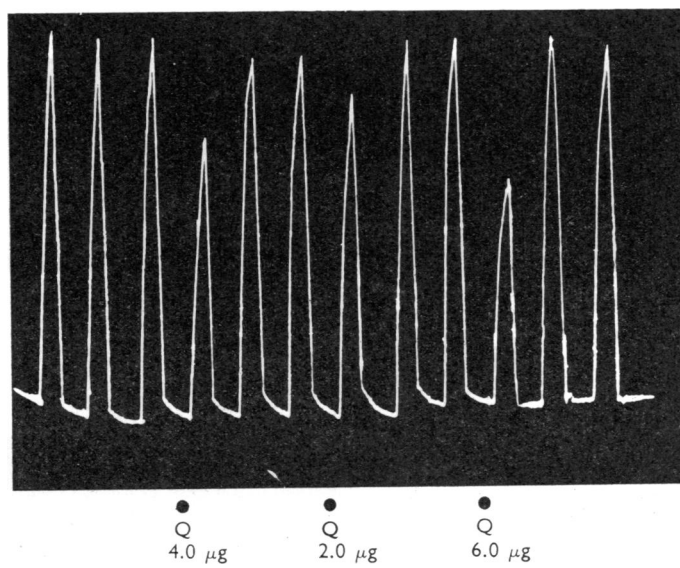


Fig. 3. Contractions of the chicken semispinalis muscle in Krebs-Henseleit solution at 35° C produced by decamethonium 0.20 μg added to the bath for 1 min every 5 min. Effect of adding quinuronium (Q) 4, 2 and 6 μg, 2 min before decamethonium at the points indicated.

Atropine (1 mg/kg) was given, because this drug had previously been shown to protect from the more severe effects of quinuronium at higher doses (Eyre, 1967). Quinuronium (1–1.5 mg/kg) given intravenously to the atropinized rabbit gave rise to transient partial neuromuscular block, produced total apnoea for a few minutes, and gave a biphasic or triphasic rise in the blood pressure (Fig. 4a). Tubocurarine 200–300 μg/kg produced approximately 50% neuromuscular block in the extensor muscles and caused a comparable reduction in respiratory volume. The injection of quinuronium 250 μg/kg during the tubocurarine effects caused immediate relief of the neuromuscular block and respiratory inhibition, and produced a large fall in blood pressure (Fig. 4b).

A therapeutic dose of quinuronium (up to 2 mg/kg) given either subcutaneously or intramuscularly produced augmentation of the twitch responses and hyperpnoea within 2–3 min. Thereafter the twitch height decreased and respiration was more shallow. There was a progressive fall in blood pressure by about 50% (Fig. 5).

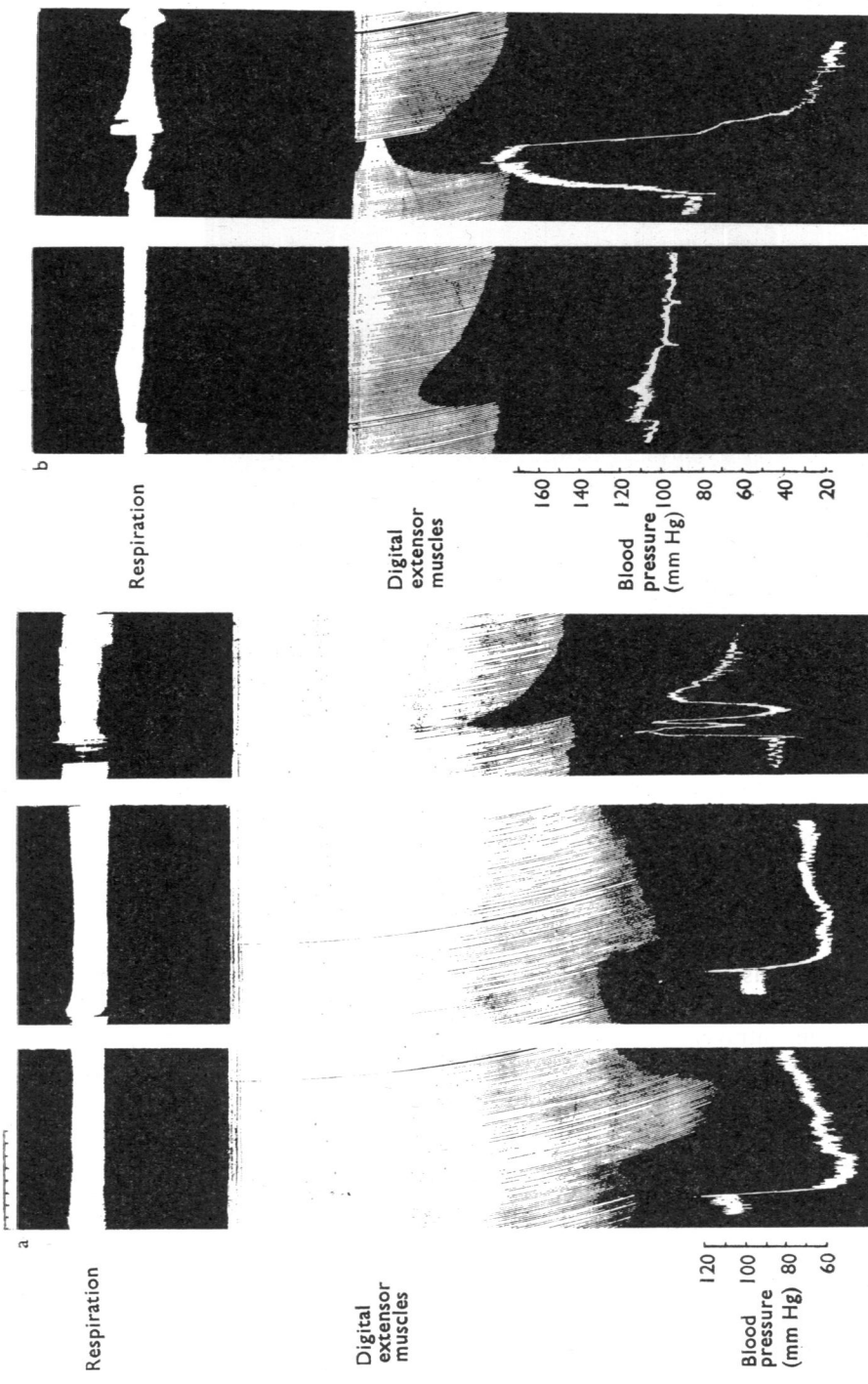


Fig. 4. Peroneal nerve digital extensor muscle preparation of two rabbits. Urethane anaesthesia. Indirect stimulation 8 shocks/min at 10 V. Top tracing: pulmonary ventilation. Middle tracing: maximal twitches of digital extensor muscles. Bottom tracing: carotid blood pressure. Time scale, 1 min. (a) Effect of intravenous injections of quinuronium (Q) 250 μg/kg, 750 μg/kg and 1.5 mg/kg. At A intravenous atropine 1 mg/kg. (b) Effect of intravenous injection of tubocurarine (tc) 200 μg/kg and the effect of quinuronium (Q) 250 μg/kg on the action of tubocurarine 300 μg/kg.

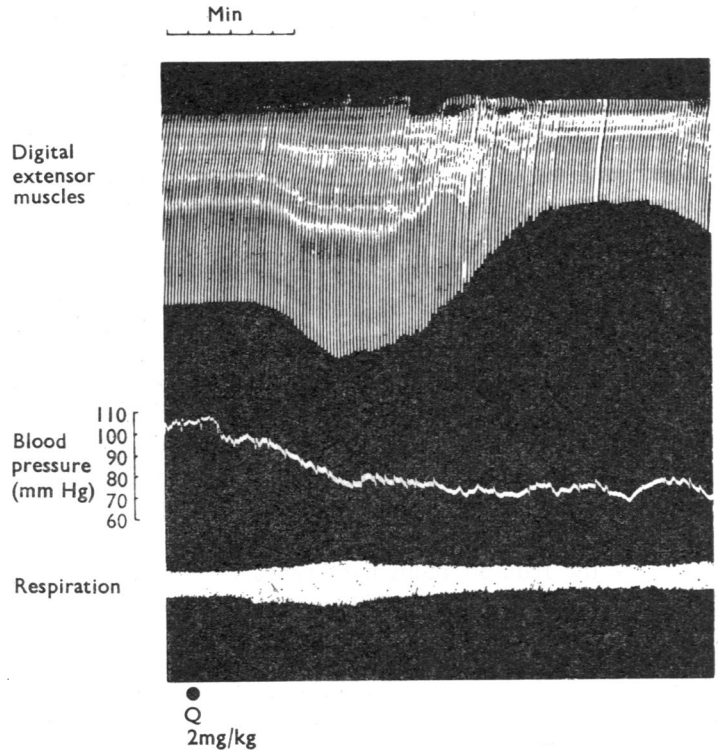


Fig. 5. Peroneal nerve digital extensor muscle preparation of a rabbit. Urethane anaesthesia. Indirect stimulation 8 shocks/min at 6 V. Top tracing, maximal twitches of extensor muscles. Middle tracing, carotid blood pressure. Bottom tracing, respiratory ventilation. Quinuronium (Q) 2 mg/kg injected subcutaneously at the point indicated.

DISCUSSION

Quinuronium sulphate in small concentrations showed signs of anticholinesterase activity (Eyre, 1966a) by augmenting the muscle contractions and antagonizing tubocurarine in mammalian skeletal nerve-muscle preparations. Increasing the concentration of quinuronium produced neuromuscular block which could not be antagonized by anticholinesterases such as eserine or neostigmine.

The curare-like activity of quinuronium in mammals is clearly sufficient to produce blockade by overcoming its own anticholinesterase effect at the neuromuscular junction, and the anticholinesterase activity of quinuronium explains the inability of eserine to antagonize the neuromuscular block produced by the babesicide, because if cholinesterase activity is already inhibited by quinuronium, eserine could not produce any further action.

Quinuronium did not readily augment neuromuscular transmission in the chick biventer muscle and had no " anti-curare " action. Moreover the drug was a more powerful blocking agent in avian than in mammalian muscles, and its action was more readily antagonized by eserine in the bird. This suggests that quinuronium has a smaller anticholinesterase action in this species. In fact quinuronium has been shown to have a lower

affinity for the chicken cholinesterases than for those of the rat and other mammals (Eyre, 1966a).

The present observations agree with those described by Bowman (1958, 1964) who showed that a number of "bis-onium" compounds, including benzoquinonium and ambenonium had little affinity for chicken cholinesterases compared with mammalian (cat); whereas there was no difference with "mono-basic" compounds (for example, eserine and neostigmine). Bowman postulated that chicken cholinesterases, because of differences in molecular structural configuration, combine less readily with compounds possessing two quaternary nitrogen groups.

The results obtained are characteristic of a non-depolarizing competitive neuromuscular blocking compound, the action of which was similar to that of tubocurarine.

The measurement of neuromuscular blocking activity and respiratory ventilation in the anaesthetized rabbit did not suggest any cause and effect relationship between neuromusclar block and "respiratory" symptoms during quinuronium "treatment." It was interesting to note that intravenous quinuronium 750 μg/kg caused a transient 50% reduction in ventilation simultaneously with marked augmentation of the twitch height in the skeletal muscles. This effect could presumably be a manifestation of cholinesterase inhibition and the reduced pulmonary ventilation may be the result either of central inhibition of respiration (Kronfeld, 1959) or of broncho-constriction in the absence of atropine (Eyre, 1967). Therapeutic doses of quinuronium (1.5 mg/kg) when administered intravenously gave rise to partial neuromuscular block (approximately 30–40%) together with apnoea lasting several minutes. Doses of tubocurarine which caused a comparable degree of neuromuscular block reduced the pulmonary ventilation only slightly. The results are consistent with the idea that the the respiratory depression produced by tubocurarine is largely (though not necessarily entirely) a result of neuromuscular block in skeletal muscles, whereas the apnoea caused by intravenous quinuronium in the rabbit is probably caused by factors distinct from neuromuscular block. This idea is further supported by the fact that injections of quinuronium by either the subcutaneous or the intramuscular route produced different responses from those given intravenously. The intravenous route was always followed by a period of tachypnoea or more usually apnoea which was consistent with previous findings in anaesthetized sheep (Eyre, 1967). Subcutaneous dosing produced hyperpnoea, however, which was sometimes followed by slight tachypnoea. The respiratory rate (which is not shown in Fig. 5) was increased by quinuronium. These findings are in agreement with the dyspnoea, hyperventilation and skeletal muscular incoordination shown by treated conscious sheep (Eyre, 1966c).

The differences shown by quinuronium when administered by different routes might be caused by the corresponding differences in plasma concentrations: the intravenous route, for example, produces a high blood level for a short period, in contrast to the subcutaneous route after which plasma concentrations would be comparatively low.

There seems little doubt that quinuronium acts as a competitive neuromuscular blocking agent in the preparations tested. The dose of quinuronium currently used in the therapy of animal babesiosis (1–2 mg/kg) produces effects on skeletal musculature which are sufficient to account for the symptoms of muscle fasciculation and skeletal incoordination in the treated animals, but are unlikely to be the main cause of the respiratory symptoms of quinuronium intoxication.

SUMMARY

1. Quinuronium methylsulphate caused augmentation of the twitch response, followed by neuromuscular block in the isolated rat phrenic nerve-diaphragm. The response to direct stimulation was unaffected.

2. Blockade was antagonized by tetanic stimulation, potassium and calcium.

3. Quinuronium showed no depolarizing action on the biventer or semispinalis muscles of the chicken, but had actions indistinguishable from those of tubocurarine.

4. The babesicide caused neuromuscular block and respiratory inhibition in the anaesthetized rabbit. It is suggested that the interference with neuromuscular transmission may account for the symptoms of muscle incoordination in animals injected with quinuronium therapeutically. Respiratory inhibition is probably caused chiefly by factors other than neuromuscular block.

I wish to thank Dr. F. Alexander for facilities and encouragement and Mr. David Goff for valuable technical assistance. I am grateful also to the following for generous gifts of quinuronium sulphate: Baywood Chemicals Ltd., Bury St. Edmunds, for Ludobal; Evans Medical Ltd., Liverpool, for Pirevan; I.C.I. Ltd., Macclesfield, for Babesan.

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