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ACTIONS OF HEMICHOLINIUM (HC-3) ON NEUROMUSCULAR TRANSMISSION

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Hemicholinium No. 3 (HC-3 ; α,α′-dimethylaminoethanol-4, 4′-biacetophenone) produced neuromuscular block of the tibialis anterior muscle-sciatic nerve preparation of the anaesthetized cat which was of slow onset, of long duration and dependent on the nerve stimulus frequency. The failure of the compound to modify the response of the tibialis muscle to close-arterial injections of acetylcholine suggested that the sensitivity of the motor endplate was not changed by it. The neuromuscular block produced by hemicholinium was antagonized by choline but only partly relieved by anticholinesterase drugs. The response to tetanic stimulation of the nerve during the neuromuscular block was well sustained and was followed by a slight posttetanic potentiation. The neuromuscular blocking action of hemicholinium could be relieved by temporary suspension of stimulation. These results suggest that the mode of action of hemicholinium at the neuromuscular junction is different from that of tubocurarine, and indicate that the action of hemicholinium is presynaptic, probably arising from a reduction in acetylcholine released by nerve stimulation.

Hemicholinium No. 3 (HC-3 ; α,α′-dimethylaminoethanol-4, 4′-biacetophenone), first described by Long & Schueler (1954) and Schueler (1955), produces neuromuscular block in the rabbit, dog and chicken of slow onset and of long duration, which depends on the frequency of nerve stimulation (Long & Reitzel, 1958 ; Reitzel & Long, 1959a ; Wilson & Long, 1959).

This paper describes the effects of hemicholinium on neuromuscular transmission in the cat tibialis anterior muscle-sciatic nerve preparation. A preliminary report of these findings has been made (Evans & Wilson, 1962).

During the course of this work some actions of hemicholinium on the tibialis anterior and soleus muscles of the cat were reported by Bowman & Rand (1961).

METHODS

Cats (2 to 5 kg of body weight) were anaesthetized with pentobarbitone sodium (35 mg/kg) intraperitoneally. Both hind-limbs were fixed horizontally on a Brown-Schuster myograph by steel drills through the lower ends of the femur and tibia. The tendon of each tibialis anterior muscle was attached to a flat steel spring and the movements were recorded on smoked paper by levers giving a tenfold magnification. The muscle was bathed continuously in warm liquid paraffin (B.P.). It was stimulated indirectly by shielded platinum electrodes placed on the lateral branch of the distal stump of the cut sciatic nerve. The stimuli were rectangular pulses of 0.05 msec duration and of twice the intensity required to produce a maximal twitch.

Stimulation of both innervated and chronically denervated muscles was achieved through fine platinum wires passed through the belly of the muscle at right angles to its long axis; the drill through the lower end of the femur acted as the earth point (Irwin & Wells, 1959). The platinum wires did not impair the ability of the muscle to contract because the response of innervated muscles to sciatic nerve stimulation was unchanged after their insertion. The direct muscle stimuli were supramaximal and of 0.1 msec duration for innervated and 0.4 msec for denervated muscles. Denervated muscles were prepared by aseptic section of the sciatic nerve during sodium pentobarbitone anaesthesia, 14 to 15 days previously.

Arterial blood pressure was recorded from a carotid artery by means of a mercury manometer after section of both vagus nerves in the neck.

Hemicholinium was prepared for injection by dissolving it in 0.9% saline of *p*H 5.3, and by adjusting the concentration so that the required dose was contained in 1 ml. The solution was injected intravenously over a period of 1 min and washed in by 0.5 ml. of 0.9% saline.

Muscle responses were assessed by measuring the heights of the twitches at 10 min intervals throughout the experiment; each response was the mean of three consecutive twitches. Changes due to hemicholinium or to control injections of 1 ml. of 0.9% saline were expressed as a percentage increase or decrease of the response immediately before the injection.

Acetylcholine chloride, choline chloride, neostigmine methyl sulphate and edrophonium chloride were administered to the tibialis muscle by close-arterial injection (Brown, 1938). The concentration of the solutions was adjusted so that the dose was contained in 0.2 ml.

RESULTS

Indirect stimulation

Preliminary experiments revealed that, at a frequency of nerve stimulation of 1 shock/sec, doses of hemicholinium ranging from 2 to 4 mg/kg caused an immediate depression of the height of the muscle response lasting approximately 1 min which, after incomplete recovery, was followed by a more gradual depression. Since these effects were accompanied by a fall in the mean blood pressure of approximately 80 mm Hg (Fig. 1) the possibility could not be excluded that they were due to

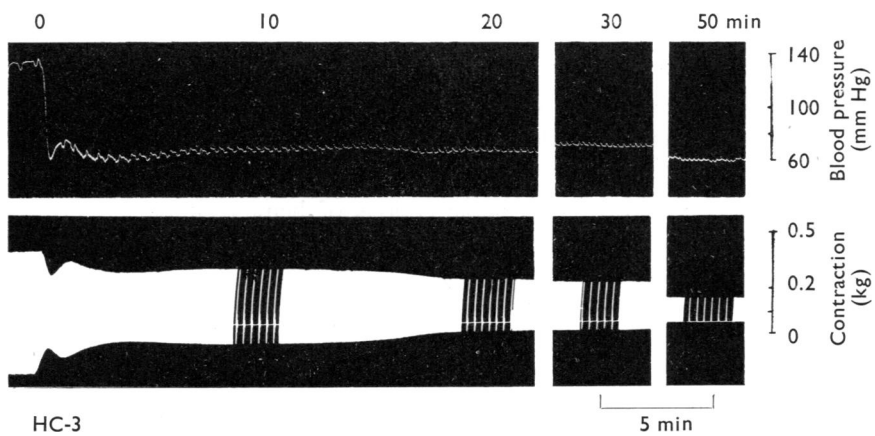

Fig. 1. Cat 2.6 kg. The effect of 4 mg/kg of hemicholinium (HC-3) on blood pressure (upper trace) and response of a tibialis anterior muscle to nerve stimulation at 1 shock/sec (lower trace). The speed of the drum was increased to record a few contractions at 10, 20, 30 and 50 min after the injection of hemicholinium.

reductions in arterial pressure (Schweitzer, 1945). These results are similar to those reported by Bowman & Rand (1961) who used doses of 1 to 2 mg/kg of hemicholinium and a stimulus frequency of 1 shock/sec.

The fall in blood pressure produced by hemicholinium could be prevented by reducing the dose to 500 μg/kg and by slow injection over a period of 1 min. Then hemicholinium produced no immediate effect on the height of the muscle response but a slowly developing depression of long duration occurred with frequencies of 1 and 2 shocks/sec.

Five control experiments were performed with stimulus frequencies of 1 and 2 shocks/sec. The reduction in height of the muscle response after a 3 hr period of continuous stimulation was $7.0 \pm 2.3\%$ (mean and standard error) at a frequency of 1 shock/sec and $16.4 \pm 2.6\%$ at 2 shocks/sec (Table 1). When a frequency of 5 shocks/sec was used, however, the height of the muscle response was decreased by 40% after 1 hr. It was concluded that stimulus frequencies of 1 and 2 shocks/sec would be suitable for investigating the effects of hemicholinium. Bowman & Rand (1961) also reported that, with frequencies greater than 2 shocks/sec, the twitches of a tibialis muscle gradually decreased, often to below 20% of their original size, after 2 hr of continuous stimulation.

The effects of three doses of hemicholinium were investigated. Five experiments were performed at each of the doses of 100, 250 and 500 μg/kg and for each of the frequencies of 1 and 2 shocks/sec. The results of these experiments are summarized in Table 1. They were represented graphically in our preliminary report (Evans & Wilson, 1962).

Responses at a frequency of 1 shock/sec. At a frequency of 1 shock/sec, 100 μg/kg of hemicholinium produced no significant effect on the tibialis muscle during

TABLE 1

EFFECTS OF HEMICHOLINIUM ON THE RESPONSE OF THE TIBIALIS MUSCLE TO STIMULATION OF THE SCIATIC NERVE AT 1 AND 2 SHOCKS/SEC

Hemicholinium was injected at zero time. Vertical joining lines indicate where values differ significantly ($P<0.05$) from those of controls. Values are means with standard errors for groups of five experiments throughout

Stimulus frequency (shocks/sec)	Time (min)	Percentage change in muscle response			
			Hemicholinium (μg/kg)		
		Controls	100	250	500
1	0	0	0	0	0
	20	+1·4±1·4	+1·1±1·5	−4·4±1·1	−4·1±1·7
	40	−3·6±1·5	−1·4±1·5	−22·3±2·0	−15·4±1·3
	90	−6·0±2·6	−8·3±2·7	−43·1±2·7	−45·5±1·7
	150	−6·8±1·2	−2·0±3·8	−19·3±3·6	−37·5±5·7
	180	−7·0±2·3	−0·5±3·1	−6·6±3·5	−28·3±3·4
2	0	0	0	0	0
	10	+1·4±1·4	−3·5±1·4	−3·7±1·5	−6·0±2·6
	20	−1·2±1·2	−23·9±2·6	−28·8±5·1	−22·0±4·2
	50	−6·6±2·1	−43·7±4·3	−63·2±2·7	−65·7±5·0
	70	−11·2±3·8	−41·1±8·9	−67·0±2·2	−78·5±2·6
	80	−14·4±2·9	−41·1±8·9	−66·0±2·9	−79·7±2·7
	110	−11·8±3·1	−29·0±5·8	−51·0±5·3	−79·7±2·7
	140	−16·4±2·6	−18·0±6·2	−35·9±5·6	−73·6±3·3
	180	−16·4±2·6	−10·1±4·3	−18·0±6·5	−57·9±2·8

a 3 hr period of nerve stimulation when compared with control readings. Doses of 250 and 500 μg/kg, however, reduced the height of the muscle responses by $4.4 \pm 1.1\%$ and $4.1 \pm 1.7\%$, 20 min after administration. The maximum depressions produced by these doses were $43.1 \pm 2.7\%$ and $45.5 \pm 1.7\%$ respectively at 90 min after injection of the compound, and were followed immediately by slow recovery. Recovery with 250 μg/kg was complete in 160 min. After the largest dose, the height of the muscle response was still reduced by $28.3 \pm 3.4\%$ 180 min after hemicholinium.

Responses at a frequency of 2 shocks/sec. At a frequency of 2 shocks/sec, doses of 100, 250 and 500 μg/kg of hemicholinium significantly reduced the height of the muscle response 10 min after administration, by $3.5 \pm 1.4\%$, $3.7 \pm 1.5\%$ and $6.0 \pm 2.6\%$ respectively when compared with control readings. The maximum depressions produced by 100 and 250 μg/kg were $43.7 \pm 4.3\%$ and $67.0 \pm 2.2\%$. These occurred at 50 and 70 min respectively after the injection of hemicholinium, and were followed by slow recovery. The responses of the muscles returned to control values at 120 min and 150 min respectively after administration of these doses. The maximum depression produced by 500 μg/kg was $79.7 \pm 2.7\%$, occurring 80 min after hemicholinium. The height of the muscle response remained at this value until recovery began at 130 min, but it was still reduced by $57.9 \pm 2.8\%$ at 180 min.

The slow onset of action of hemicholinium was not due to its activation in the body because the time of onset could not be reduced by preliminary incubation of the compound with whole blood or plasma for periods of up to 1.5 hr at 37° C.

Direct stimulation

In a preparation set up for indirect and direct muscle stimulation at 2 shocks/sec (Fig. 2), the response to nerve stimulation (I) was reduced by 82%, 70 min after the

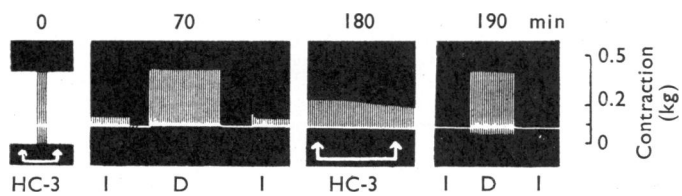

Fig. 2. Cat 3.2 kg. Response of a tibialis anterior muscle to nerve stimulation (I) and to direct stimulation (D) at 2 shocks/sec. Hemicholinium was injected at HC-3, 500 μg/kg at 0 min and 1 mg/kg at 180 min.

administration of 500 μg/kg ot hemicholinium at zero time, while undiminished responses were still obtained on direct stimulation (D). The full response to direct stimulation (D) was also obtained at 190 min when the effects of nerve stimulation (I) were abolished by 1.0 mg/kg of hemicholinium injected 180 min after the previous dose (Fig. 2). Similar observations were made on four preparations.

The effects of hemicholinium on chronically denervated muscle stimulated at 1 shock/sec were compared with those produced on the normal contralateral muscle

stimulated indirectly at the same rate. Fig. 3 shows that a dose of 250 μg/kg of hemicholinium at zero time reduced the response of the normal muscle to nerve stimulation by 50% (upper record) 65 min after the injection without affecting that of the denervated muscle (lower record).

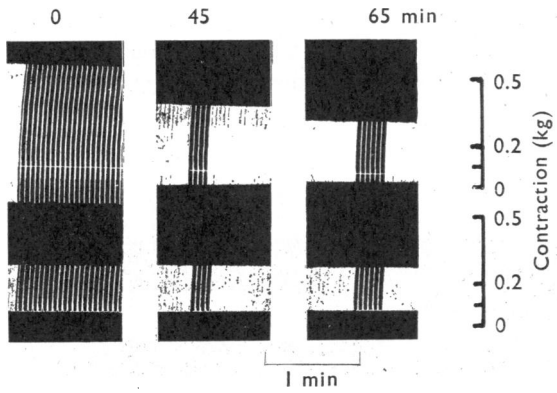

Fig. 3. Cat 2.7 kg. Upper trace, response of the right tibialis anterior muscle to nerve stimulation at 1 shock/sec. Lower trace, response of the left tibialis anterior muscle, denervated 15 days previously, to direct stimulation at the same frequency. Hemicholinium (250 μg/kg) was injected at 0 min.

The response to acetylcholine

The responses of the tibialis muscle to close-arterial injections of acetylcholine before and after the neuromuscular block produced by hemicholinium were observed in experiments in which the sciatic nerve was stimulated at 2 shocks/sec. Doses of 3 μg of acetylcholine (at Ach) given 20 and 5 min before the administration of 250 μg/kg of hemicholinium (at HC-3) produced similar responses (Fig. 4). No change in the height of the response to the same dose of acetylcholine was observed 80 min after hemicholinium when the height of the muscle response to indirect stimulation had been reduced by 58%. Similar results were obtained when doses of 500 μg/kg of hemicholinium were used.

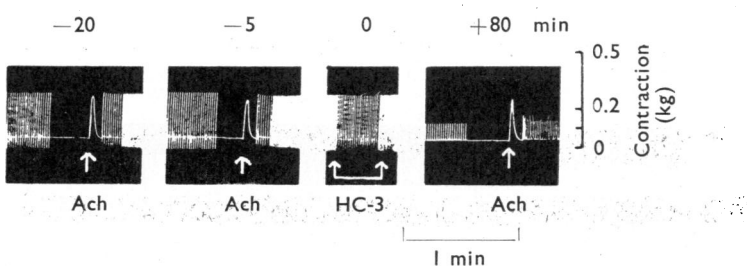

Fig. 4. Cat 2.8 kg. Effects produced by 250 μg/kg of hemicholinium (HC-3) on the response of a tibialis anterior muscle to nerve stimulation at 2 shocks/sec and to close-arterial injections of 3 μg of acetylcholine chloride (Ach). Nerve stimulation was temporarily suspended during the acetylcholine injections.

The effect of choline

Reitzel & Long (1959a) first demonstrated that neuromuscular block produced in the rabbit by hemicholinium could be antagonized by choline injected intravenously. A similar anatagonism of short duration was also observed by Bowman & Rand (1961) in the cat. Since the dose of choline (5 mg/kg, intravenously) required to produce this antagonism reduced the blood pressure immediately by 10 mm Hg and later caused a rise of 60 mm Hg lasting approximately 5 min, it was considered advisable to avoid these effects by administering choline by close-arterial injection.

Fig. 5 shows the effects produced by 100 μg/kg of choline injected close-arterially (at Ch) 110 min after the intravenous administration of 250 μg/kg of hemicholinium (at HC-3) in a preparation stimulated by the motor nerve at 2 shocks/sec and when

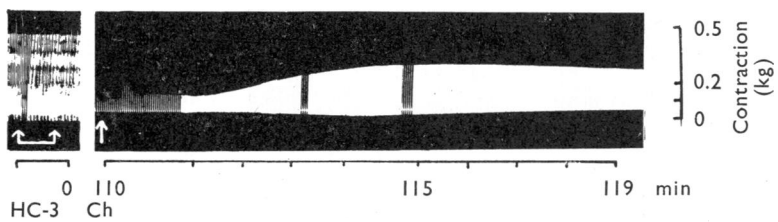

Fig. 5. Cat 4.8 kg. Response of a tibialis anterior muscle to 100 μg/kg of choline chloride (Ch) administered by close-arterial injection during a neuromuscular block by 250 μg/kg of hemicholinium (HC-3). The nerve to the muscle was stimulated at 2 shocks/sec.

the height of the muscle response was reduced by 81%. The immediate response consisted of a transient increase in height of the muscle twitch. This was followed by a more gradual increase starting about 1 min later which attained a maximum approximately 5 min after the injection and then gradually declined.

The action of anticholinesterase drugs

Reitzel & Long (1959a) demonstrated that the neuromuscular block produced by small doses of hemicholinium was only partly antagonized by neostigmine. If the neuromuscular blocking action of hemicholinium is due to a depression in the amount of acetylcholine released, then neostigmine and edrophonium would be expected to antagonize this action.

The effects of neostigmine and edrophonium injected close-arterially were observed in preparations stimulated indirectly at 2 shocks/sec. Fig. 6 shows that

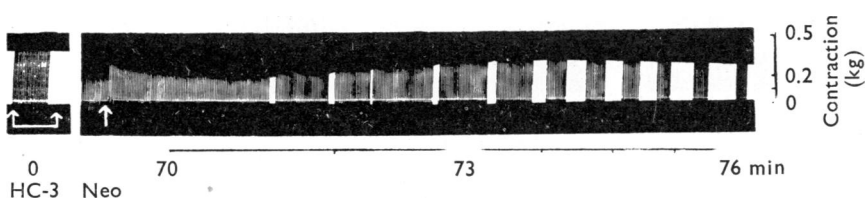

Fig. 6. Cat 2.9 kg. Effects of 33 μg/kg of neostigmine (Neo) given by close-arterial injection during a neuromuscular block produced by 250 μg/kg of hemicholinium (HC-3). The nerve to the muscle was stimulated at 2 shocks/sec.

a dose of 33 μg/kg of neostigmine (Neo) injected, when the height of the muscle response had been reduced to 50% by a dose of 250 μg/kg of hemicholinium administered at HC-3 between 69 and 70 min previously, produced a transient increase in twitch height accompanied by a small but brief contracture of the muscle. The height of the muscle response returned to that seen before neostigmine after approximately 20 sec, when it was followed by a more gradual and sustained increase in twitch height which reached a maximum 3 min after the injection. The height of the muscle response then gradually declined and in 10 min it had returned to that observed immediately before the injection of neostigmine.

An injection of 2.5 μg/kg of edrophonium (Edroph, in Fig. 7), administered 80 min after a dose of 500 μg/kg of hemicholinium (at HC-3) when the twitch

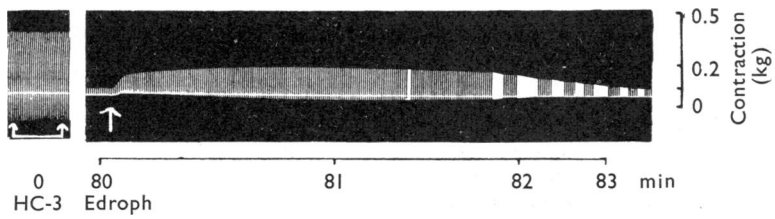

Fig. 7. Cat 2.6 kg. Response of a tibialis anterior muscle to 2.5 μg/kg of edrophonium (Edroph) given by close-arterial injection during a neuromuscular block produced by 500 μg/kg of hemicholinium (HC-3). The nerve to the muscle was stimulated at 2 shocks/sec.

height had been reduced by 84%, caused an immediate increase in the height of the muscle response accompanied by a small but sustained contracture lasting approximately 15 sec. The height of the twitch continued to increase for 1 min after the injection and then declined, reaching the height seen before edrophonium in 3 min (Fig. 7). Attempts to produce a greater antagonism by edrophonium were not successful because even small increases in dosage led to a marked contracture of the muscle followed by a decrease in twitch height.

The effect of tetanic stimulation

The response of the tibialis muscle to tetanic stimulation (50 shocks/sec) of the sciatic nerve for periods of 10 sec was recorded before and during the neuromuscular block produced by hemicholinium. Fig. 8 (*b*, *c* and *d*) shows that the response to

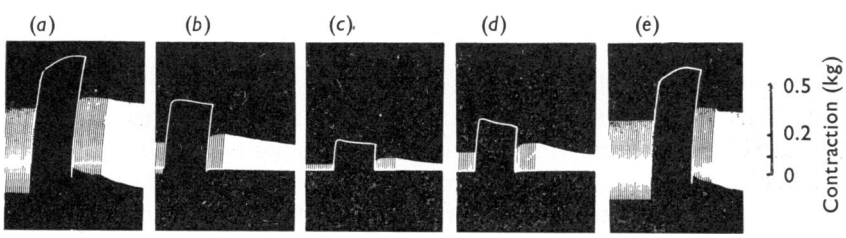

Fig. 8. Cat 3.2 kg. Response of a tibialis anterior muscle to nerve stimulation at 2 shocks/sec and to bursts of tetanic stimulation (50 shocks/sec for 10 secs). (*a*), before hemicholinium (500 μg/kg) and (*b*), (*c*), (*d*) and (*e*) 30, 80, 125 and 200 min after hemicholinium.

a tetanus was well maintained after partial neuromuscular block by 500 μg/kg of hemicholinium and that it was followed by a slight transient posttetanic potentiation of muscle twitches.

These responses to bursts of tetanic stimulation resemble those seen during a block by decamethonium, in that the tetanus is well sustained (Paton & Zaimis, 1949), but the posttetanic potentiation is reminiscent of the response obtained after tubocurarine.

The effects produced by interrupting the stimulation

Reitzel & Long (1959a) showed in rabbits that interruption of the stimulus to the sciatic nerve for 10 to 15 min antagonized the neuromuscular block produced by hemicholinium. A similar effect by resting the muscle was observed in our investigations but the recovery in neuromuscular transmission was maintained for periods of only a few minutes.

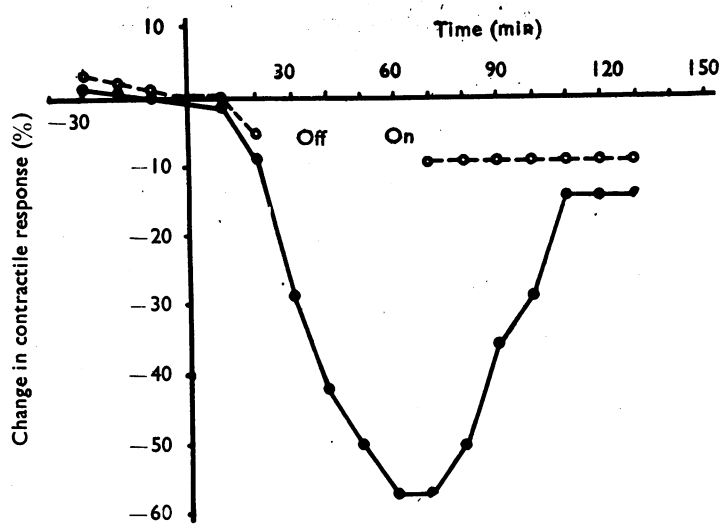

Fig. 9. Cat 2.5 kg. Effects produced by interrupting the stimulus (at Off) to the left tibialis anterior muscle (—O— —O—) as the action of 250 μg/kg of hemicholinium (injected at 0 min) appeared and by reapplication of the stimulus 50 min later (at On) when the effect on the continuously stimulated right tibialis muscle (— ●—— ●—) had reached a maximum. Both muscles were stimulated indirectly at 2 shocks/sec.

Observations were made of the effects of interrupting the stimulus when the neuromuscular block produced by hemicholinium was just beginning, and also when it had reached its maximum. Fig. 9 (broken lines) shows the result of discontinuing stimulation of the nerve in one preparation at 20 min just as the effects of 250 μg/kg of hemicholinium had begun to depress the muscle twitch. When stimulation was recommenced at 2 shocks/sec 50 min later (at 70 min), the response of the rested muscle was only reduced by 9% whilst that of the continuously stimulated contralateral muscle was depressed by 57% (Fig. 9, full line). During the next hour the

response of the rested muscle remained unaltered whilst that of the unrested muscle gradually recovered until it was within 14% of the pre-injection value.

In Fig. 10 (broken line) the effect of 500 μg/kg of hemicholinium approached its maximum (at 50 min). The stimulus to the nerve was then switched off. When the response of the continuously stimulated contralateral muscle began to recover (at 100 min), the nerve to the rested muscle was again stimulated. The initial response of the rested muscle to stimulation was greater than at the time of injection of hemicholinium but during the next 40 min the response declined at the same rate as previously observed and it attained a maximum depression of 47%. Thereafter, recovery of both muscles proceeded at the same rate.

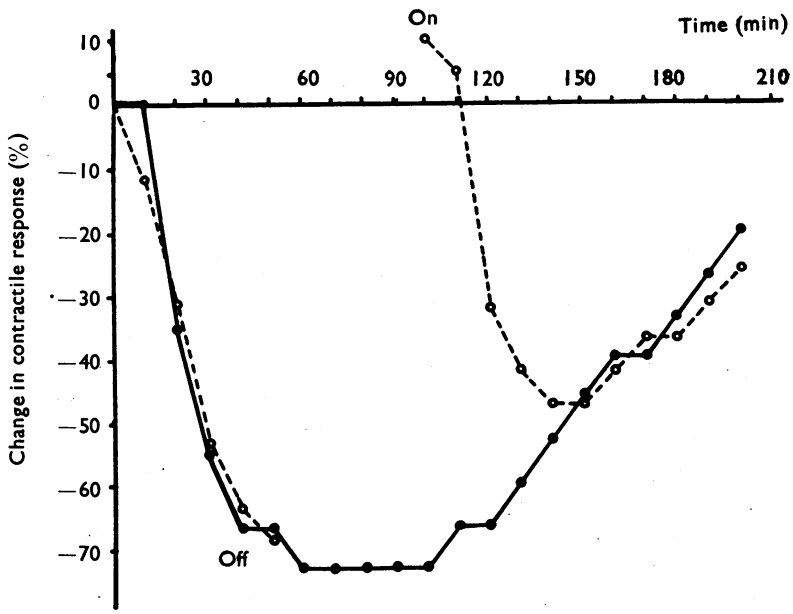

Fig. 10. Cat 4.3 kg. Effects produced by interrupting the stimulus (at Off) to the left tibialis anterior muscle (—O— —O—) when the response to 500 μg/kg of hemicholinium (injected at 0 min) had reached a maximum, and by reapplication of the stimulus (at On) 50 min later as the continuously stimulated right tibialis muscle (— ●—— ●—) was recovering. Both muscles were stimulated indirectly at 2 shocks/sec.

DISCUSSION

These results have shown that the neuromuscular block produced by three different doses of hemicholinium on the tibialis muscle of the cat in response to sciatic nerve stimulation was slow in onset, of long duration and related to the stimulus frequency. These features are similar to those observed by Reitzel & Long (1959a) and Wilson & Long (1959) on the gastrocnemius muscle of the rabbit.

At 1 shock/sec, 100 μg/kg of hemicholinium was ineffective but 250 and 500 μg/kg produced a neuromuscular block which was more prolonged with the higher dose. When the nerve was stimulated at 2 shocks/sec the three doses of

hemicholinium produced a progressively deeper and more prolonged neuromuscular block. These doses were not followed by a block, however, if the stimulus frequency was 0.5 shocks/sec. Increasing the frequency of nerve stimulation has also been reported to increase the activity of tubocurarine and decamethonium (Preston & Van Maanen, 1953; Gesler & Hoppe, 1956), but the effects are not so marked with these compounds as with hemicholinium.

The striking effects produced on the action of hemicholinium by increasing the stimulus frequency, together with the failure of the compound to modify the response of the tibialis muscle to close-arterial injections of acetylcholine, are in favour of a presynaptic action. The idea of a presynaptic site of action of hemicholinium is suggested by the findings of MacIntosh, Birks & Sastry (1956), who showed that hemicholinium caused a reduction in the amount of acetylcholine liberated from the perfused superior cervical ganglion of the cat in response to preganglionic nerve stimulation, an effect which was antagonized by choline. The reduction in acetylcholine release by hemicholinium is probably due to the compound competing with choline for transport by a specific carrier system to intraneuronal sites of acetylation (MacIntosh *et al.*, 1956; MacIntosh, 1959; Gardiner, 1957, 1961). Antagonism by choline of the neuromuscular block produced by hemicholinium suggests that the failure in transmission at this site may also be due to a reduction in acetylcholine formation or release. This conclusion is supported indirectly by the observations of Reitzel & Long (1959b), who found the structure of choline appeared to be specific for the antagonism of this action of hemicholinium. It is of interest to note that the tertiary amine dimethylaminoethanol, which has been postulated to be a choline precursor, produced no antagonism (Pfeiffer, 1959; quoted by Schueler, 1960).

Relief of the block in ganglionic and neuromuscular transmission by reducing the frequency of nerve stimulation or by rest provides further evidence for an action of hemicholinium on acetylcholine release (MacIntosh, Birks & Sastry, 1958; MacIntosh, 1959; Reitzel & Long, 1959a; MacIntosh, 1961). The extent of the recovery following rest appeared to depend on the stage at which stimulation was interrupted. Thus if it was discontinued as the effects of hemicholinium appeared, and later resumed when the block in the continuously stimulated contralateral muscle was deepest, recovery was sustained. This suggests that there was sufficient acetylcholine available at the nerve endings to maintain transmission. When, however, the stimulus was discontinued as the block produced by hemicholinium was approaching its maximum and reapplied as the contralateral muscle was recovering, the rested muscle showed a transient recovery which was immediately followed by a block. The block developed at the same rate as previously until it reached a value similar to that of the contralateral muscle, when both muscles recovered together. These findings suggest that the more prolonged stimulation had caused a depletion of acetylcholine at the nerve endings and that the amount which accumulated as a result of rest was only sufficient to maintain transmission for a short time when the stimulus was reapplied. The lesser block which followed the rest period in Fig. 10 and the similarity of the rates of recovery of both muscles after 150 min is probably due to the falling concentration of hemicholinium.

Antagonism of the effects of hemicholinium by neostigmine and edrophonium indicates a resemblance to the activity of tubocurarine. If however, the apparently greater antagonism of neostigmine is due to its more prolonged anticholinesterase activity the neuromuscular block produced by hemicholinium is probably due to a reduction in the acetylcholine at this site. It would be interesting to know from direct electrical recordings whether the increase in height of the muscle twitch is due to repetitive firing of unblocked fibres or to an increase in the number of fibres responding to nerve stimulation.

A difference between the modes of action of hemicholinium and tubocurarine is indicated, however, by the response of the tibialis muscle to bursts of tetanic stimulation. The finding that the tension with tetanic stimulation was maintained during the block by hemicholinium indicates that the tetanus caused no further depletion of acetylcholine. An interesting feature of the response to tetanic stimulation was the slight posttetanic potentiation. If posttetanic potentiation is due to an increase in the amount of aceylcholine liberated by single nerve shocks following tetanic stimulation (Hutter, 1952; Liley & North, 1953; Del Castillo & Katz, 1954a,b; Liley, 1956a,b; Hubbard & Schmidt, 1963), its presence during the block produced by hemicholinium indicates that some reserve of acetylcholine is still available. A posttetanic potentiation was also reported after hemicholinium by Desmedt (1958), who applied test trains of 3 shocks/sec to the motor nerve every minute before and after a tetanus, and compared the reduction of the electrical response of the muscle to the fifth response with respect to the first. In contrast to the potentiation produced by tubocurarine, however, Desmedt (1958) found that with hemicholinium there was an increase in the neuromuscular block lasting approximately 15 min which he attributed to a depletion of stores of preformed acetylcholine in nerve endings.

By direct measurement, Bhatnagar (1961; quoted by MacIntosh, 1961) found that hemicholinium reduced the amount of acetylcholine extractable from cat muscle when the motor nerve was stimulated. Thus the amount of acetylcholine was reduced by 60% after nerve stimulation at 20 shocks/sec for 30 min whereas in the absence of hemicholinium it was only reduced by 25%. Cheymol, Bourillet & Ogura (1962) showed that hemicholinium (1×10^{-4}) reduced the amount of acetylcholine released from the rat isolated diaphragm in response to nerve stimulation.

We conclude that the site of action of hemicholinium on the cat tibialis anterior muscle-sciatic nerve preparation is presynaptic. Its effects could be accounted for by a reduction in acetylcholine release on nerve stimulation, but only direct measurement of acetylcholine release could provide the basis for acceptance of this view.

We are grateful to Professor Andrew Wilson for his interest and advice throughout the course of these investigations. We would like to thank Dr J. P. Long, of the Department of Pharmacology of the State University of Iowa, U.S.A., for a generous supply of hemicholinium No. 3.

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