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Post-tetanic and drug-induced repetitive firing in the soleus muscle of the cat

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1. The effects of a high frequency indirect tetanus on the responses to subsequent infrequently applied nerve shocks have been compared in the tibialis anterior and soleus muscles of cats and rabbits.

2. Post-tetanic augmentation of twitches in the cat soleus muscle was shown to be partly due to repetitive firing and partly due to increased synchronization of the muscle fibre response. Post-tetanic repetitive firing was not evident in the responses of the other three muscles studied.

3. Post-tetanic repetitive responses in the cat soleus muscle and nerve did not originate in the nerve trunk and were not produced by direct muscle stimulation; they were abolished by doses of tubocurarine smaller than those necessary to reduce the twitch tension below the pre-tetanic level. These findings support the conclusion of others that the repetitive firing originates at the neuromuscular junction.

4. The repetitive firing could not be explained by an increase in the sensitivity of the motor endplates to acetylcholine, suggesting that an increase in and/or a prolongation of the output of transmitter from the motor nerve contributes to it.

5. The cat soleus muscle was shown to be more sensitive to neostigmine than were the other three muscles studied, and acetylcholinesterase determinations showed that this muscle possesses less enzyme activity.

6. It is concluded that an increase in transmitter output, coupled with a weaker cholinesterase activity, probably accounts for the post-tetanic repetitive activity in the cat soleus muscle.

7. Post-tetanic repetitive firing was absent in cat soleus muscles which had been cross-innervated with the nerve formerly innervating a fast-contracting muscle.

After a period of high frequency stimulation of the motor nerve innervating the slow-contracting soleus muscle of the cat, augmented contractions associated with repetitive firing of the muscle fibres are temporarily produced in response to each

of a train of infrequently applied nerve shocks. At the same time repetitive discharges in response to individual post-tetanic nerve shocks may be recorded antidromically in the ventral roots (Feng, Li & Ting, 1939 ; Werner, 1960 ; Standaert, 1963, 1964). This type of post-tetanic augmentation, which has been attributed to an action on the motor nerve endings, is not evident in fast-contracting mammalian muscles such as the gastrocnemius or tibialis anterior. In the latter muscles, the post-tetanic increase in contractions is not associated with repetitive firing (Brown & von Euler, 1938 ; Feng *et al.*, 1939 ; Bowman, Goldberg & Raper, 1962 ; Standaert, 1964) and has been ascribed to an action on the muscle fibres through which contractility is increased. Even in the soleus muscle of the cat, post-tetanic repetitive responses are not produced unless prolonged tetani of high frequency are applied. After brief tetani within the physiological range of frequencies (Denny-Brown, 1928) the soleus response is opposite to that of a fast-contracting muscle, twitch tension being slightly decreased for a short time as a result of an action on the muscle fibres (Brown & von Euler, 1938 ; Bowman *et al.*, 1962).

The similarity between the repetitive responses to anticholinesterase drugs (Masland & Wigton, 1940) and the post-tetanic repetitive responses of the cat soleus muscle and nerve has long been realized (Feng & Li, 1941), and more recently has been used to support theories, extensively modified from the classically held ones, to explain neuromuscular transmission and the actions of anticholinesterase drugs (Riker, 1960, 1966 ; Werner, 1960 ; Standaert, 1963). In the experiments described in this paper, a further study has been made of the relationship between the effects of a tetanus and the effects of neostigmine on neuromuscular transmission in the cat soleus muscle. The results support the idea that a similar mechanism underlies both types of repetitive response, but they also suggest that an explanation involving cholinesterase may account for both.

Methods

The experiments were performed on forty-three cats and sixteen rabbits. Cats were anaesthetized with a mixture of chloralose (80 mg/kg) and sodium pentobarbitone (6 mg/kg) injected intravenously or intraperitoneally. Rabbits were anaesthetized similarly except that the dose of chloralose was 110 mg/kg.

Contractions of the soleus muscles and/or the tibialis anterior muscles were recorded on smoked paper by means of flat steel spring myographs, or on an oscilloscope (Tektronix 502) by means of isometric strain gauges made from RCA 5734 mechano-electric transducer valves. The methods used have been fully described before (Bowman *et al.*, 1962). The resting length of the muscle was that giving the maximum twitch tension. Muscle action potentials in response to nerve stimulation were recorded on the oscilloscope by means of platinum wires inserted through the belly and the tendon of the muscle, or by concentric needle electrodes. Indirectly evoked contractions of the muscles were elicited by rectangular pulses of 100 μsec duration applied through bipolar platinum electrodes either to the muscle nerves, in which case the main trunk of the sciatic nerve was sectioned at the level of the trochanter, or to the severed L7 and S1 ventral roots (Bowman *et al.*, 1962). Directly evoked contractions were produced by stimulating the fully curarized muscles with rectangular shocks of 1 msec duration applied between a platinum wire inserted through the musculotendinous junction and an indifferent electrode attached to the drill in the lower end of the femur. The strength of the shocks was

such as to give twitches of comparable size and time course to those of the indirectly elicited maximal twitches recorded before curarization. Full curarization of innervated muscles was produced by an intravenous injection of tubocurarine 1 mg/kg followed by a continuous infusion of 0.3 mg/kg per hr. In three cats the muscles were denervated by sectioning the sciatic nerve aseptically under sodium pentobarbitone anaesthesia. Degeneration was allowed to proceed for 10 days. The denervated muscles were stimulated directly using stimulus parameters similar to those used to excite fully curarized muscles. In all cases, the muscles were bathed in pools of warm mineral oil (heavy liquid paraffin, B.P.) formed from the skin flaps. Muscle temperature was recorded by means of a small thermocouple (Cambridge Instruments) and was maintained at 36°–37° C by means of a small heating device placed under the surface of the mineral oil.

In cats, nerve action potentials in response to stimulation with pulses of 10 μsec duration were recorded under mineral oil from the small branch of the tibial nerve which supplies the soleus muscle. The stimulating electrodes were placed high on the sciatic nerve at the level of the trochanter and the nerve was cut between ligatures above the stimulating electrodes. The soleus branch of the tibial nerve was exposed between the soleus and the plantaris and followed upwards between the two heads of the gastrocnemius into the popliteal space. All branches of the sciatic nerve between the stimulating electrodes and the soleus muscle were cut between ligatures. Bipolar platinum recording electrodes were placed on the soleus branch of the nerve, close to the muscle, and the nerve was crushed at the point where it entered the muscle. The more distal pole of the recording electrode was placed in contact with the crushed part of the nerve. In similar experiments, also on cats, action potentials were recorded from the distal end of the common peroneal nerve which includes the supply to tibialis anterior and other fast-contracting muscles (for example the extensor longus digitorum) but which does not include nerve fibres to the soleus muscle.

In three experiments on cats, soleus muscle action potentials in response to stimulation of the soleus branch of the tibial nerve were recorded simultaneously wth antidromic nerve action potentials recorded from the peripheral end of a severed L7 or S1 ventral root. The method used was similar to that described by Blaber & Bowman (1963).

In four experiments on cats, venous outflow from the soleus muscle was recorded on smoked paper simultaneously with the contractions, by the method described by Bowman & Zaimis (1958).

In four young cats (5–8 months old), cross-innervation between the soleus and the fast-contracting flexor digitorum longus muscle was achieved as described previously (Bowman & Raper, 1962), following the method described by Buller, Eccles & Eccles (1960). The cats were anaesthetized with intravenous sodium pentobarbitone and the nerve cross union was carried out aseptically, the central stump of the cut soleus nerve being tied to the peripheral stump of the cut flexor digitorum longus nerve near the muscles, and vice versa. Two months later responses of the cross-innervated muscles were compared, under chloralose anaesthesia, with those of the contralateral normal muscles.

Drugs were injected intravenously in both cats and rabbits through a cannula in an external jugular vein, and close-arterially to the soleus or tibialis anterior muscles of cats by the methods described by Brown (1937) and (1938) respectively.

The acetylcholinesterase activity of the tibialis anterior and soleus muscles of both cats and rabbits was estimated in the Warburg apparatus by a method similar to that described by Blaber (1960). For comparison at one substrate concentration the muscles were freed as completely as possible from connective tissue and tendon and weighed. They were then finely cut up and placed in about 10 ml. bicarbonate buffer (4.2 g $NaHCO_3$ in 1 litre of distilled water adjusted to pH 7.6 by dropwise addition of HCl). The muscles were homogenized in an all glass homogenizer, and both tibialis and soleus homogenates were diluted with buffer to contain 150 mg of muscle per ml. The substrate used was acetylcholine chloride, the final concentration being 0.0138M (Aldridge, 1950). Thirteen Warburg flasks were used. Flasks 1 to 4 contained 2ml. tibialis homogenate together with 0.5 ml. distilled water in the main compartment and 0.5 ml. acetylcholine solution in the side arm. Flasks 5 to 9 contained 2 ml. soleus homogenate together with 0.5 ml. distilled water in the main compartment and 0.5 ml. acetylcholine solution in the side arm. Flask 10 contained 2 ml. tibialis homogenate together with 0.5 ml. distilled water in the main compartment and 0.5 ml. distilled water in the side arm. Flask 11 contained 2 ml. soleus homogenate together with 0.5 ml. distilled water in the main compartment and 0.5 ml. distilled water in the side arm. Flasks 10 and 11 were " tissue blanks " to record the amount of gas given off by the muscle itself. Flask 12 contained 2 ml. of buffer solution together with 0.5 ml. distilled water in the main compartment and 0.5 ml. acetylcholine solution in the side arm, to record the amount of aqueous hydrolysis. The remaining flask, containing 3 ml. distilled water, was used as a thermometric barometer. The total volume of fluid in each flask was 3 ml. The flasks were gassed with a mixture of nitrogen and 5% carbon dioxide. After tipping in the substrate the flasks were shaken on the apparatus for 10 min at which time zero readings were taken. Readings were subsequently taken at 10 min intervals for 30 min.

When acetylcholine concentration/activity curves were plotted for tibialis and soleus, the following substrate (acetylcholine chloride) concentrations were used 10^{-1}M, 3×10^{-2}M, 10^{-2}M, 3×10^{-3}M and 10^{-3}M. For each muscle type, four flasks were used at each substrate concentration, two containing muscle and two containing buffer, the latter acting as aqueous hydrolysis controls. A tissue blank was also included. The different substrate concentrations for each type of muscle were divided between two runs, the second run including one of the substrate concentrations (10^{-2}M) from the first run to enable any corrections to be made.

The drugs used were: tubocurarine chloride (Burroughs Wellcome), neostigmine methylsulphate (Roche), acetylcholine chloride (Roche), and atropine sulphate (British Drug Houses).

Results

Post-tetanic changes

Cat

In the cat, prolonged high frequency stimulation applied either to the nerve close to the muscle, or to the ventral roots, produced a strong augmentation of the subsequent maximal twitches of the soleus muscle (Figs. 1, 2a, 4a and 5). As shown also by Standaert (1964), the lowest frequency of stimulation to give rise to this effect was 100/sec and it was necessary to apply it for at least 0.5 to 1 min (Fig. 1). At

this frequency, a tetanus of 2–3 min duration produced the maximum increase in the post-tetanic twitches, but longer lasting tetani (up to 12 min—the longest studied) produced a more prolonged augmentation. Briefer tetani at higher frequencies of stimulation produced a similar effect, confirming the results of Feng *et al.* (1939) and Standaert (1964).

The marked post-tetanic augmentation of the soleus twitch which occurred after high frequency stimulation of the motor nerve, was accompanied by repetitive firing of the muscle fibres (Figs 1 and 2a). The peak voltage of the initial main negative deflection was sometimes, but not always, reduced during this repetitive firing, and the fact that it was not always reduced, confirms the conclusion of Feng *et al.* (1939) that the repetitive discharges could not be attributed simply to temporal dispersion of the individual components. During the period that repetitive muscle responses were recorded, antidromic repetitive firing was also evident in the ventral root (Fig. 3) confirming the observation of Feng *et al.* (1939) and Standaert (1964).

When the first post-tetanic twitch was elicited within 4–5 sec after the tetanus, only slight or no augmentation was evident and repetition in both muscle and nerve was rarely detectable in the action potential records associated with such a twitch (Fig. 2a). The amplitude and duration of the muscle action potential in these

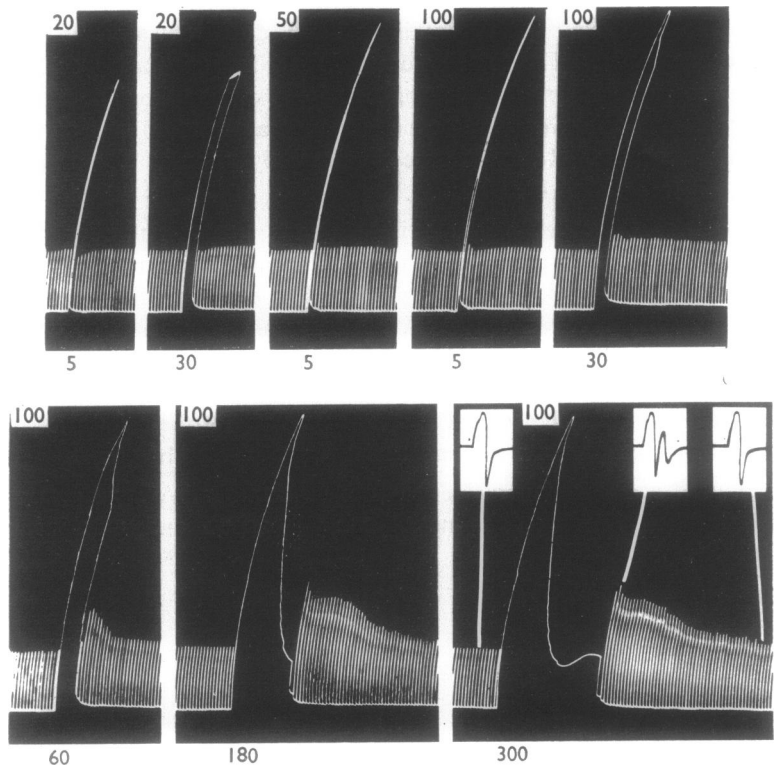

FIG. 1. Cat. Maximal twitches of the soleus muscle elicited indirectly once every 10 sec with tetani interposed. The number at the top of each panel gives the frequency/sec of the tetanus. The number below each panel is the duration of the tetanus in sec. Towards the end of the experiment (last panel) gross muscle action potentials were recorded simultaneously with the twitches. The white lines indicate the twitches associated with the action potentials shown.

circumstances were reduced by about 20–25%. Despite the small increase in the amplitude of the twitch, the time to peak tension was unaltered or even slightly reduced because the rate of rise of tension was increased; and the time from the tension crest to half-decay was reduced by about 25–30%. A twitch elicited 10 sec after a high frequency tetanus was always markedly augmented and the action potential records showed the occurrence of repetitive firing of the muscle fibres. Repetitive discharges were no longer evident some minutes before the twitch tension had declined to the pre-tetanic level. As the repetition waned, the amplitude of the gross action potential increased up to about 125% of the pre-tetanic level. At the same time the duration of each action potential was reduced by 15–20%. This might well have been caused by better synchronization due to the individual fibres responding more quickly, and the increase in the rate of rise of tension adds support to this possibility. This effect was observed both immediately before and after the appearance of repetitive discharges, so it seems likely that it occurred throughout the augmentation of twitches. The augmented indirectly elicited twitch following a high frequency tetanus could therefore be ascribed to at least two factors—better synchronization of the contractions of the individual fibres, and repetitive firing.

Although greatly augmented, the post-tetanic twitches had often become submaximal as shown by the additional increase in twitch which resulted on increasing the strength of the stimulus applied to the nerve (Fig. 4a). As the augmentation decayed the extent of the increase in twitch tension, which could be produced by increasing the stimulus strength, began to diminish so that by the time the twitch tension had returned to the pre-tetanic level, the original stimulus strength was again supra-maximal. The post-tetanic twitches did not become submaximal if the stimulus strength before the tetanus was more than two or three times maximal.

Recording of action potentials from the severed soleus nerve of the cat, showed that the repetitive firing of the muscle fibres did not originate in the main nerve

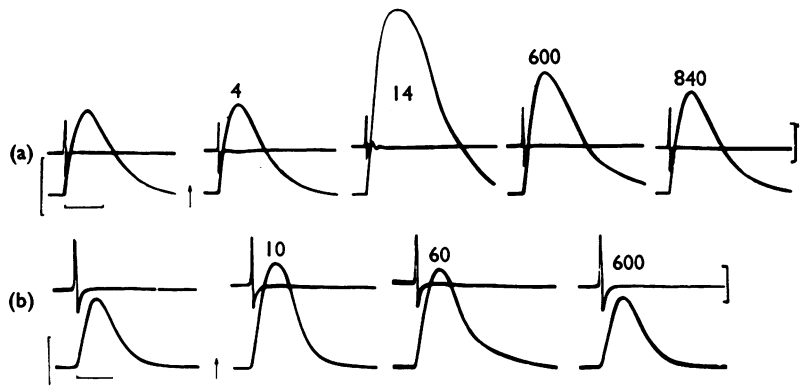

FIG. 2. Cats. (a) Isometric maximal twitches and gross muscle action potentials of a soleus muscle were elicited indirectly once every 10 sec. The first panel is one of a series of control responses. At the arrow, a tetanus (100/sec for 150 sec) was elicited. The subsequent responses were recorded after the tetanus; the numbers denote the time in sec after the tetanus. Note that no repetitive firing is evident in the twitch recorded 4 sec after the tetanus but is present after 14 sec. Calibrations: tension on the left, 0.25 kg; time below, 100 msec; action potential on the right, 40 mV. (b) Similar experiment to (a) except that the soleus muscle had been cross-innervated with the nerve to flexor digitorum longus 2 months previously. Note that the twitch speed is faster than in (a) (different sweep speeds were used for (a) and (b)) and the post-tetanic augmentation is not associated with repetitive firing. Calibrations: tension on the left, 0.1 kg; time below, 50 msec; action potential on the right, 10 mV.

trunk. When the stimulus strength remained supramaximal throughout, the only change in the nerve action potentials following prolonged high frequency stimulation, was an increase in their amplitude (Fig. 4b). The period during which this occurred was similar in duration to that during which repetitive firing was recorded from the muscle. There was no change in the duration of the nerve action potential,

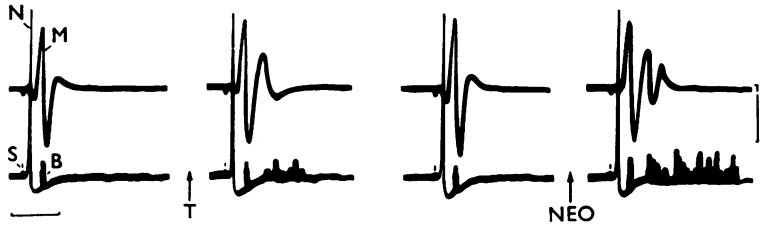

FIG. 3. Cat. Soleus muscle action potential (M) recorded with a concentric needle electrode, and nerve action potential (N) recorded antidromically from the ventral root in response to stimulation of the soleus nerve near the muscle. B is the ephaptic back response in the nerve (see Brown & Matthews, 1960), and S is the stimulus artifact. At T, a tetanus was elicited (100/sec for 3 min) and the subsequent response was recorded 20 sec later. The third panel was recorded 15 min after the tetanus and immediately before neostigmine (NEO, 50 μg/kg intravenously). The last response was recorded 4 min after neostigmine at the height of the repetitive firing. Voltage calibration, 10 mV for the muscle and 50 μV for the nerve. Time calibration, 5 msec.

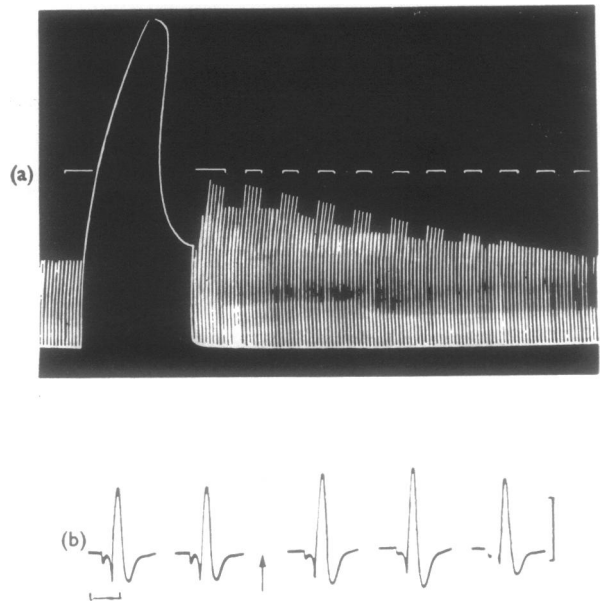

FIG. 4. Cats. (a) Twitches of a soleus muscle elicited indirectly once every 10 sec with a tetanus (100/sec) interposed. During the periods marked by the horizontal bars (including the tetanus) the voltage output of the stimulator was set to read 1.5 V. During the remaining periods the voltage output read 0.75 V. Note that before the tetanus, both stimulus strengths produced maximal twitches, but after the tetanus, the weaker stimulus produced submaximal twitches. (b) Maximal action potentials were recorded from the distal end of the soleus branch of the tibial nerve once every 10 sec, and a tetanus (100/sec for 3 min) was interposed. The panels show action potentials before, and 10 sec, 100 sec and 8 min after the tetanus. The stimulus strength was such as to remain supramaximal throughout. Time calibration, 2 mec. Voltage calibration, 1 mV.

and no evidence of repetitive firing. In each experiment on the soleus nerve, the smallest stimulus strength necessary to elicit a just perceptible action potential was determined both before and after a period of 2.5–3 min supramaximal stimulation at 100/sec. Such high frequency stimulation always caused about 100% increase in the threshold. The threshold then gradually returned to normal over a period similar in duration to that of the post-tetanic increase in twitch tension.

The post-tetanic repetitive firing in response to a single nerve shock was abolished in both nerve and muscle by intravenous doses of tubocurarine (50 μg/kg) smaller than those necessary to reduce the amplitude of the main muscle action potential. Standaert (1963) also recorded this effect. The same doses of tubocurarine markedly reduced but did not abolish the post-tetanic increase in twitch tension, and electrical recording of the twitches showed that the times to the tension crest and to half-decay were shorter than those of the pretetanic controls. These tension changes recorded in the indirectly stimulated muscle in the presence of sub-blocking doses of tubocurarine resembled the weak post-tetanic augmentation of contractions produced in the directly stimulated fully-curarized or chronically denervated soleus muscle.

The post-tetanic augmentation of twitches produced by shorter periods of stimulation in the tibialis anterior muscle was not accompanied by repetitive firing (Brown & von Euler, 1938 ; Bowman *et al.*, 1962), and after prolonged high frequency stimulation comparable with that necessary to produce repetitive firing in the soleus muscle, the twitches of the tibialis were often depressed ; again there was no evidence of repetitive firing. Nerve action potentials recorded from the common

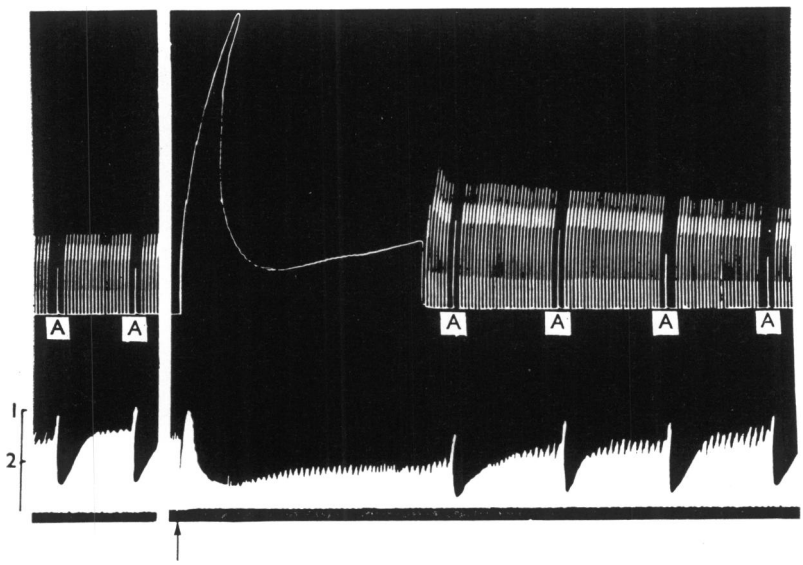

FIG. 5. Cat. Simultaneous recording of venous outflow from and contractions of a soleus muscle. Maximal twitches were elicited once every 10 sec and a tetanus of 11 min duration and 100/sec frequency was interposed beginning at the arrow. At A, 5 μg acetylcholine was injected close-arterially. Electrical stimulation was stopped from shortly before to shortly after each injection. Note that an increase in the height of the blood flow recording denotes a decrease in flow. The small decrease in flow preceding each vasodilatation produced by acetylcholine was caused by brief occlusion of the arterial supply during the injection. Flow calibration in ml./min.

peroneal nerve of cats, in response to single shocks delivered at 10 sec intervals, showed no change after short periods of stimulation at 50–100/sec. After stimulation at 100/sec for 2 min or more, the subsequent action potentials in response to single shocks were sometimes reduced in amplitude but were never increased. The reduction was not caused by a change in threshold of the nerve, since increasing the strength of stimulus did not increase the amplitude of the action potential. Post-tetanic augmentation of the twitches of the tibialis anterior muscle was not affected by sub-blocking doses of tubocurarine.

The contractions of the soleus muscle of the cat produced by close-arterially injected acetylcholine were always augmented during the post-tetanic increase in tension resulting from prolonged high-frequency stimulation. In these experiments, the venous outflow from the soleus muscle was recorded simultaneously with the contractions of the muscle. Figure 5 illustrates such an experiment. The post-tetanic increase in the response to acetylcholine occurred during the post-tetanic hyperaemia, but the post-tetanic increase in twitch tension outlasted both of these effects. In Fig. 6 the percentage increases in twitch tension, in acetylcholine-response and in blood flow, obtained in another experiment, are expressed graphically. It can be seen that the curve representing the increase in acetylcholine-response followed that representing the increase in blood flow but was quite different from that representing the increase in twitch tension. The response of the tibialis anterior muscle to close-arterially injected acetylcholine was briefly augmented during the post-tetanic augmentation produced by shorter periods of stimulation. Post-tetanic

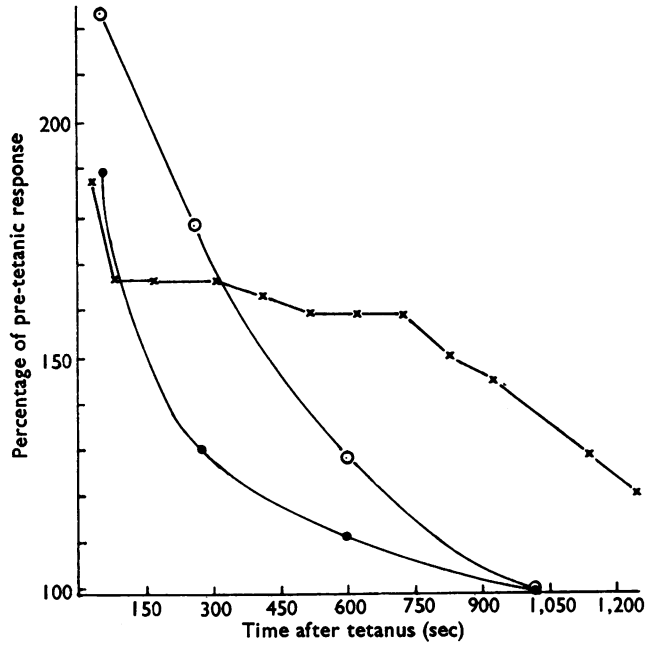

FIG. 6. Graphical representation of the post-tetanic changes in twitch tension (×——×), venous outflow (●——●) and acetylcholine response (⊙——⊙) in a similar experiment to that illustrated in Fig. 5. Note that the time course of the increase in acetylcholine response resembled that of the post-tetanic hyperaemia but differed from that of the increase in twitch tension.

hyperaemia is also shorter-lasting in this muscle than in the cat soleus (Bowman, 1959).

In cat soleus muscles that had been cross-innervated with the nerve to flexor digitorum longus, high frequency stimulation of the nerve gave rise to post-tetanic responses which were non-repetitive (Fig. 2b). The characteristic depressant effect (Fig. 1) following low frequency tetani (Brown & von Euler, 1938 ; Bowman *et al.*, 1962) was also absent in cross-innervated soleus muscles, which exhibited non-repetitive post-tetanic augmentation resembling that of the tibialis anterior muscle and of the normally innervated contra-lateral flexor digitorum longus muscle. Although the speed of contraction was slowed, cross-innervation did not change the type of post-tetanic response produced in the flexor digitorum longus muscle, which continued to respond in the same way as the normally innervated contra-lateral muscle.

Rabbit

The tibialis anterior muscle of the rabbit resembled the same muscle in the cat. Post-tetanic augmentation of twitches was produced by relatively low frequencies of stimulation and there was no evidence of repetitive firing of the muscle fibres (Bowman *et al.*, 1962).

The soleus muscle of the rabbit differed from that of the cat in that high frequency stimulation did not produce a post-tetanic augmentation of twitches and there was no evidence of repetitive firing (see also Bowman *et al.*, 1962).

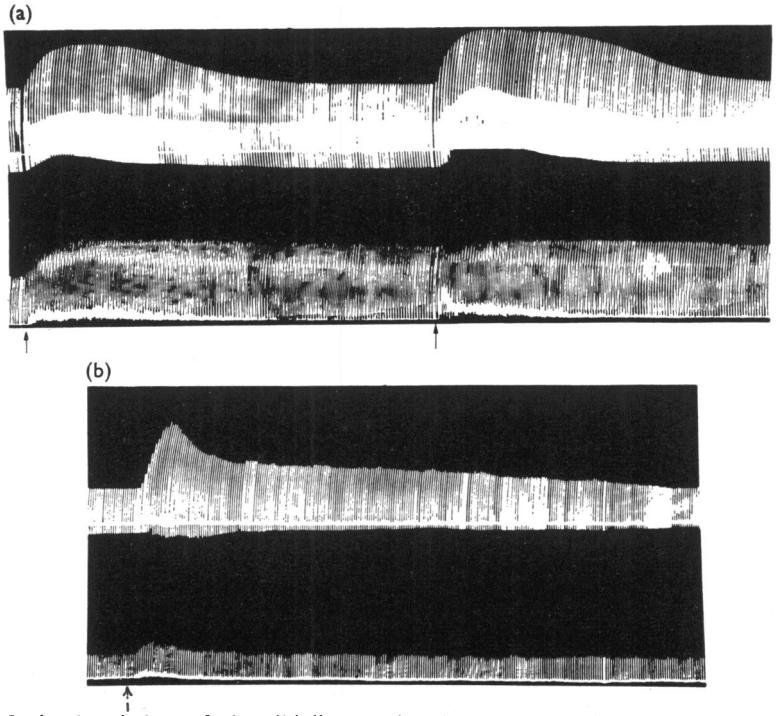

FIG. 7. Maximal twitches of the tibialis anterior (upper record in each panel) and soleus muscles (lower record in each panel) of a cat (upper panel) and a rabbit (lower panel) elicited indirectly once every 10 sec. At the arrows, neostigmine 50 μg/kg was injected intravenously.

Effects of neostigmine

In the tibialis anterior and soleus muscles of both cats and rabbits, neostigmine caused the well known augmentation of the indirectly excited maximal twitches which was associated with repetitive firing of the muscle fibres. Brown, Dale & Feldberg (1936) and Bacq & Brown (1937) first demonstrated this ability of an anticholinesterase to convert the muscle response to a single nerve shock into a brief tetanus. This effect in the cat was shown to be accompanied by repetitive discharges in the ventral root (Fig. 3) thus confirming the observations of Masland & Wigton (1940) and numerous other workers.

The soleus muscle of the cat differed from the tibialis anterior muscle of both the cat and the rabbit, and from the soleus muscle of the latter species, in its reaction to neostigmine, although the difference was mainly one of degree. The soleus muscle of the cat was more sensitive than the tibialis and with a dose that affected both muscles, the increase in twitch tension produced in the soleus was always longer lasting than that produced in the tibialis anterior muscle. With doses of the order of 50 μg/kg administered intravenously, the increase in the tibialis twitches lasted about 15–20 min. This dose could be repeated, at intervals of 30–40 min, up to at least five times, and the response was approximately the same each time. In the cat soleus muscle, however, the same dose produced maximal augmentation, and powerful fasciculations of the muscle occurred. These effects persisted undiminished for more than 1 hr. A second dose, administered before the effect of the first had disappeared, did not cause further augmentation of the contractions but merely increased the fasciculations for a short time. The upper records of Fig. 7 illustrate the effects of two doses of neostigmine on the tibialis anterior and soleus muscles of the cat. Figure 8 illustrates an experiment in which gross muscle action potentials were recorded simultaneously from the tibialis anterior and soleus muscles of the cat. In this experiment, repetitive firing after neostigmine appeared first in the tibialis anterior muscle but occurred soon afterwards in the soleus. With this dose, repetition persisted for about 150 msec after each main muscle spike in the tibialis

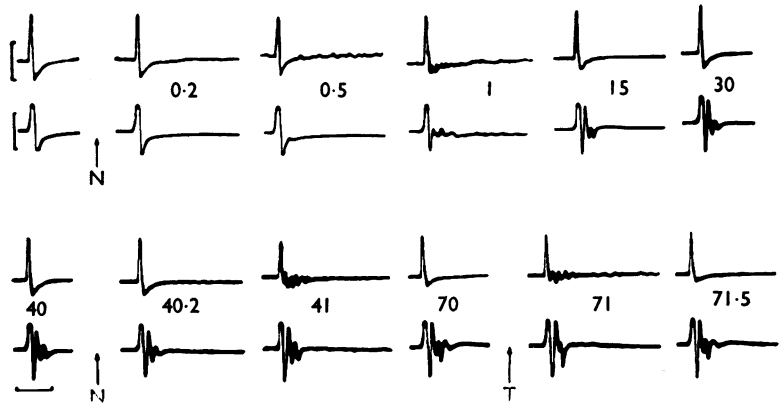

FIG. 8. Cat. Gross muscle action potentials of the tibialis anterior (upper trace in each panel) and soleus muscle (lower trace in each panel) simultaneously elicited indirectly once every 10 sec. The first panel in the upper row is one of a series of constant control responses. At N, neostigmine 50 μg/kg was injected intravenously, and at T, a tetanus (100/sec for 5 sec) was elicited. The numbers denote the times in min after the first dose of neostigmine. Voltage calibrations; 10 mV for tibialis and 20 mV for soleus. Time calibration, 40 msec.

anterior, but remained small in amplitude. In the soleus muscle the repetition became more synchronized and resolved itself into two or three potentials of large amplitude. The repetition in the soleus record then persisted unchanged long after the action potential in the tibialis anterior muscle had returned to normal. With a second injection of neostigmine at this stage, the effect on the tibialis anterior muscle was repeated, but the soleus muscle action potential was barely altered, and the same amount of original repetition continued. When the effect on the tibialis action potential had again disappeared, a short lasting tetanus applied to the motor nerve temporarily restored the repetitive discharge in this muscle, but did not produce much change in that still present in the soleus. After the effect of neostigmine had disappeared, a brief motor nerve tetanus restored the repetitive discharges in the soleus muscle. Although repetitive firing was more pronounced after neostigmine that after a tetanus in the cat soleus, the increase in twitch tension produced by a tetanus was greater than that produced by neostigmine.

In the rabbit, the twitch augmentation produced by neostigmine in the soleus muscle was similar in extent and duration to that produced in the tibialis anterior. The lower records of Fig. 7 illustrate an experiment on both muscles in the rabbit.

Thus the drug-induced repetition was analogous to that produced by a tetanus in that it occurred to the greatest extent in the soleus muscle of the cat. Motor nerve tetani did not themselves result in repetitive discharges in the other three muscles except in the presence of subthreshold amounts of anticholinesterase.

Cholinesterase activity

Figure 9 illustrates concentration/activity curves for tibialis and soleus muscles of both cats and rabbits.

In another series of experiments (five on cat muscles and four on rabbit muscles) cholinesterase activities were determined at only one acetylcholine concentration

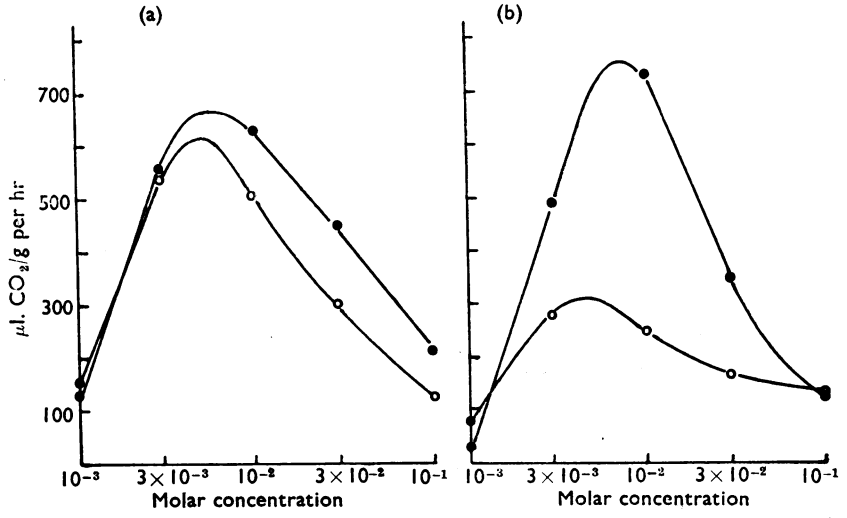

FIG. 9. - Acetylcholine-concentration/activity curves for cholinesterase of the tibialis anterior and soleus muscles of the rabbit (a) and the cat (b). Activity in μl. CO_2/g of muscle per hr was plotted against log molar concentration of acetylcholine. ●, Tibialis anterior ; ○, soleus.

(0.0138M). The mean results obtained, expressed as μl. CO_2 per gram of muscle per hour were: for cat tibialis, 469 (with a standard deviation of 123); for cat soleus, 220 (with a standard deviation of 77); for rabbit tibialis, 569 (with a standard deviation of 97); for rabbit soleus, 446 (with a standard deviation of 97). For each species the t value was calculated from the differences between the means for the two muscles. For the cat, the calculated t value was 3.8; for the rabbit the calculated t value was 1.8. With 8 degrees of freedom (for the cat) the value significant at the 5% level is 2.31, and with 6 degrees of freedom (for the rabbit) the value significant at the 5% level is 2.45. This indicates that the difference in cholinesterase activity of the tibialis and soleus muscles of the cat is significant. By weight, the cat soleus possessed only about half the enzyme activity of the cat tibialis, but there was little difference between the enzyme activity of the two muscles in the rabbit.

Discussion

The results demonstrate that an indirectly elicited high frequency tetanus produces changes both in the transmission process and in the contractility of the muscle fibres of the cat soleus. When the first post-tetanic twitch was elicited within 5 sec of the tetanus, there was little augmentation of tension and the gross action potential was reduced in amplitude suggesting that fewer fibres were contributing to it. Feng *et al.* (1939) showed that, after a high frequency tetanus, each post-tetanic twitch is followed by a period of junctional inhibition lasting 70–100 msec, and it seems probable that after the tetanus itself such junctional inhibition would last for much longer so that even 4 or 5 sec later some of the muscle fibres fail to respond to a nerve impulse. Despite fewer fibres contributing, however, the tension of the twitch was often slightly increased. This could be explained if there was better synchronization of the responses of the active fibres after the tetanus. That this was so was indicated by the faster rate of rise of tension and the reduced duration of the gross action potential. This effect was evident both before and after the period of repetitive firing, and also occurred in directly stimulated fully curarized or chronically denervated muscle.

The results confirm the finding of others (Feng *et al.*, 1939; Standaert, 1963) that the post-tetanic repetitive firing in the soleus muscle of the cat originates at the neuromuscular junction. Thus nerve action potential recording showed that it did not originate in the nerve trunk near the stimulating electrodes; it did not occur when the muscle was stimulated directly, and in the indirectly excited muscle it was blocked by small doses of tubocurarine.

One possibility is that the excitability of the motor endplate to acetylcholine is increased after a tetanus, and the post-tetanic augmentation of contractions produced by close-arterially injected acetylcholine could be interpreted as support for this. The period during which acetylcholine responses were increased was, however, shown to match the period of post-tetanic hyperaemia rather than the augmentation of twitches. The response of a muscle to close-arterially injected acetylcholine is strongly dependent on the rate of injection. Such is the rapidity of action of cholinesterase, that even with close-arterial injection some of the acetylcholine may be destroyed *en route* to its site of action. The amount of destruction will therefore be less with the more rapid injection occurring when the vessels are dilated. Furthermore, the more rapid the injection, the more synchronous the responses of the

various units and the greater the summed tension of the whole muscle. Thus when the vessels are dilated after a tetanus, the resistance to injection is reduced, and synchronization consequently increased. It is therefore concluded that the post-tetanic increase in acetylcholine response is mainly the result of the post-tetanic hyperaemia and owes little if anything to increased motor endplate excitability. A more likely explanation of the post-tetanic repetitive firing is therefore that it involves an increase in and/or a prolongation of the output of acetylcholine from the nerve endings.

Post-tetanic repetitive firing did not occur in the tibialis anterior muscles of the cat or rabbit, or in the soleus muscle of the latter species. Part of the explanation for this difference may be that prolonged stimulation causes a depletion of transmitter in these muscles. Indirect evidence that the cat soleus nerve terminals may contain more stored transmitter than those of tibialis anterior was provided by Bowman & Rand (1961) who showed that hemicholinium and triethylcholine, which interfere with acetylcholine synthesis, were less effective in causing transmission failure in the cat soleus muscle than in the tibialis anterior muscle.

The repetitive firing in the cat soleus muscle was associated with an increased amplitude of the nerve action potential. This effect was consistently observed, yet it was never seen when similar experiments were made on the nerve to tibialis anterior indicating that it was not an artefact arising from polarization phenomena in the electrodes. The increased amplitude of the soleus nerve action potential, which was accompanied by an increase in the threshold stimulus necessary for excitation, may have been the result of hyperpolarization of the nerve fibre membranes. Hyperpolarization and an increase in the presynaptic spike amplitude have been demonstrated following repetitive activity in several pathways in the spinal cord (Lloyd, 1949 ; Eccles & Rall, 1951 ; Wall & Johnson, 1958 ; Eccles & Krnjević, 1959a, b ; Curtis & Eccles, 1960), and in slow-conducting non-myelinated fibres (Brown & Holmes, 1956). Eccles, Eccles & Lundberg (1958) showed that the hyperpolarization occurring after a single impulse in soleus motoneurones is long-lasting (more than 140 msec), and summation of the positive after potentials after a prolonged high frequency tetanus would therefore probably give rise to a prolonged hyperpolarization of the nerve endings. Hyperpolarization of motor nerve endings by anodal currents has been shown to increase the amount of acetylcholine released by a nerve impulse (del Castillo & Katz, 1954 ; Hubbard & Willis, 1962), and post-tetanic hyperpolarization would be expected to produce a similar effect.

The difference between the cat soleus and the other muscles studied is probably quantitative rather than qualitative, because with tetani of lower frequency, post-tetanic facilitation of transmission occurs in all muscles studied. In muscles other than the cat soleus, this effect on transmission, which again is believed to be pre-junctional in origin, is not powerful enough to give rise to repetitive firing ; it does not seem to contribute to the post-tetanic augmentation of twitches in these muscles in normal circumstances (Brown & von Euler, 1938 ; Bowman *et al.*, 1962), but does account for the anti-curare effect of a tetanus (Hutter, 1952).

Another quantitative difference between the soleus muscle of the cat and the other muscles studied was demonstrated by experiments with neostigmine and by cholinesterase determinations. The results showed that the acetylcholinesterase activity per unit weight of the cat soleus muscle was less than that of the other three muscles. These results by themselves would be meaningless since, in order to

account for differences in the transmission process by differences in the amounts of cholinesterase, it would have to be shown that the amount of enzyme per motor endplate was less in the cat soleus muscle than in the other muscles. The demonstration that at a given dose level the effects of the anticholinesterase drug, neostigmine, were most pronounced on the cat soleus muscle, however, provides circumstantial evidence that the cholinesterase activity per unit weight broadly reflects the activity at each junction. Furthermore, according to Gerebtzoff (1959) and Csillik (1965), histological examination shows that cat soleus junctions do in fact contain less enzyme than the junctions in mammalian fast-contracting muscles, and a consideration of the function of this muscle makes this not unlikely. In physiological conditions, the rates of firing in soleus motoneurones are relatively low ; according to Denny-Brown (1928), postural tone in the cat's soleus is maintained by rates of firing in the various motor units of 10–25/sec. Inactivation of transmitter need not therefore be so rapid as in a phasic muscle in order to avoid accumulation between one impulse and the next. With a given extracellular concentration of anticholinesterase drug, the percentage inhibition of the enzyme in all muscles would probably be the same. If the cat soleus junctions contain less enzyme to start with, however, a relatively small dose of neostigmine could reduce the enzyme activity to a level below the safety margin for transmission, while leaving it above this level in other muscles.

It is concluded that the two factors taken together, a greater post-tetanic increase in transmitter release coupled with a smaller cholinesterase content, probably account for the post-tetanic repetitive firing in the cat soleus. The fact that post-tetanic repetitive firing is also produced in other muscles after the injection of a sub-effective dose of anticholinesterase drug, emphasizes that the difference between the cat soleus and other muscles is mainly quantitative rather than qualitative. The observation, confirmed here, that the antidromic repetitive firing in the ventral roots as well as that in the muscle, whether produced by an anticholinesterase or by a tetanus, is blocked by small doses of tubocurarine indicates that acetylcholine is involved. And the more recent demonstration that acetylcholine (Hubbard, Schmidt & Yokota, 1965 ; Riker, 1966) as well as other depolarizing drugs (Standaert & Adams, 1965) can act on motor nerve endings, as they can on other non-myelinated nerve endings, suggests that the drug-induced and the post-tetanic repetitive responses may be explained on this basis. That is, when cholinesterase is inhibited by an anticholinesterase drug, or when the transmitter release mechanism is greatly facilitated by a previous tetanus and this is coupled with a relatively low cholinesterase content, the excess acetylcholine may act at some point near the nerve endings to initiate repetitive firing in the nerve. The finding that externally applied acetylcholine is more likely to produce conduction block than repetitive firing (Hubbard *et al.*, 1965 ; Riker, 1966) need not negate this conclusion, because the response evoked by flooding the junctional region with exogenous acetylcholine might well differ from that produced by a relatively brief and localized release from nerve endings.

It is known that the junctional cholinesterase is induced and maintained by the muscle nerve (Gerebtzoff, 1959 ; Miledi, 1963), and it was therefore of interest that cat soleus muscles, which had been cross-innervated with the nerves formerly innervating a fast-contracting muscle, lost their characteristic post-tetanic repetitive response and resembled a fast-contracting muscle in their reaction. Nerve cross

union has been shown to result in the appropriate change in cholinesterase pattern (Csillik, 1965), and this is therefore compatible with the conclusion that the repetitive soleus muscle response is related to its cholinesterase content. In our experiments the cross-innervated flexor digitorum longus muscle did not come to resemble the soleus muscle in its post-tetanic response, and we have no convincing explanation to offer for this except to point out that the soleus nerve, being much thinner than the nerve to the fast muscle, may not contain sufficient neurones to form an adequate number of functional new junctions for a change to be detectable.

This work was started while the authors were members of the Department of Pharmacology, School of Pharmacy, University of London.

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