

A COMPARISON BETWEEN THE EFFECTS OF A TETANUS AND THE EFFECTS OF SYMPATHOMIMETIC AMINES ON FAST- AND SLOW-CONTRACTING MAMMALIAN MUSCLES

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The effects of adrenaline and isoprenaline on the tension and time-course of the contractions of the tibialis anterior and soleus muscles of cats and rabbits have been compared with the effects of previous high-frequency stimulation. Like a tetanus, adrenaline possessed a facilitating action on neuromuscular transmission and an action exerted directly on the muscle fibres. Isoprenaline possessed only the second of these two actions. The effect of adrenaline and isoprenaline on the muscle fibres was blocked by dichloroisoprenaline and by 1-(2 naphthyl)-2-isopropylaminoethanol, whereas the effect of adrenaline on neuromuscular transmission was blocked by phentolamine and by phenoxybenzamine. In the soleus muscle, both the catechol amines and a previous tetanus caused similar decreases in maximal twitch tension and in the times to peak tension and to half-relaxation. The muscle action potentials were unaltered or slightly increased in amplitude. In the tibialis anterior muscle, a previous tetanus and the catechol amines caused an increase in twitch tension and an increase in the overall duration of the twitch. The muscle action potentials were either unchanged or were slightly decreased in amplitude. In this muscle the effect of a tetanus differed from that of the catechol amines in that the large post-tetanic change was associated with a marked increase in the rate of rise of twitch tension.

Adrenaline and some other sympathomimetic amines have been shown to increase the maximal twitch tension of unfatigued fast-contracting skeletal muscles in various mammalian species (Oliver & Schäfer, 1895 ; Gruber, 1922a and b ; Brown, Goffart & Vianna Dias, 1950 ; Goffart, 1952 ; Bowman & Zaimis, 1958) but to decrease that of the slow-contracting soleus muscle of the cat (Bowman & Zaimis, 1955, 1958 ; Bowman & Raper, 1962 ; Jurna & Rummel, 1962). Like adrenaline, a previous tetanus causes an increase in the maximal twitch tension of fast-contracting skeletal muscles (Rosenblueth & Morison, 1937 ; Brown & von Euler, 1938 ; Feng, Li & Ting, 1939 ; von Euler & Swank, 1940), but in the soleus muscle of the cat, a low-frequency tetanus causes a short-lasting decrease in the tension of the subsequent maximal twitches (Brown & von Euler, 1938). A further similarity is evident in partially curarized muscles, where both a tetanus (Boyd, 1932 ; Brown & von Euler, 1938 ; Hutter, 1952) and adrenaline (Panella, 1907 ; Rosenblueth, Lindsley & Morison, 1936 ; Wilson & Wright, 1937 ; Maddock, Rankin & Youmans, 1948 ; Brown *et al.*, 1950) exert an anticurare action.

In the present experiments a more detailed comparison of the effects of adrenaline, isoprenaline and tetanic stimulation has been made on the tibialis anterior and soleus muscles of cats and rabbits.

METHODS

The experiments were carried out on 47 cats and 26 rabbits. Four cats were decerebrated under preliminary ether anaesthesia and the remainder were anaesthetized by intravenous injection of a mixture of chloralose (80 mg/kg) and pentobarbitone sodium (5 mg/kg). Ten rabbits were anaesthetized with intravenous urethane (6 ml/kg of a 25% solution) and the remainder with an intravenous mixture of chloralose (110 mg/kg) and pentobarbitone sodium (5 mg/kg).

Kymograph recording. In 7 experiments on cats and in 8 experiments on rabbits contractions of the tibialis anterior and soleus muscles were recorded simultaneously on smoked paper. A hind-limb was clamped in a horizontal position to a Brown-Schuster myograph stand and the tendons of insertion of the tibialis anterior and soleus muscles were attached to flat steel spring myographs. Care was taken to free the muscles as completely as possible from muscles in their vicinity. For indirect stimulation the sciatic nerve was ligated and cut high in the thigh and shielded bipolar platinum electrodes were placed on the peripheral portion of the nerve so that the cathode was nearest the muscle. As far as possible, all branches of the peripheral portion of the nerve, other than those supplying the muscles under study, were ligated and cut. Twitches and tetani of the muscles were elicited by rectangular pulses of 50 to 100 μ sec duration and of twice the voltage required to produce a maximal twitch.

Electrical recording. In 40 experiments on cats and in 18 experiments on rabbits, contractions of the tibialis anterior and soleus muscles were recorded isometrically on a Tektronix (type 502) double-beam oscilloscope by means of RCA 5734 mechano-electric transducer valves. In these experiments only one muscle was studied at a time. The sciatic nerve was ligated and cut in the popliteal space. In experiments on the tibialis anterior muscle, stimulating electrodes were placed on the common peroneal nerve and all other branches of the sciatic nerve were cut. In experiments on the soleus muscle of the cat, small stimulating electrodes were placed close to the muscle on the soleus branch of the nerve so that no other muscle was excited. The soleus muscle of the rabbit can be, and was, completely separated from all other muscles in its vicinity right up to its tendon of origin; it was therefore unnecessary to undertake the difficult procedure of identifying and selectively stimulating the soleus branch in this animal. For experiments on the soleus muscle of the rabbit, therefore, the stimulating electrodes were placed on the sciatic nerve, and all main branches, other than that containing the supply to the soleus, were ligated and cut. The tension records showed that the shape of the twitches was not distorted by the contractions of neighbouring muscles. Gross muscle action potentials in response to nerve stimulation were recorded simultaneously with the contractions by means of platinum wires inserted through the belly and the tendon of the muscle or by means of concentric needle electrodes. The nerve was excited with rectangular pulses as described under kymograph recording.

In three cats under chloralose anaesthesia, contractions of the tibialis anterior or soleus muscles, together with the gross muscle action potentials, were elicited by stimulation of the ventral roots. After preparing the muscles for recording, the animal was laid face downwards and the spinal cord was exposed from the level of L.6 to S.1. Each ventral root, L.6, L.7 and S.1, was laid across a platinum hook and the three hooks were connected to the cathode of the stimulator. Stimulation was applied under paraffin between this electrode and a steel crocodile clip placed on the skin near by. All branches of the sciatic nerve was ligated and cut, other than those containing the supply to the muscle under study.

Direct stimulation. Direct electrical stimulation was applied to the fully curarized or chronically denervated muscles of cats under chloralose anaesthesia by means of a platinum wire inserted through the tendon of the muscle and an indifferent electrode attached to the drill in the lower end of the femur.

Denervation was carried out in 6 cats by sectioning the sciatic nerve under pentobarbitone sodium anaesthesia. Degeneration of the nerve was allowed to proceed for 5, 6, 8, 10 or 14 days, two experiments being carried out after 8 days. In experiments on fully curarized muscles, maximal indirect twitches were first recorded and sufficient tubocurarine (about 1 mg/kg intravenously) to block them completely was then injected. Full curarization was maintained by a slow intravenous infusion of tubocurarine (0.3 mg/kg/hr). The electrodes on the nerve were left in position and indirect stimulation was frequently applied throughout the experiments to check that curarization was complete. Contractions were elicited directly by rectangular pulses of 1 msec duration and of sufficient voltage to produce twitches of a tension and time-course similar to those of the indirectly elicited maximal twitches previously recorded. Higher voltages usually produced greater twitches which showed a flattening of the tension crest probably due to repetitive responses from some muscle fibres. Chronically denervated muscles were excited by rectangular pulses of 1 msec duration and 50 V strength. The shape of the twitches did not give the appearance of repetitive firing, but we were unable to ascertain that repetitive firing was completely absent or that the responses were the summed effects of single contractions of all of the individual fibres.

Muscle temperature was maintained by heating lamps and by bathing the muscles in pools of warm liquid paraffin previously equilibrated with Tyrode solution. The operating table was also heated. Muscle temperature was recorded by means of a small copper-constantan thermocouple inserted into the belly of the muscle. Except where otherwise stated, the muscle temperature was between 34 and 36° C.

Drugs were injected intravenously through a cannula in a jugular vein. The drugs used were (—) adrenaline bitartrate (B.D.H.), (±) isoprenaline sulphate (Bayer), dichloroisoprenaline hydrochloride (Lilly), 1-(2 naphthyl)-2-isopropylaminoethanol hydrochloride (I.C.I.) and (+) tubocurarine chloride (B. W. & Co.). The doses of adrenaline and isoprenaline refer to the base; those of the other drugs refer to the salts.

RESULTS

All of the experiments on cats under chloralose anaesthesia were repeated on four decerebrate cats. The results obtained were the same in both preparations and therefore no distinction is made in the following description.

Kymographic recording

Cat. Fig. 1*a* illustrates an experiment on a cat in which adrenaline and a tetanus both caused an increase in the indirectly elicited maximal twitches of the tibialis anterior muscle but a decrease in those of the soleus. The dose of adrenaline used in this experiment (5 µg/kg intravenously) produced only a small effect on the tibialis anterior muscle, but produced the maximal effect obtainable on the soleus. As reported previously (Bowman & Zaimis, 1958; Bowman & Raper, 1962), the smallest effective intravenous doses of adrenaline were of the order of 0.05 µg/kg for the soleus and 3 to 5 µg/kg for the tibialis anterior muscle. Thus, when the action of adrenaline was studied on both muscles simultaneously, it was always necessary to administer doses which produced the maximal effect on the soleus muscle. The effect of a small dose of adrenaline on the tibialis anterior muscle was augmented when it was administered during, or shortly after, the post-tetanic potentiation (Fig. 1*a*). In the soleus muscle it was more difficult to demonstrate potentiation of the adrenaline effect by a tetanus, but when a brief tetanus was applied shortly after a subthreshold dose of adrenaline the resulting post-tetanic decrease in twitch tension was slightly greater and longer-lasting than that occurring

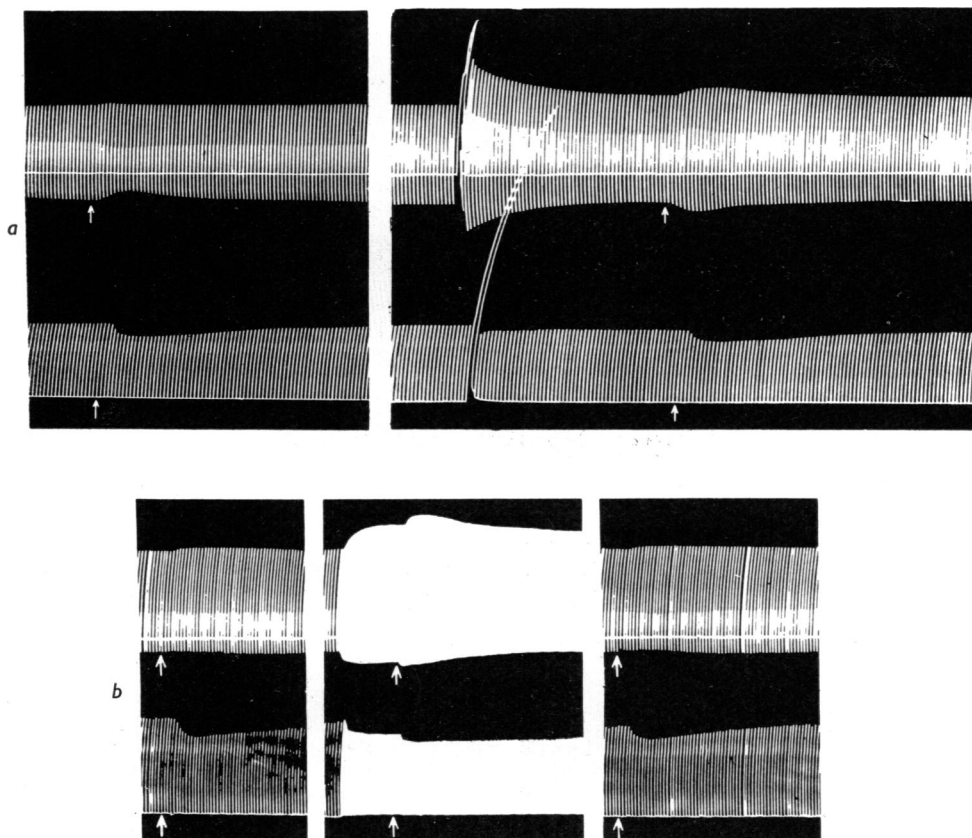


Fig. 1. Cats, chloralose anaesthesia. Kymograph recording of maximal twitches of the tibialis anterior (upper records) and soleus muscles (lower records) elicited indirectly once every 10 sec. At the arrows in *a*, 5 μ g/kg adrenaline was injected intravenously before and after a tetanus (50/sec for 3 sec). In *b*, the frequency of stimulation was increased to 1/sec. At the arrows in *b*, 5 μ g/kg isoprenaline injected intravenously.

in the absence of adrenaline. Isoprenaline, in slightly smaller doses, produced effects similar to those of adrenaline in both muscles (Fig. 1*b*). Maximal post-tetanic potentiation in the tibialis anterior muscle was produced by stimulation at a frequency of 50/sec for about 5 sec. In the soleus muscle, maximum depression of the twitches occurred after stimulation at 10 to 20/sec for 2 to 3 sec. Post-tetanic depression was still usually evident after stimulation at 50/sec, as in Fig. 1*a*, but with higher frequencies of stimulation the post-tetanic twitches remained unchanged or were slightly increased in tension. These results agree with those of Brown & von Euler (1938).

When the frequency of stimulation was increased from 1/10 sec to 1/sec, the twitches of the tibialis anterior muscle increased in tension during the first 1 to 2 min and then became constant. This treppe phenomenon in fast-contracting skeletal muscles is well known (Eccles & O'Connor, 1939). In contrast, the twitches of

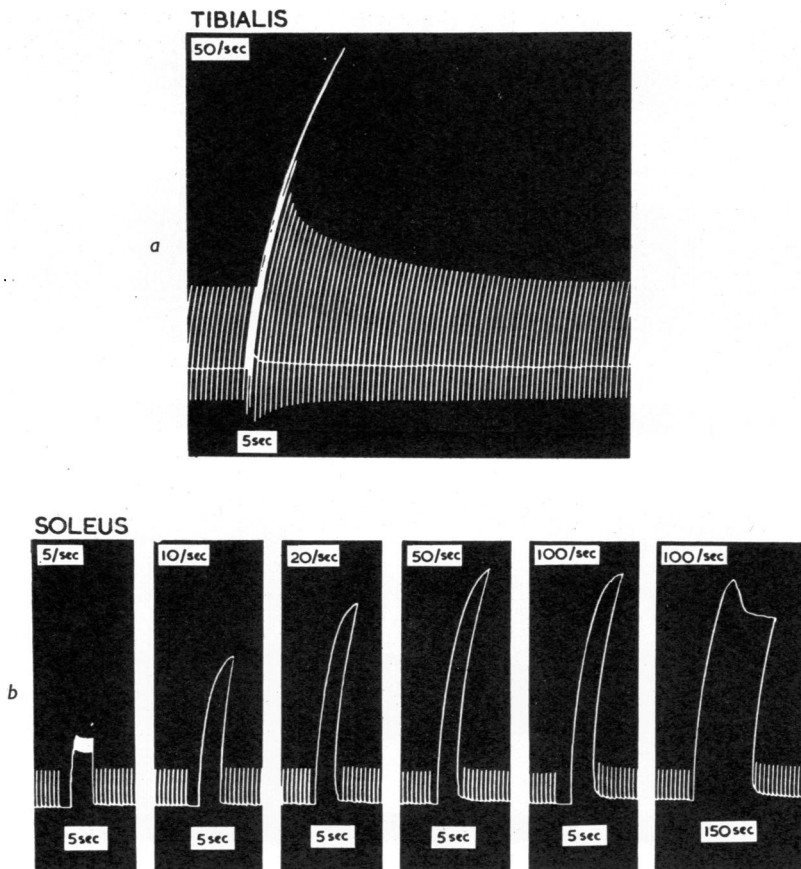


Fig. 2. Rabbits, urethane anaesthesia. Kymograph recording of maximal twitches of the tibialis anterior and soleus muscles elicited indirectly once every 10 sec with tetani, of the frequencies and durations shown, interposed. The kymograph speed was increased during the first five tetani of the soleus muscle.

the soleus rapidly decreased in tension during the first 10 to 20 sec stimulation at 1/sec, after which they become constant at the lower level. Fig. 1*b* illustrates these effects. Adrenaline and isoprenaline were more effective in increasing the twitches of the tibialis anterior muscle when administered during the higher rate of stimulation (Fig. 1*b*).

Brown & von Euler (1938) showed that post-tetanic potentiation still occurred in the directly stimulated tibialis anterior muscle of the cat after the neuromuscular transmitting apparatus had been excluded by degeneration of the nerve or by tubocurarine. This result was confirmed and it was also shown that a post-tetanic decrease in twitch tension occurred in the directly stimulated soleus muscle. Adrenaline and isoprenaline also produced the same effects in the directly stimulated muscle as in the indirectly stimulated muscles. These results confirm those of Bowman & Zaimis (1958).

Rabbit. In rabbits anaesthetized with urethane or with chloralose, the effects of the catechol amines and of tetanic stimulation on the tibialis anterior muscles were similar to those described for the cat. However, in the soleus muscle of the rabbit under urethane anaesthesia, both the catechol amines and tetanic stimulation were usually without any appreciable effect on the maximal twitches. Occasionally a slight decrease in twitch tension occurred when the rate of stimulation was increased from once every 10 sec to once every sec, but it was always a small and insignificant effect compared with that seen in the cat. Fig. 2*a* illustrates post-tetanic potentiation in the indirectly excited tibialis anterior muscle of the rabbit under urethane anaesthesia, and Fig. 2*b* illustrates the absence of effect of tetani of various frequencies and durations on the post-tetanic twitches of the soleus muscle. In the rabbit under chloralose anaesthesia the catechol amines and tetanic stimulation produced effects on the soleus muscle similar to those described for the same muscle in the cat, but the effects in the rabbit were always less pronounced than those seen in the latter species.

Oscilloscope recording

Indirect stimulation

Tibialis anterior muscle. In experiments on 24 cats the maximal twitches of the tibialis anterior muscle elicited indirectly 1/10 sec showed times from the start of the contraction to the tension crest ranging from 20 to 32 msec and from the tension crest to half-relaxation ranging from 21 to 32 msec. The corresponding times for the same muscle in 12 rabbits were 13.5 to 17.5 msec and 13.5 to 16.5 msec respectively. In any one animal these times remained constant for a given frequency of stimulation providing the muscle temperature did not change.

After an indirectly elicited tetanus of 50/sec frequency and 5 sec duration the marked increase in the twitch tension of the tibialis anterior muscles of both cats and rabbits was associated with an increase in the time to peak tension and an increase in the rate of tension development. Figs. 3*a* and 4*a* illustrate typical results in the cat and the rabbit respectively. In these experiments, the time to peak tension was increased in the cat from 25 msec to 30 msec and in the rabbit from 15 msec to 18.6 msec. These changes in time-course accompanied increases in tension of 139% in the cat and 82% in the rabbit.

When the frequency at which maximal twitches of the tibialis anterior muscles were elicited was increased from 1/10 sec to 1/sec, the increase in twitch tension which occurred was always associated, in both cats and rabbits, with decreases in the times to peak and to half-relaxation (Fig. 4*b*). In the experiment of Fig. 4*b*, the twitches gradually increased up to 51% over their original tension during 80 sec stimulation at 1/sec; they then became constant at this increased level. The times to peak and to half-relaxation were decreased from 15 to 11.8 msec and from 14.1 to 9.5 msec respectively. Although in the present experiments the post-tetanic twitches were associated with increases in their times to peak while the more rapidly elicited twitches were associated with decreases in their times to peak, the difference might simply be due to the fact that the increase in twitch tension was considerably greater after a tetanus. In fact, the changes in the post-tetanic twitches of the cat tibialis anterior muscle recorded by Brown & von Euler (1938) resembled those

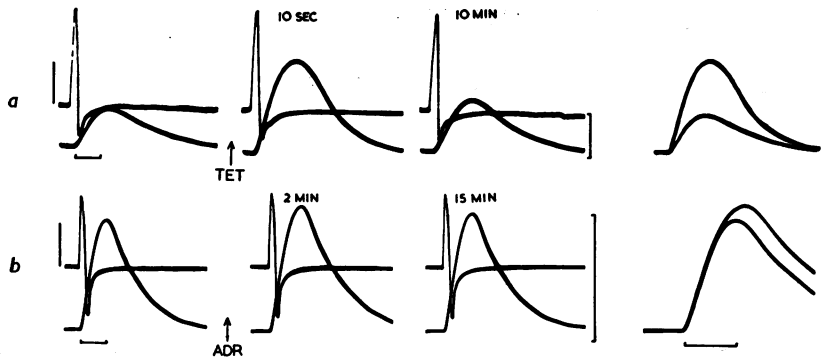


Fig. 3. Cats, chloralose anaesthesia. Oscilloscope recordings of maximal twitches and gross action potentials of tibialis anterior muscles elicited indirectly once every 10 sec. The first record in each row is one of a series of identical control responses. In *a*, a tetanus (50/sec for 5 sec) (TET) was interposed; and in *b*, adrenaline (10 μ g/kg) (ADR) was injected intravenously. The times above the records indicate the time after the tetanus or after adrenaline. The last record in *a* is the twitches of the first two superimposed. The last record in *b* is a superimposition of two twitches, recorded at double the sweep speed, 10 sec after each of the first two shown. Calibrations: action potentials on the left, 20 mV; tension on the right, 1 kg; time below, 25 msec.

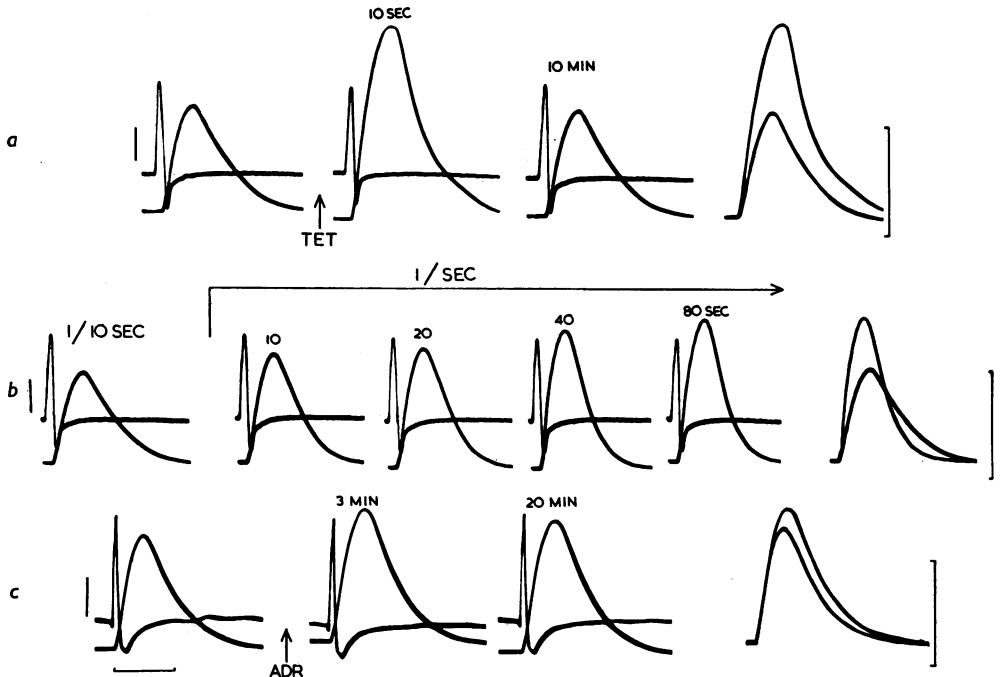


Fig. 4. Rabbits, urethane anaesthesia. Tibialis anterior muscles. Rows *a* and *c* as in Fig. 3. Row *b* shows the effect of increasing the frequency of stimulation from 1/10 sec to 1/sec on the tension and time-course of the indirectly excited maximal twitches. The numerals above the records denote the time in sec after increasing the frequency of stimulation. The last record in *b* is the twitch after 80 sec stimulation at 1/sec superimposed on the control twitch recorded when the frequency was 1/10 sec. Calibrations: action potentials on the left, 10 mV; tension on the right, 0.5 kg; time below, 30 msec.

recorded in the present experiments merely by increasing the rate of stimulation to 1/sec. Their experiments were carried out at a low muscle temperature, and this may have accounted for the smaller increase in tension which they recorded. The most outstanding change in the potentiated twitches was the marked increase in the rate of rise of tension. This is clearly illustrated in Figs. 3*a*, 4*a*, and 4*b*.

Macpherson & Wilkie (1954) showed in frog muscle that the rate of rise of tension in a maximal tetanus was initially the same as that in a twitch, and this was also found to be so in the present experiments on mammalian muscle. Fig. 5 illustrates the rising phase of the tension curve of a maximal tetanus superimposed on that of

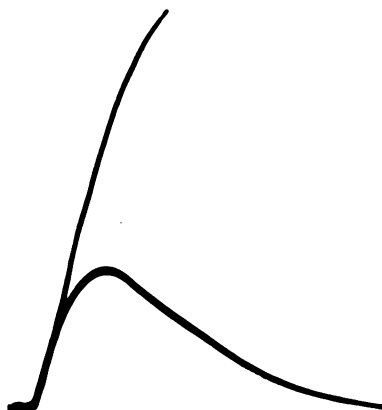


Fig. 5. Cat, chloralose anaesthesia. Oscilloscope recording of one of a series of maximal twitches of the tibialis anterior muscle, elicited indirectly once every 10 sec, superimposed on the rising phase of a maximal tetanus (120/sec) subsequently elicited indirectly with the same gain and time base. Note that the two curves separate only near the peak of the twitch.

a twitch. It can be seen that the two curves initially run together and separate only near the peak of the twitch. However, the rate of rise of tension in a post-tetanic twitch was much greater than that which had occurred in the tetanus itself. As might be expected, a second tetanus, initiated during the period of potentiation following the first, showed a rate of rise of tension initially the same as that of a potentiated twitch. The rate of relaxation of a potentiated twitch was also increased. This is clearly seen if the slopes of the declining phases of the superimposed twitches in Figs. 3*a*, 4*a* and 4*b* are compared.

The potentiation of the maximal twitches of the tibialis anterior muscles of both cats and rabbits produced by adrenaline or by isoprenaline was much smaller than that occurring after a tetanus or during an increase in stimulation frequency. In 30 experiments, intravenous doses of 10 to 15 $\mu\text{g/kg}$ of adrenaline caused increases of 6 to 20% in the twitch tension. The rate of rise of tension of the twitch after adrenaline or isoprenaline was the same as that of the control twitches, but the tension continued to rise to reach a peak 2 to 6 msec later. The rate of fall of tension was also about the same after the catechol amines as before, so that the declining curves of the control and the potentiated twitches were parallel over most of their

lengths. These results confirm previous reports by Bowman & Zaimis (1958) and Bowman & Raper (1962), who experimented on cats, and those of Goffart & Ritchie (1952), who studied the effects of adrenaline on the isolated rat diaphragm preparation. Figs. 3 and 4 compare the effects of repetitive stimulation with the effects of adrenaline on the tension and time-course of the maximal twitches of the tibialis anterior muscle of the cat and of the rabbit respectively.

The changes in the gross muscle action potentials produced by a tetanus, by an increase in stimulation frequency or by the catechol amines were very slight in both the cat and the rabbit. In 15 experiments, marked post-tetanic potentiation of the twitches was unaccompanied by any detectable change in the gross muscle action potentials. Fig. 3*a* illustrates such an experiment. In the remaining 5 experiments, the post-tetanic potentiation was accompanied by a slight diminution (2 to 5%) in the peak voltage of the muscle action potential, but its width remained unaffected. Fig. 4*a* illustrates a slight decrease in the amplitude of the muscle action potential during post-tetanic potentiation, and Fig. 4*b* illustrates a similar slight diminution when the frequency of stimulation was increased from once every 10 sec to once every sec. Care was taken to maintain the muscle temperature as near to normal as possible; we were not always successful in this respect, and we gained the impression that a diminution in post-tetanic action potentials was more likely to occur at lower muscle temperatures. Brown & von Euler (1938) always recorded a diminution in the gross muscle action potential, but the muscle temperature in their experiments was low.

The increase in twitch tension produced by adrenaline or by isoprenaline was also accompanied either by no change or by a slight (up to 2%) diminution in the gross muscle action potential. In the experiment of Fig. 3*b* no change is evident, while in that of Fig. 4*c* a slight reduction in amplitude is detectable. Goffart (1952), who used much larger doses, also recorded a small reduction in the voltage of the muscle action potential during the adrenaline effect. These findings apply only to the unfatigued muscle of a fresh preparation. When a slight degree of transmission failure had developed through prolonged periods of stimulation, then adrenaline, but not isoprenaline, often caused a small increase in the amplitude of the action potential. The increase in voltage was probably caused by recruitment of those muscle fibres which had been failing to respond to the supramaximal nerve shocks (cf. Krnjević & Miledi, 1958). This effect of adrenaline appears to be due to an action on neuromuscular transmission and is discussed later; it is independent of the effects on the contractions of a fresh preparation.

The effects of the catechol amines were more clearly seen when tetanic contractions were elicited in place of single twitches. When brief unfused tetani of the muscle were elicited at intervals less than 5 min, each of the first 5 to 10 contractions showed an increase in tension and in degree of fusion over the preceding one. Subsequent contractions were maintained at a constant elevated level. Fig. 6*a* illustrates this effect in the tibialis anterior muscle of the cat. When a single tetanus of higher frequency was interposed, the subsequent lower-frequency tetani were further potentiated for 1 to 2 min (Fig. 6*b*). Adrenaline or isoprenaline produced similar changes. Fig. 6*c* illustrates the increase in tension and in degree of fusion produced

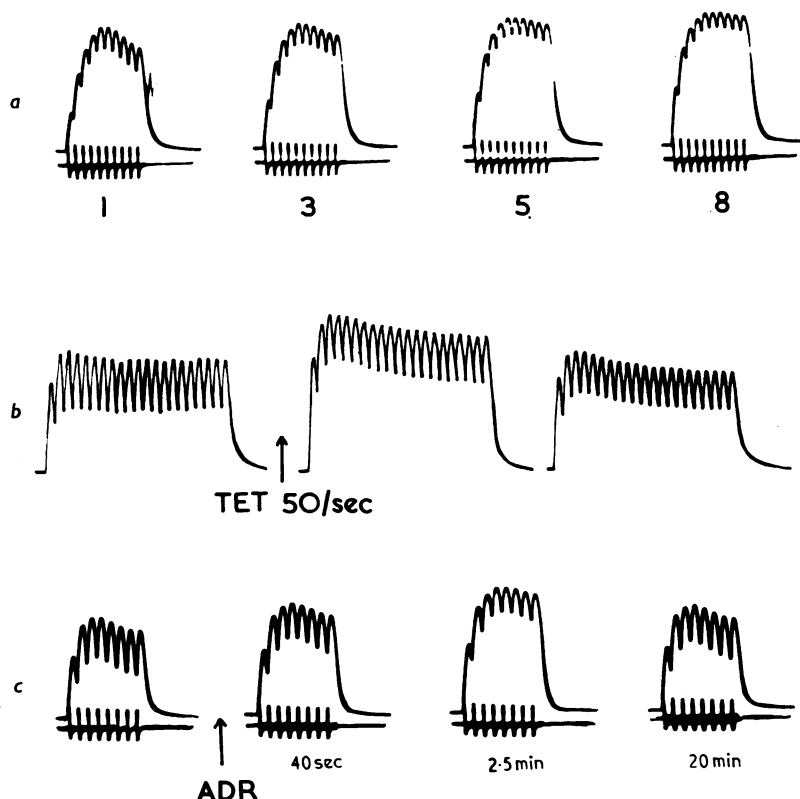


Fig. 6. Cats, chloralose anaesthesia. Tibialis anterior muscles stimulated indirectly at 40/sec for 0.25 sec every 10 sec in *a*, at 20/sec for 1 sec every 10 sec in *b*, and at 32/sec for 0.25 sec every 10 sec in *c*. *a* and *c* include records of the gross muscle action potentials. *a* shows the increase in tension and in degree of fusion which gradually occurs in a series of contractions; the numeral below each record indicates the number of the contraction in the series. After the eighth contraction, tension and fusion remained constant. In *b*, one of a series of constant contractions was replaced by a tetanus of higher frequency (50/sec for 5 sec) (TET). The figure shows the first subsequent lower-frequency contraction and another 4 min later when the potentiation had disappeared. In *c*, adrenaline (8 $\mu\text{g}/\text{kg}$) (ADR) was injected intravenously during a series of constant contractions. The times below the records show the times after adrenaline.

by 8 $\mu\text{g}/\text{kg}$ of adrenaline injected intravenously during a series of constant contractions elicited by stimulation of the motor nerve at a frequency of 32/sec for 0.25 sec every 10 sec.

Soleus muscle. In experiments on 20 cats, maximal twitches of the soleus muscle elicited indirectly 1/10 sec showed times from the start of the contraction to the tension crest of 72 to 112 msec and from the tension crest to half-relaxation of 70 to 136 msec. The times were slightly longer in the corresponding muscle of the rabbit; in experiments on 8 rabbits the soleus showed times to peak of 100 to 125 msec and times to half-relaxation of 117 to 160 msec.

The decrease in twitch tension produced by adrenaline or by isoprenaline in the soleus muscle of the decerebrate or chloralosed cat and of the rabbit under chloralose

anaesthesia was associated with a diminution in the times to peak twitch tension and to half-relaxation; the rate of rise of tension was unaffected. The effect of isoprenaline was slightly greater than that of adrenaline. These results on the cat under chloralose anaesthesia confirm previous reports (Bowman & Zaimis, 1958; Bowman & Raper, 1962). In experiments on 15 cats, doses of 1 $\mu\text{g}/\text{kg}$ of adrenaline given intravenously caused decreases in twitch tension which ranged from 4 to 12%, decreases in time to peak of 6 to 26% and decreases in time to half-relaxation of 12 to 26%. The effects in the rabbit under chloralose anaesthesia were less pronounced than they were in the cat. In experiments on 6 rabbits, the same doses caused decreases in twitch tension of 1 to 7%, decreases of 2 to 8% in time to peak and decreases of 10 to 20% in time to half-relaxation. The smallest effective intravenous doses of adrenaline and isoprenaline were of the order of 0.05 $\mu\text{g}/\text{kg}$. With threshold doses, sometimes the only effect seen was a small decrease in the time from the tension crest to half-relaxation; that is, the rate of relaxation had been increased. The maximal effect in the cat and in the rabbit under chloralose anaesthesia was produced by doses of the order of 3 $\mu\text{g}/\text{kg}$ intravenously. Increase in the dose merely prolonged the effect. The greatest effect recorded on the soleus muscle of the cat was a decrease in tension of 14%, accompanied by decreases of 17% and 29% in the times to peak tension and to half-relaxation respectively. This effect was produced by 2 $\mu\text{g}/\text{kg}$ adrenaline intravenously. In the rabbit under urethane anaesthesia, small doses of adrenaline (1 $\mu\text{g}/\text{kg}$) were without effect on the soleus muscle, whilst larger doses (4 to 10 $\mu\text{g}/\text{kg}$), which would have produced a maximal effect under chloralose anaesthesia, caused only a slight speeding in relaxation.

High-frequency indirect stimulation of the soleus muscle of the cat (100/sec for 2 to 10 min) caused pronounced post-tetanic potentiation which was associated with repetitive firing of the muscle fibres. This effect has been recorded by others and has been shown to be due to an action on neuromuscular transmission (Feng *et al.*, 1939; Werner, 1960). It was not studied in detail in the present experiments.

The changes in the twitches of the soleus muscle following a low-frequency tetanus, or occurring during an increase in frequency from once every 10 sec to once every sec, were qualitatively identical with those produced by the catechol amines. In the cat and in the rabbit under chloralose anaesthesia, the catechol amines were capable of producing much greater effects than those produced by a tetanus of any frequency or duration, whereas in the rabbit under urethane anaesthesia slight post-tetanic effects were observed even when adrenaline and isoprenaline had no effect on the tension or time-course of the twitches.

The weak effects of the catechol amines and of a tetanus on the soleus muscle of the rabbit under urethane anaesthesia are probably explained by the discharge of adrenaline from the adrenal medulla caused by this anaesthetic (Elliot, 1912; Hökfelt & McLean, 1950). Thus, under urethane anaesthesia, the sensitive soleus muscle is probably already almost maximally affected by the released adrenaline and little further effect can be produced. However, the amounts of adrenaline released from the adrenals are probably too small to affect the tibialis anterior muscle and the injection of catechol amines or tetanic stimulation still produces marked effects.

Emmelin & Strömblad (1951) have shown that there is no appreciable discharge of adrenaline from the adrenal medulla under chloralose anaesthesia.

During the depression of the twitches produced by the catechol amines or by repetitive stimulation, the gross muscle action potentials remained unchanged or were slightly increased in amplitude. In six experiments with catechol amines and in nine experiments in which post-tetanic effects were studied, no change could be detected in the muscle action potentials. In five experiments with catechol amines and in two in which post-tetanic effects were studied, the decrease in twitch tension was accompanied by a slight increase (0.4 to 3%) in the amplitude of the action potentials. Fig. 7 compares the effects of a tetanus with those of adrenaline on the twitches and action potentials of the soleus muscle of the cat. As in the tibialis

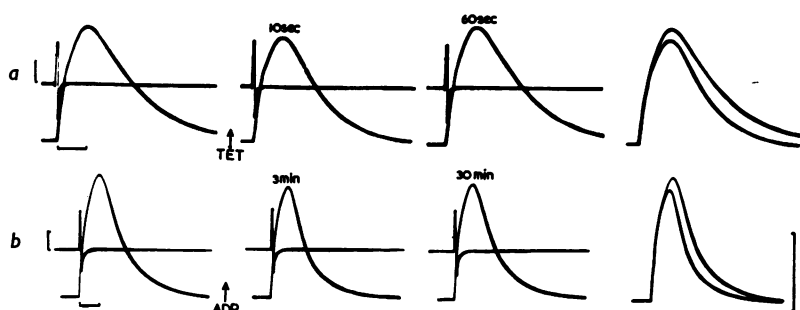


Fig. 7. Cats, chloralose anaesthesia. Oscilloscope recordings of maximal twitches and gross action potentials of soleus muscles elicited indirectly once every 10 sec. In *a*, a tetanus (20/sec for 2 sec) (TET) was interposed. In *b*, adrenaline (1 $\mu\text{g}/\text{kg}$) (ADR) was injected intravenously. The times above the records denote the times after the tetanus or after adrenaline. The last records in each row are the twitches of the first two superimposed. Calibrations: action potentials on the left, 20 mV; tension on the right, 0.25 kg; time below, 100 msec.

anterior muscle, there was no correlation between the effect on tension and the effects on the action potential. Marked tension changes were not always accompanied by detectable changes in the muscle action potentials.

In both the cat and the rabbit the first three or four of a series of low-frequency tetani of the soleus muscle showed a progressive decrease in tension and degree of fusion; subsequent tetani remained constant. Adrenaline and isoprenaline injected during the constant stage in a series of tetani caused a further marked reduction both in the tension and in the degree of fusion. Fig. 8 illustrates these effects in the soleus muscle of the cat. The effect in the cat was always more marked than that in the rabbit. The smallest dose of adrenaline found to affect submaximal tetani of the soleus muscle of the cat was 0.0125 $\mu\text{g}/\text{kg}$ intravenously.

In both the tibialis anterior and the soleus muscles the catechol amines produced more pronounced changes in the tension of unfused tetani than would be expected from their effects on the maximal twitch. This is because two factors contribute to the pronounced effect on submaximal tetanic tension. These are the change in tension of the individual responses and the change in fusion which occurs because

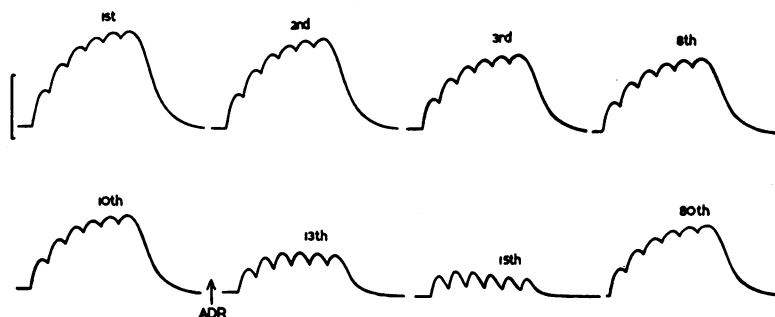


Fig. 8. Cat, chloralose anaesthesia. Oscilloscope recordings of contractions of the soleus muscle elicited indirectly at a frequency of 6/sec for 1 sec every 10 sec. After the 8th contraction, the tension and degree of fusion were constant. Adrenaline ($2 \mu\text{g/kg}$) (ADR) was injected intravenously immediately after the 10th contraction. Tension calibration, 1 kg.

of the altered duration of each twitch. In contrast, the effects of conditioning tetani on the tension and fusion of subsequent tetani were less pronounced than might have been expected from their effects on the maximal twitch. This was possibly because the change in contractility had already occurred to a large extent during the first tetanus of the series so that subsequent tetani showed only a small further change. This may also be the explanation for the finding that, in both muscles, the effects of conditioning tetani and of the catechol amines were more marked the lower the stimulation frequency of each tetanus in the series. A series of maximal tetani (120/sec for tibialis anterior and 80/sec for soleus) did not exhibit staircase effects, nor were they altered by the catechol amines possibly because the maximal change in contractility had already occurred during the first tetanus.

The pulse widths and voltages used to stimulate the motor nerves in these experiments were unlikely to have been sufficiently great to excite the non-myelinated post-ganglionic autonomic fibres running in the sciatic trunk. Nevertheless, in experiments on three cats, muscle contractions were elicited by stimulation of the motor roots. The effects of tetanic stimulation and of the catechol amines both on the tibialis anterior and on the soleus muscle were identical with those already described, and it is therefore concluded that any stimulation of autonomic fibres which might have occurred in other experiments did not influence the results obtained.

When the blood flow to the muscles was prevented by occluding the femoral artery, a tetanus or an increase to 1/sec in the frequency of stimulation caused the same changes in tension and time-course of the twitches as those produced in the normal muscles. These results confirm those of Brown & von Euler (1938) and show that the effects were not a consequence of the hyperaemia of contraction which is known to occur in both the tibialis anterior and the soleus muscles (Bowman, 1959).

Direct stimulation

In the directly stimulated fully curarized or chronically denervated muscles of the cat, a tetanus, an increase in frequency from 1/10 sec to 1/sec and the injection of

the catechol amines produced changes in the tension and time-course of the twitches which were similar to those produced in the indirectly stimulated muscles. In the chronically denervated muscles, the effects of repetitive stimulation were less pronounced when more than 8 days had elapsed since the nerve section. Brown & von Euler (1938), who also found that post-tetanic potentiation in the tibialis anterior became much smaller after a similar period of denervation, concluded that the diminished effect was a consequence of atrophic changes in the muscle. In the chronically denervated muscles, adrenaline and isoprenaline often produced an increase in the background tension of the muscle (Bowman & Zaimis, 1961), but their effects on the directly elicited twitches could be seen superimposed on this increased tension.

Effect of temperature

Post-tetanic potentiation is accompanied by a rise in muscle temperature (Brown & von Euler, 1938), and the calorogenic effects of adrenaline and isoprenaline are well known (Lundholm, 1949; Griffith, 1951). The possibility that the tension changes might be secondary to temperature changes was therefore tested in two decerebrate cats as follows. All heating was removed, and, in one experiment, the temperature of the tibialis anterior muscle and, in the other, that of the soleus muscle were allowed to fall to 30° C and 29° C respectively. The twitch tension of both muscles was reduced by the fall in temperature and the times to peak tension and to half-decay were prolonged. In the tibialis anterior muscle, the twitch tension decreased by 21%, the time to peak increased from 21 to 25 msec and the time to half-relaxation from 20 to 23 msec. In the soleus muscle the twitch tension decreased by 11%, the time to peak increased from 98 to 135 msec and the time to half-decay from 75 to 95 msec. In both of the cooled muscles, tetanic stimulation and adrenaline produced effects on the tension and time-course of the twitches which were similar to those produced in the warm muscles. The increases in muscle temperature produced by a tetanus and by adrenaline were slight (less than 1° C) and too small to be measured accurately by the available apparatus. The muscles were then gradually re-warmed to 36° C while continually recording the isometric twitches. Re-warming increased the tension, the rate of rise of tension and the rate of relaxation in both muscles. This effect of increase in temperature in the soleus muscle was therefore quite different from the effects of a tetanus and of adrenaline; it was also different from the effect of adrenaline in the tibialis anterior muscle. A tetanus of the tibialis anterior muscle produced qualitatively similar effects to those produced by raising the muscle temperature, but even a 6° C rise in temperature was less effective than a tetanus. Furthermore, Brown & von Euler (1938) showed that the small rise in muscle temperature produced by a tetanus of the tibialis anterior muscle reached its peak 2 to 3 min after the tetanus, but post-tetanic potentiation was maximal immediately after the tetanus and then gradually declined. These results show that the effects of tetanic stimulation and those of the catechol amines on the maximal twitches of the tibialis anterior and soleus muscles cannot be a direct consequence of the small increase in muscle temperature which they produce.

Anticurare action

Experiments in which the anticurare actions of the catechol amines and of indirectly elicited tetani were studied were carried out in cats under chloralose anaesthesia. When the maximal indirect twitches of the tibialis anterior or the soleus muscles were partially blocked by tubocurarine, both adrenaline (Bowman & Zaimis, 1955) and a tetanus exerted an anticurare action. Isopropylnoradrenaline did not antagonize tubocurarine paralysis, and its effects on the partially blocked twitches resembled those in the unblocked muscles. During partial tubocurarine paralysis both adrenaline and a tetanus were capable of producing more powerful increases in the twitch tension of the tibialis anterior muscle than they were in the unblocked muscle. The anticurare effect of adrenaline was less pronounced in the soleus muscle than in the tibialis anterior. The increase in the tension of the partially blocked twitches of both muscles, produced either by a tetanus or by adrenaline, was accompanied by a corresponding increase in the amplitude of the gross muscle action potential. These results indicate that the effect was largely a true antagonism caused by recruitment of previously blocked muscle fibres. Fig. 9 illustrates an experiment

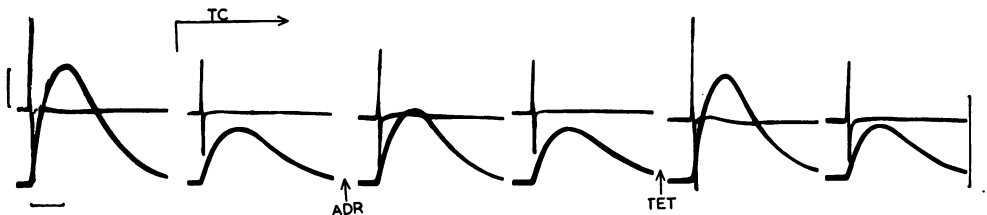


Fig. 9. Cat, chloralose anaesthesia. Oscilloscope recording of maximal twitches and gross action potentials of soleus muscle elicited indirectly once every 10 sec. The first record shows a twitch of the muscle before tubocurarine. The second record illustrates the constant degree of block produced by a continuous infusion of tubocurarine (0.25 mg/kg/hr intravenously) (TC). The third record was taken 50 sec after the intravenous injection of 5 μ g/kg adrenaline (ADR) at the height of its anticurare effect. After 3 min (fourth record) the effect of adrenaline had worn off. The remaining two records were taken 10 sec and 100 sec after a tetanus (30/sec for 2 sec) (TET). Calibrations: action potentials on the left, 10 mV; tension on the right, 0.25 kg; time below, 100 msec.

on the soleus muscle in which a tetanus (30/sec for 2 sec) and adrenaline (5 μ g/kg intravenously) both caused an anticurare effect. In the same cat before tubocurarine was administered, the same dose of adrenaline and the same tetanus caused the characteristic decrease in maximal twitch tension already described.

Effect of antiadrenaline drugs

The anticurare effect of adrenaline was completely blocked by the previous administration of phenoxybenzamine (10 mg/kg) or phentolamine (3 mg/kg). These findings confirm those of Maddock *et al.* (1948) and Brown *et al.* (1950). However, as reported by Brown *et al.* (1950) and Bowman & Zaimis (1958), the direct effects of adrenaline and of isoprenaline on the maximal twitches of the non-curarized tibialis anterior and soleus muscles of the cat were not abolished by the previous administration of the same doses of phenoxybenzamine or phentolamine.

Experiments were also carried out using dichloroisoprenaline, a blocking agent which has been shown to prevent many of the inhibitory actions of adrenaline and isoprenaline on smooth muscle, their inotropic and chronotropic actions on the heart, and some of the metabolic effects of the amines (Powell & Slater, 1958 ; Moran & Perkins, 1958 ; Claasen & Noach, 1960 ; Mayer, Moran & Fain, 1961). Fig. 10

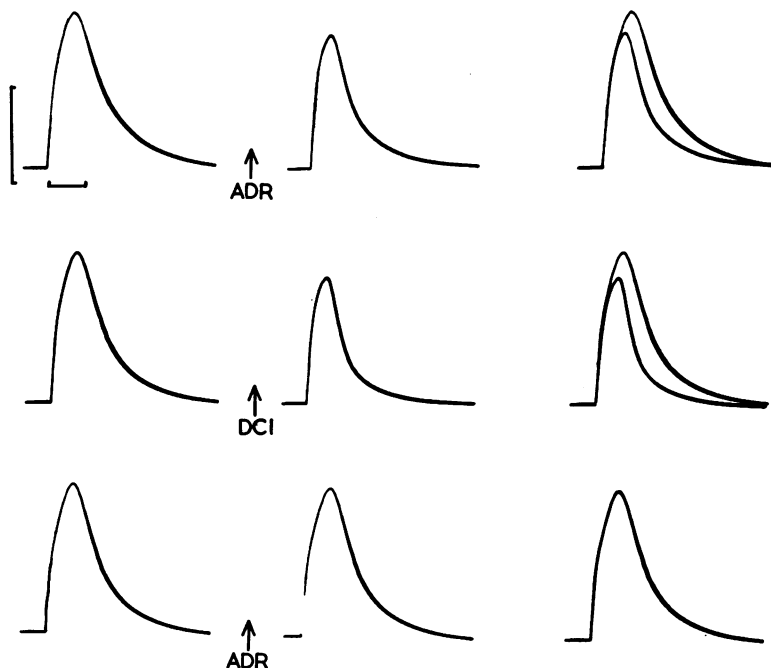


Fig. 10. Cat, chloralose anaesthesia. Oscilloscope recording of maximal twitches of soleus muscle elicited indirectly once every 10 sec. Upper row, before and 50 sec after adrenaline ($1 \mu\text{g/kg}$) (ADR). Middle row (20 min later) before and 50 sec after dichloroisoprenaline (DCI) (10 mg/kg intravenously). Lower row (20 min after dichloroisoprenaline), before and 50 sec after adrenaline ($1 \mu\text{g/kg}$) (ADR) intravenously. The last record in each row shows the first two superimposed. Calibrations: tension on the left, 0.25 kg ; time below, 100 msec .

shows the effect of dichloroisoprenaline on the response of the non-curarized soleus muscle to adrenaline. Dichloroisoprenaline, in a dose of 10 mg/kg intravenously, itself produced an effect like that of the catechol amines in the soleus muscle. This sympathomimetic effect of dichloroisoprenaline began to wear off about 5 min after the injection, but the twitches did not completely regain their normal tension. By about 15 min after injection they had reached a constant level slightly below that of the original tension. Adrenaline or isoprenaline, administered when the twitches had recovered to this constant level, no longer produced any detectable effect on their tension or time-course. Thus 10 mg/kg of dichloroisoprenaline blocked the effect of $1 \mu\text{g/kg}$ of adrenaline on the soleus muscle. Dichloroisoprenaline did not itself affect the twitches of the tibialis anterior muscle to any great extent, but blocked or considerably reduced the effects of the much larger doses of adrenaline or

isoprenaline necessary to affect this muscle. In the same doses, dichloroisoprenaline did not prevent the anticurare action of adrenaline.

A few experiments were carried out using 1-(2 naphthyl)-2-isopropylaminoethanol hydrochloride (nethalide), a drug which appears to possess antiadrenaline properties similar to those of dichloroisoprenaline (Black & Stephenson, 1962). Doses of 10 mg/kg of this drug effectively prevented the direct effects of adrenaline and isoprenaline on the non-curarized tibialis anterior and soleus muscles of cats and rabbits but did not reduce the anticurare action of adrenaline. The drug had less sympathomimetic activity than dichloroisoprenaline and did not itself affect the

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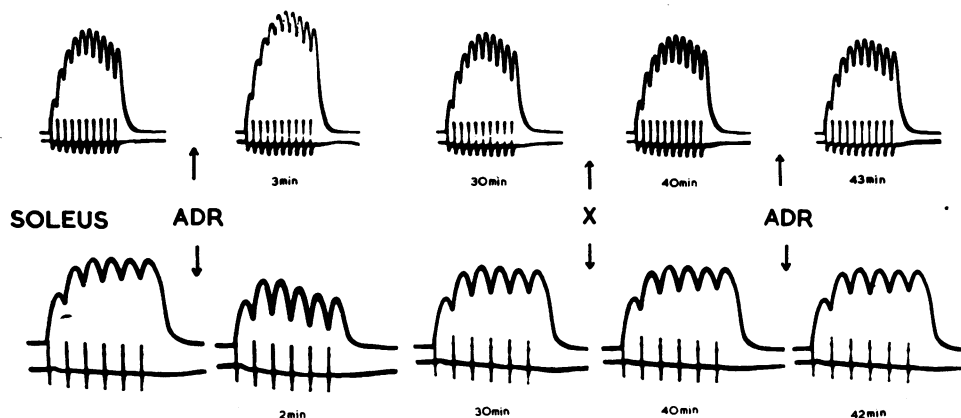


Fig. 11. Cats, chloralose anaesthesia. Upper records, tetani and gross action potentials of the tibialis anterior muscle elicited indirectly at a frequency of 40/sec for 0.25 sec every 10 sec. Lower records, the same for the soleus muscle elicited indirectly at a frequency of 6/sec for 1 sec every 10 sec. The dose of adrenaline (ADR) was 10 μ g/kg intravenously in the experiment on the tibialis anterior muscle and 1 μ g/kg intravenously in the experiment on the soleus muscle. The dose of 1-(2 naphthyl)-2-isopropylaminoethanol hydrochloride (X) was 10 mg/kg intravenously in both experiments. The times below the records indicate the times after the first injection of adrenaline.

twitches of either muscle. Fig. 11 illustrates blockade by nethalide of the effects of adrenaline on unfused tetani of the tibialis anterior and soleus muscles of the cat.

None of the antiadrenaline drugs prevented the effects of increasing the frequency of stimulation or of tetanic stimulation on the twitches of either muscle.

DISCUSSION

The experiments confirmed the finding of others (Brown *et al.*, 1950; Goffart, 1952; Bowman & Zaimis, 1958) that the effects of adrenaline on the contractions of the non-curarized, non-fatigued muscles are due to actions on the muscle fibres themselves, since the same changes were produced during direct stimulation. Bowman & Zaimis (1958) showed that these effects were independent of the concomitant vascular changes produced in the individual muscles. The anticurare action of adrenaline is clearly different from its direct action on the muscle fibres.

During partial tubocurarine paralysis, adrenaline increased the contractions both of the tibialis anterior and of the soleus muscle, and recordings of the gross muscle action potential showed that this effect arose through an increase in the number of contracting muscle fibres. This action of adrenaline on neuromuscular transmission was prevented by the previous administration of phenoxybenzamine or of phentolamine, but was unaffected by dichloroisoprenaline and 1-(2 naphthyl)-2-isopropylaminoethanol. Isoprenaline did not possess an anticholinergic action but was slightly more active than adrenaline in its action on the muscle fibres themselves; both amines increased the contractions of the tibialis anterior muscle but decreased those of the soleus. These effects were blocked by dichloroisoprenaline and by 1-(2 naphthyl)-2-isopropylaminoethanol, but not by phenoxybenzamine or by phentolamine. On the classification of Ahlquist (1948), therefore, the anticholinergic action of adrenaline can be considered an α -receptor effect whereas the actions of the amines on the muscle fibres themselves correspond to β -receptor effects.

Like adrenaline, a tetanus affected the subsequent contractions of the muscles in two ways. Post-tetanic potentiation in the tibialis anterior muscle and post-tetanic depression in the soleus muscle were produced equally well during indirect and during direct stimulation of the muscles. These effects were therefore independent of neuromuscular transmission. That an indirectly applied tetanus has an additional effect on neuromuscular transmission was illustrated by the striking post-tetanic relief of tubocurarine paralysis which occurred in both muscles. In the soleus muscle, parameters of tetanic stimulation which depressed the subsequent maximal twitches of the non-curarized muscle nevertheless exerted an anticholinergic action when the same muscle was partially curarized. These conclusions confirm those of Brown & von Euler (1938).

There is substantial evidence that, for a short time after a tetanus, the motor nerve endings release a greater quantity of acetylcholine in response to a nerve impulse than they did before the tetanus (Hutter, 1952; Liley & North, 1953; Liley, 1956). There is also evidence that adrenaline increases the release of acetylcholine from cholinergic nerve endings (Krnjević & Miledi, 1958; Birks & MacIntosh, 1961) so that the decurarizing actions of both appear to be pre-junctional effects. However, there is as yet no evidence to show whether or not the increased transmitter release produced by a tetanus and by adrenaline arises through similar effects on the nerve endings.

In the non-curarized muscles the changes in the gross muscle action potentials produced by a tetanus or by adrenaline and isoprenaline were very slight and there was no correlation between them and the effects on the muscle tension. Thus marked effects on tension were sometimes unaccompanied by any detectable change in the action potentials, and, conversely, changes in the action potentials were occasionally associated with relatively small tension changes. These results suggest that the changes in tension are not a direct consequence of membrane effects and point to a site of action at some stage after membrane depolarization in the series of events leading to contraction.

Goffart & Ritchie (1952), who experimented on the tibialis anterior muscle of the cat and on the isolated diaphragm muscle of the rat, reached the conclusion that

adrenaline increased the maximal twitch tension by prolonging the active state of the stimulated muscle. Ritchie & Wilkie (1955) later showed in frog muscle at 0° C that a tetanus also caused a prolongation of the active state. When a pre- and post-tetanic twitch of the frog muscle were superimposed, the two curves ran together over most of the rising phase, but the post-tetanic twitch reached its peak later. These results showed that, although the time-course of activity had been altered by the tetanus, the force-velocity curve had not. In our experiments on the tibialis anterior muscle, however, superimposition of a pre- and post-tetanic twitch showed that the two rising phases separated immediately after the onset of contraction. This effect could not be ascribed to a prolongation of the active state, since the post-tetanic rate of rise of twitch tension was much greater than the rate of rise of tension in a preceding maximal tetanus. In a tetanus the active state is prolonged by the repeated stimuli, but the rate of rise of tension is initially the same as that in the preceding twitches. The two curves separate only at the time which corresponds to the decline from the plateau of full activity in a twitch (Macpherson & Wilkie, 1954). This increase in the rate of rise of tension must be the most important factor in post-tetanic potentiation in the mammal, since, even if the duration of the active state remained unaltered, a marked increase in twitch tension would result. This marked effect on contractility in the mammal probably accounts for the fact that post-tetanic potentiation is much more pronounced than it is in the frog.

In the soleus muscle adrenaline, isoprenaline or a preceding sub-maximal tetanus reduced both the tension and the contraction time of the maximal twitch but did not alter the rate of rise of tension. These results suggest that the plateau of the active state of this muscle is curtailed, and Jurna & Rummel (1962) recently reached similar conclusions from experiments with adrenaline on the soleus muscle of the cat.

It therefore appears that, while both a tetanus and the catechol amines may affect the active states of the stimulated muscles, a tetanus produces an additional powerful action on contractility in the fast-contracting mammalian muscles which is not shared by the catechol amines.

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