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THE EFFECTS OF SYMPATHOMIMETIC AMINES ON CHRONICALLY DENERVATED SKELETAL MUSCLES

BY

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Adrenaline has been shown to cause a slowly developing reversible increase in the tension of chronically denervated mammalian striated muscles both *in vivo* (Euler & Gaddum, 1931; Bülbring & Burn, 1936; Luco & Sanchez, 1956, 1959; Bowman & Zaimis, 1961) and *in vitro* (Montague, 1955; Cambridge, 1961; Bhoola & Schachter, 1961; Paterson, 1963). The membrane potential of chronically denervated muscles undergoes spontaneous rhythmic oscillations which trigger propagated spikes whenever the depolarizations reach a critical level (Li, Shy & Wells, 1957). The propagated spikes, which may be recorded with concentric needle electrodes as an irregular series of diphasic action potentials (Brown, 1937), are associated with contractions of the muscle fibres so that the denervated muscle is in a state of continuous fibrillary activity (Langley & Kato, 1915a, b). The contractile response of denervated striated muscle to adrenaline is associated with an increase in the frequency of the fibrillary potentials (Luco & Sanchez, 1959; Bowman & Zaimis, 1961). In the cat isoprenaline is slightly more potent than adrenaline in producing these effects, while noradrenaline is less potent (Luco & Sanchez, 1959; Bowman & Zaimis, 1961). In the experiments described in this paper, the actions of (−)-adrenaline, (−)-noradrenaline and (−)-isoprenaline (levisoprenaline) have been further studied on the chronically denervated tibialis anterior and soleus muscles of the cat.

METHODS

The left sciatic nerves of forty-seven cats were sectioned aseptically in the popliteal space during pentobarbitone sodium anaesthesia. Degeneration of the nerve was allowed to proceed for from 8 to 21 days after which the cats were anaesthetized with a mixture of chloralose (80 mg/kg) and pentobarbitone sodium (6 mg/kg) injected intravenously or intraperitoneally.

Kymographic recording. The tendons of insertion of the tibialis anterior and soleus muscles of the denervated limb were freed and the muscles were separated from neighbouring muscles. The cat was placed on its back and the limb clamped in a horizontal position by means of drills inserted in the lower ends of the femur and the tibia and fibula. The tendons were attached, via pulley wheels, to frontal-writing levers weighted with 30 g and magnifying the muscle movements ten-times. Movements of the two muscles were recorded simultaneously on smoked paper. Electrical stimulation was not applied in these experiments.

In six cats, the venous outflow from the denervated soleus muscle was recorded on smoked paper by the method described by Bowman & Zaimis (1958). In three of these experiments the muscle was stimulated directly at a frequency of 6 shocks/min with supramaximal rectangular pulses of 0.5 msec duration applied between a silver-silver chloride wire inserted through the musculotendinous junction and a second electrode attached to the drill in the femur. In the remaining three experiments, the muscle was not stimulated

electrically. Muscle tension was recorded by means of a flat steel-spring myograph simultaneously with the blood flow. Arterial blood pressure was recorded from a common carotid artery.

Electrical recording. When electrical recording was used, only one muscle was studied at a time. The cat was laid on its back for recordings from the tibialis anterior muscle, and face downwards for recordings from the soleus muscle. The exposed muscles were bathed in pools of warm medicinal liquid paraffin retained by the skin flaps. Isometric muscle tension was recorded in all experiments by attaching the tendon to an R.C.A. 5734 mechano-electric transducer valve in the mounting described by Kuffler & Vaughan Williams (1953). In some experiments fibrillary potentials, and in others demarcation potential, were recorded simultaneously. Fibrillary potentials were recorded by means of a concentric needle electrode (26 S.W.G.) inserted into the denervated muscle. The action potentials were fed to a Tektronix (type 122) battery powered preamplifier (frequency response 0.2 cycles/sec to 40 kcycles/sec) and thence to a Tektronix double-beam oscilloscope (type 502) with which they were recorded simultaneously with the tension changes. In some experiments, the oscilloscope beams were triggered to sweep at intervals of 5 sec and photographed on stationary film; in others, moving film was used to photograph the stationary beams. The frequency of the fibrillary potentials was continuously counted by connecting the output from the oscilloscope preamplifier to a second preamplifier (Panax type 425OH) and thence to a Panax scaler (type 100 C), the discriminator of which was set to 15 V. The total amplification was 10^6 so that only potentials larger than 15 μV at the recording electrode were counted by the scaler. Demarcation potential was recorded on one beam of the oscilloscope between nonpolarizable silver-silver chloride leads which made contact with the muscle under paraffin, through fine cotton wicks soaked in 3 M-potassium chloride solution containing 2% agar. The wicks were in contact, one with a small cut in the muscle and the other with an uninjured part of its surface. The output was fed through a cathode follower circuit to the oscilloscope. The apparatus included a switching device so that regular checks of instrumental drift could be made. Tension was simultaneously recorded on the other beam of the oscilloscope. Both beams were free running and demarcation potential and tension were read off from the calibrated oscilloscope screen. The muscles were not stimulated electrically in any of the experiments in which electrical recording was employed.

Drugs were injected intravenously through a cannula in a jugular vein or intra-arterially through a fine polyethylene cannula inserted retrogradely into a branch of the femoral artery, usually the small branch supplying the gracilis muscle. The drugs used were (−)-adrenaline (B.D.H.), (−)-noradrenaline bitartrate (Light & Co.), (−)-isoprenaline bitartrate (levisoprenaline, Wyeth), acetylcholine chloride (Roche), tubocurarine chloride (Burroughs Wellcome), phentolamine (Ciba), phenoxybenzamine (S.K.F.), tolazoline (Ciba), dichloroisoprenaline hydrochloride (Lilly), pronethalol (I.C.I.) and β-erythroidine hydrobromide (supplied by Professor Z. M. Bacq). The drugs were dissolved in 0.9% w/v sodium chloride solution. The doses of sympathomimetic amines refer to the base.

RESULTS

The response of the denervated muscles to the sympathomimetic amines (0.5 to 5 μg/kg, intravenously) was not always a simple increase in tension. Frequently the response was triphasic as illustrated in Fig. 1; the contraction was preceded by a small and short-lasting reduction in tone and was followed by a further small but long-lasting fall below the base line. In other experiments the response was biphasic, relaxation preceding contraction, or contraction preceding relaxation. In four out of a total of forty-seven experiments, all effective doses (0.1 to 1 μg/kg and above, intravenously) produced only a relaxation. Very large doses (20 μg/kg and above, intravenously) often produced only a relaxation even when previously injected small doses had caused an increase in tension. The different types of response could not be correlated with the time of denervation within the range 1 to 3 weeks.

Experiments in which the venous outflow from the muscle was recorded showed that all these tension responses were independent of concomitant blood flow changes since they were sometimes accompanied by vasoconstriction, sometimes by vasodilatation, and some-

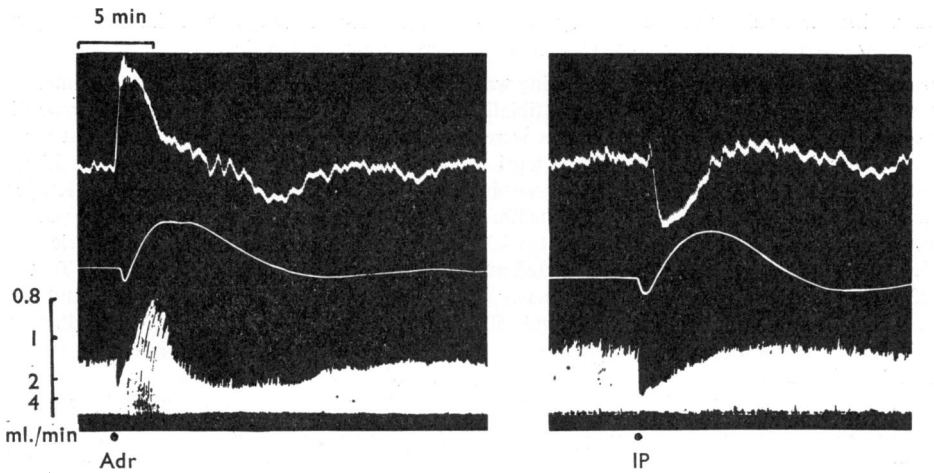

Fig. 1. Simultaneous recording of blood pressure, movements of the chronically denervated soleus muscle and venous outflow from the soleus muscle. At Adr, 5 μg/kg of adrenaline and at IP, 5 μg/kg of levisoprenaline were injected intravenously. The contractile responses are maximal. Time calibration above, 5 min; blood flow calibration on the left, ml./min. The muscle had been denervated for 14 days.

times by biphasic blood-flow changes. Levisoprenaline caused vasodilatation, whereas adrenaline and noradrenaline caused an increase in flow, a decrease in flow, or a biphasic response according to the dose and the route of injection. Fig. 1 illustrates similar triphasic tension responses produced by adrenaline and levisoprenaline, the response to adrenaline being accompanied by vasoconstriction but that to levisoprenaline by vasodilatation. After phentolamine (2 mg/kg, intravenously) the vasoconstrictor action of noradrenaline and adrenaline was reduced, abolished or reversed, but the tension changes remained the same. With very large doses of adrenaline and noradrenaline (20 μg/kg, intravenously) the contractile part of the response was often temporarily interrupted by a fall in tension so that the increase in tension showed two peaks. This transient fall in tension, occurring during the contractile response, was the only component which appeared to be due to blood flow changes. It was always accompanied by a powerful vasoconstriction and was more pronounced with noradrenaline, the more potent vasoconstrictor. It was not produced by levisoprenaline which caused only vasodilatation. After phentolamine (2 mg/kg, intravenously), which abolished the vasoconstriction, the contractile response to adrenaline and noradrenaline became a smooth curve like that to levisoprenaline (Fig. 2). Bülbring & Burn (1936), who recorded a similar double-peaked contractile response to large doses of adrenaline, found that after ergotoxine the transient depression was abolished.

The smallest effective doses and ratios of potency of the amines differed in the two muscles. In the tibialis anterior muscle the smallest effective doses of adrenaline were 0.5 to 2 μg/kg intravenously. Levisoprenaline was about twice as potent by weight and noradrenaline about two-thirds as potent. In the soleus muscle the smallest effective intravenous doses of adrenaline were 0.1 to 0.5 μg/kg; again levisoprenaline was about twice as potent, but noradrenaline was about twenty-times less potent in this muscle. Maximal contractile responses to adrenaline were produced in the tibialis anterior muscle by intravenous doses of 10 to 20 μg/kg, and in the soleus by doses of 5 μg/kg. In both

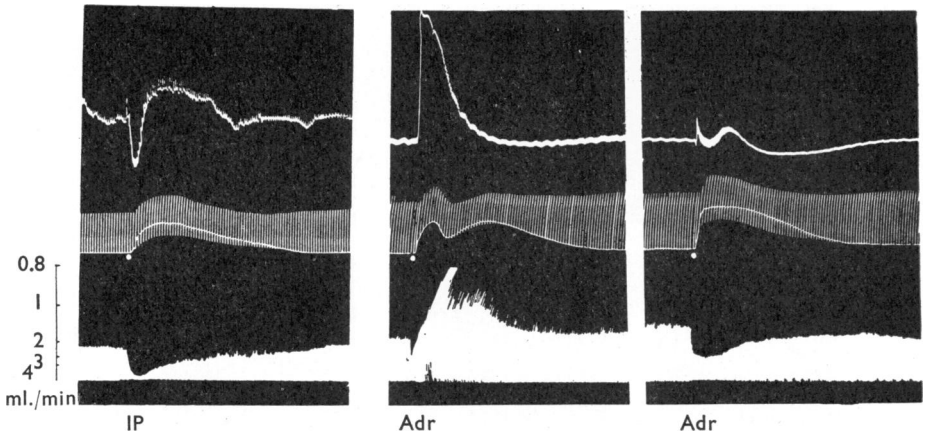

Fig. 2. Recordings as in Fig. 1 except that maximal twitches of the denervated soleus muscle were elicited once every 10 sec. At IP, 20 μg/kg of levisoprenaline and at Adr, 20 μg/kg of adrenaline were injected intravenously. Between the two doses of adrenaline, 3 mg/kg of phentolamine were injected intravenously. The muscle had been denervated for 17 days.

muscles, similar effects to those produced by intravenous injections were produced by intra-arterial injections of one-twentieth to one-tenth the dose.

Qualitatively similar responses to the amines were obtained from both muscles studied, but the effects on the soleus were always more pronounced. Simultaneous recordings from both muscles of the same cat showed that they did not always respond in the same way at the same time. For example, a contractile response from the soleus might be accompanied by only a relaxation of the tibialis anterior muscle, and, when contractions formed part of the responses of both muscles, they were often separated in time. In four of the experiments in which isotonic recording was used, the spontaneous fibrillations of the soleus muscle were sufficiently vigorous and synchronized at the start of the experiment to cause pronounced deflections of the lever (Fig. 3, *a*). The triphasic change in the muscle tone produced by adrenaline in the experiment of Fig. 3, *a*, was accompanied by corresponding changes in the spontaneous activity; during the relaxation, the spontaneous contractions were inhibited and during the contractile part of the response, the frequency of the rhythmic contractions was increased. In two other experiments on the soleus muscle, powerful oscillatory contractions were induced by large doses of adrenaline or levisoprenaline. Fig. 3, *b*, illustrates one of these experiments. Such powerful oscillatory activity was never observed in the tibialis anterior muscle. When, as in the present experiments, denervation is accomplished by sectioning the sciatic nerve, the nerve supply to the soleus muscle will degenerate sooner, because of its closer proximity, than that to the tibialis anterior muscle. However, the more vigorous spontaneous activity and more pronounced responses to the amines of the soleus muscle did not appear to be the result of a longer time for degeneration, since soleus muscles denervated for only 8 days were more reactive than tibialis anterior muscles denervated for any time between 8 and 21 days.

When large doses of the amines (10 μg/kg and above) were injected at small time intervals the contractile responses became smaller and were often converted to relaxations. With further dosage, the relaxations also became smaller. The rate of development of tachy-

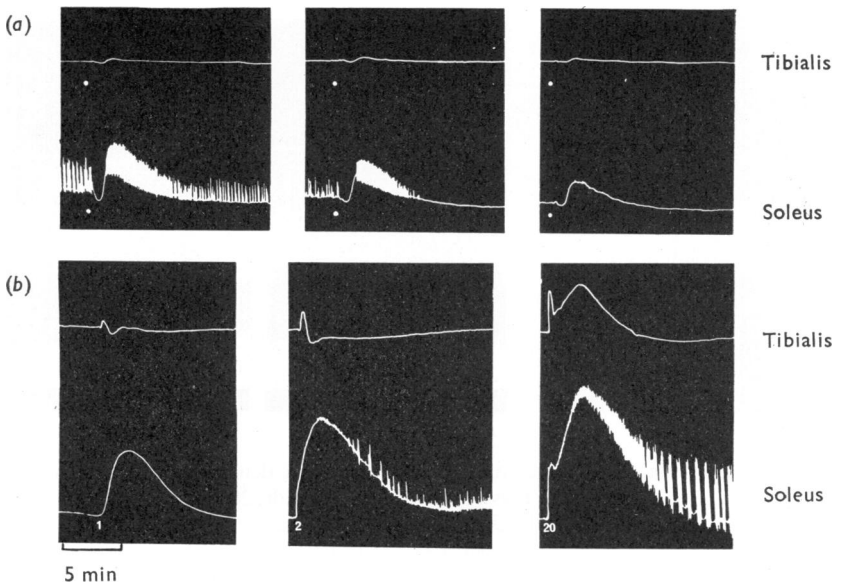

Fig. 3. Simultaneous recording of movements of the chronically denervated tibialis anterior and soleus muscles with isotonic frontal writing levers. In (*a*), 1 μg/kg of adrenaline was injected intravenously at hourly intervals. The numerals in (*b*) denote intravenous doses of adrenaline in μg/kg. Doses were injected at hourly intervals. The muscles had been denervated for 15 days in (*a*) and for 11 days in (*b*). Time calibration, 5 min.

phylaxis varied widely in different animals but in all experiments constant responses were produced by small doses (1 μg/kg) injected intravenously every 45 min for up to 10 to 15 hr, and this dosage programme was therefore used in most experiments.

Electrical recordings showed that the fibrillary potentials were accompanied by irregular oscillations of the isometric tension record. The spontaneous activity of the soleus muscle was always more pronounced than that of the tibialis anterior muscle, but with isometric recording the spontaneous contractions were never as marked as those occasionally seen in the soleus muscle when isotonic recording was used. Throughout the experiments it was observed that, in general, the effects of the amines were more pronounced the greater the degree of spontaneous fibrillary activity present to start with. This is partly determined by the muscle temperature and the applied resting tension (Bowman & Raper, 1964a). The changes in tension produced by the amines were accompanied by corresponding changes in the frequency of the fibrillary potentials. This is evident in the oscilloscope records of Fig. 4, and Fig. 5 is a graphical representation of the changes in tension and frequency of fibrillary potentials produced by a small and a large dose of adrenaline. Bowman & Zaimis (1961), who used large doses of the amines, observed that the increases in tension and in frequency of the potentials were not always in phase with each other, the peak of the increase in frequency sometimes occurring at a time when the tension was returning to its resting level. This lack of correlation was also often evident in the present experiments, but was probably an artifact arising from the fact that the strain gauge recorded the algebraic sum of the tensions in all the constituent fibres, while the needle electrode recorded electrical activity from only a small sample of the fibres.

Simultaneous recording of isometric tension and demarcation potential from either muscle showed that, whatever the dose of amine and the pattern of the tension change produced, there was always an increase in the demarcation potential. Brown, Goffart & Vianna Dias (1950), who used large doses of adrenaline injected intra-arterially, also recorded an increase in demarcation potential from the denervated tibialis anterior muscle of

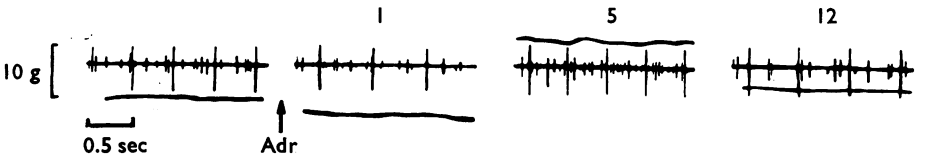

Fig. 4. Simultaneous oscilloscope recording of fibrillary potentials (upper trace) and isometric tension (lower trace) of a soleus muscle denervated for 11 days. Resting tension, 26 g. At Adr, 1 μg/kg of adrenaline was injected intravenously. The numerals above the records denote the time in min after adrenaline.

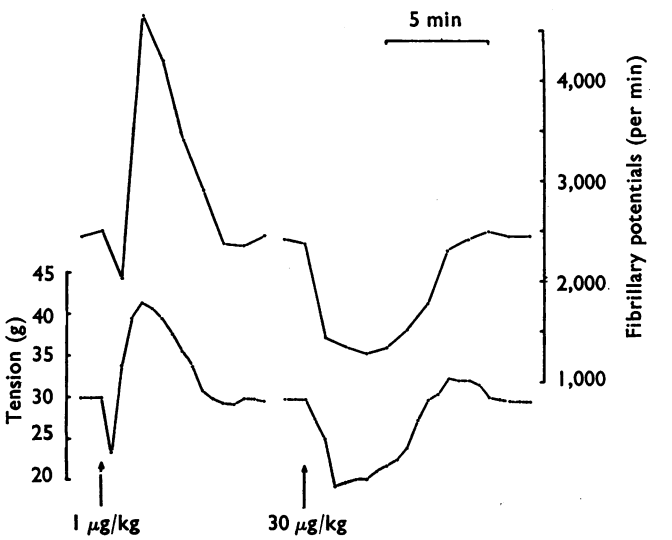

Fig. 5. Graph of frequency of fibrillary potentials (upper curves; count/min) and isometric tension (in g) recorded simultaneously from a tibialis anterior muscle denervated for 15 days. The responses are to adrenaline injected intravenously in the doses shown.

the cat. Fig. 6 illustrates the increase in the demarcation potential accompanying a triphasic tension change produced by a small dose of adrenaline and Fig. 7 illustrates the effects of larger doses. The doses necessary to affect the demarcation potential were the same as those necessary to affect tension. When a second dose of a sympathomimetic amine was injected before the demarcation potential had returned to normal, the increases in demarcation potential summed and the contractile response was reduced or converted to a relaxation. The effect on demarcation potential exhibited tachyphylaxis when large doses were repeatedly injected.

Effects of antiadrenaline drugs. The changes in tension, in frequency of fibrillary potentials and in demarcation potential produced by the sympathomimetic amines, were unaltered

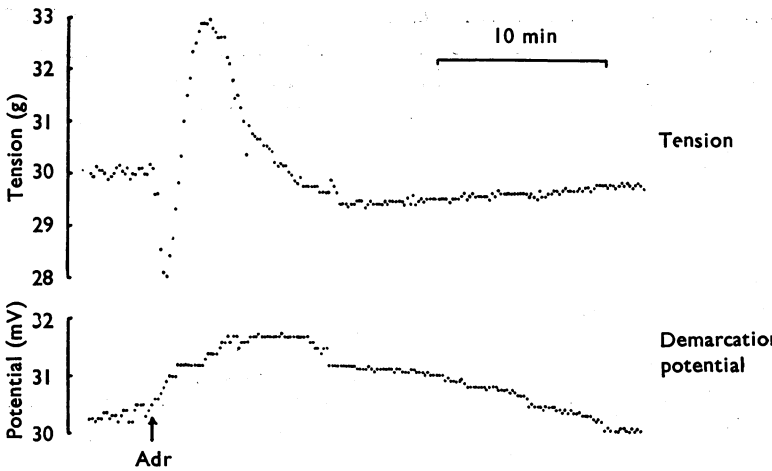

Fig. 6. Graph of tension changes in g and demarcation potential change in mV recorded from a tibialis anterior muscle (denervated for 10 days) in response to 1 μg/kg of adrenaline intravenously.

or slightly potentiated by the previous intravenous injection of the α-receptor blocking agents, phentolamine (2 to 3 mg/kg), tolazoline (2 to 3 mg/kg) or phenoxybenzamine (5 to 10 mg/kg). Fig. 8 illustrates the absence of effect of phentolamine on the increases in tension and in frequency of fibrillary potentials produced by adrenaline. In these doses, phentolamine had a weak, and tolazoline a powerful, adrenaline-like effect. The effects of the sympathomimetic amines on tension, frequency of fibrillary potentials and demarcation potential were completely abolished by the previous intravenous injection of the β-receptor

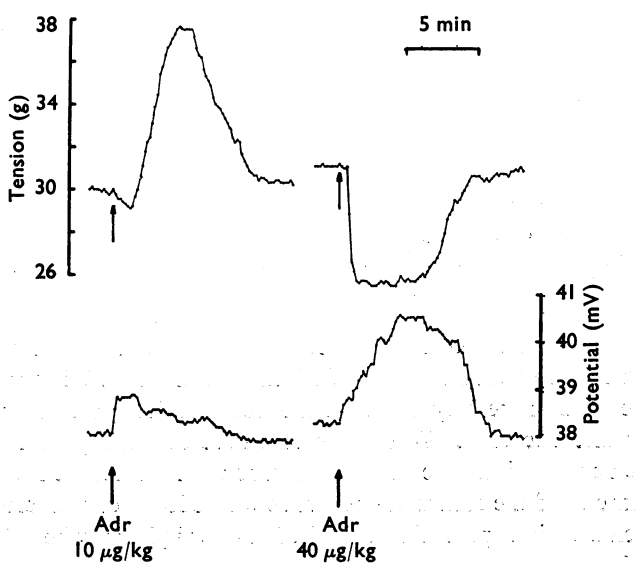

Fig. 7. Graph of tension changes and demarcation potential changes recorded from a tibialis anterior muscle (denervated for 15 days) in response to two different intravenous doses of adrenaline.

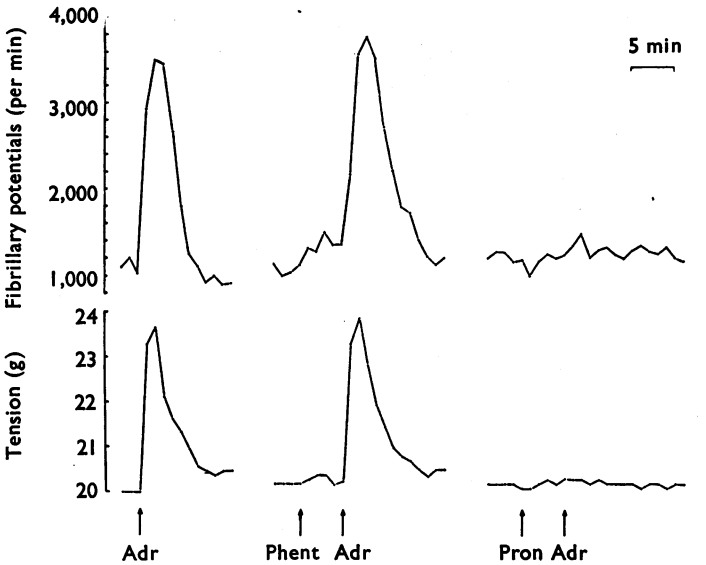

Fig. 8. Graph of frequency of fibrillary potentials in count/min and tension in g recorded simultaneously from a soleus muscle denervated for 12 days. Adrenaline (1 μg/kg intravenously at Adr) was injected at hourly intervals. At Phent, 3 mg/kg of phentolamine, and at Pron, 7.5 mg/kg of pronethal were injected intravenously.

blocking agents, dichloroisoprenaline (7.5 to 10 mg/kg) and pronethalol (7.5 to 10 mg/kg). The effect of pronethalol is illustrated in Fig. 8. Dichloroisoprenaline itself produced a weak adrenaline-like effect.

Effects of neuromuscular blocking drugs. Luco & Sanchez (1959) found that tubocurarine depressed the contractile response of denervated muscles to adrenaline, and they concluded that the effect of adrenaline resembled that of acetylcholine. We have confirmed this observation but have found that its occurrence depends on the number and size of the doses of tubocurarine injected.

The first large dose of tubocurarine (1 mg/kg) produced a pronounced stimulant action in the denervated muscles and electrical recording showed that the response was similar to that produced by depolarizing drugs, an initial abrupt contraction and burst of action potentials being followed by a slower contracture during which propagated fibrillary potentials ceased. This result confirms previous reports (McIntyre, King & Dunn, 1945; Luco & Sanchez, 1959; Bowman & Raper, 1964a). Demarcation potential recording showed that this effect of tubocurarine was accompanied by a powerful depolarization. The stimulant action of tubocurarine appeared to be most pronounced in animals which had previously received control injections of adrenaline. This observation resembles that made by Dale & Gaddum (1930) who found that adrenaline enhanced the stimulant action of acetylcholine and tetramethylammonium in denervated muscle. The abolition of fibrillary potentials following the injection of a large dose of tubocurarine long outlasted the contracture, and it was only when adrenaline was injected during this " electrical silence " that its contractile effect was abolished. The response of the muscle to intra-arterially injected acetylcholine (0.5 μg) was also abolished by tubocurarine.

Once the spontaneous fibrillary activity of the denervated muscle had returned, a second injection of tubocurarine was usually without effect. The response to acetylcholine remained blocked but adrenaline now produced its normal contractile effect. When tubocurarine was injected in a series of small doses it was possible to administer a very large total dose without abolishing the spontaneous fibrillary potentials, and under these circumstances the response to the sympathomimetic amines was not depressed, although that to acetylcholine was. Fig. 9 illustrates an experiment on the denervated soleus muscle in which the response to

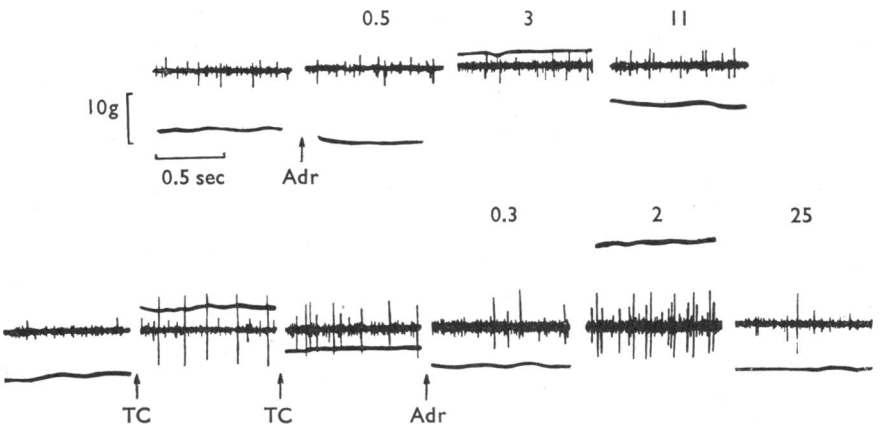

Fig. 9. Simultaneous recording of fibrillary potentials and tension from a soleus muscle denervated for 16 days. At Adr, 3 μg/kg of adrenaline were injected intravenously. At the first TC, 50 μg/kg of tubocurarine were injected intravenously and the effect on tension and fibrillary potentials 20 sec later is illustrated. At the second TC, further small doses of tubocurarine were injected to a total dose of 3 mg/kg. There was a sustained small increase in tension and frequency, but no abolition of propagated action potentials. The next dose of adrenaline was injected during the sustained increase in activity produced by tubocurarine. The numbers above the records denote the time in min after injection of adrenaline. Tension calibration on left, 10 g. Resting tension 30 g.

adrenaline was potentiated by tubocurarine. The upper records show a control response to adrenaline. Tubocurarine was injected, first in a dose of 50 μg/kg, and its only effect was to increase the tension and fibrillary activity. Subsequent small injections of tubocurarine were then made to a total dose of 3 mg/kg and there was still no electrical silence but only a sustained increase in frequency and tension. Adrenaline injected after tubocurarine and during the increase in frequency produced a much greater effect than formerly. This dose of tubocurarine was greatly in excess of that required to block the response to acetylcholine and in this experiment acetylcholine was still blocked 1 hr later.

The tertiary blocking agent, β-erythroidine, was also used in some experiments. This drug produced a much less powerful stimulant effect than tubocurarine. Even large doses caused only a small, short-lasting contraction and an increase in the frequency of fibrillary potentials. β-Erythroidine in an intravenous dose of 20 mg/kg completely abolished the response to acetylcholine but did not affect the response to adrenaline.

The depolarizing drugs, decamethonium (10 μg/kg, intravenously), suxamethonium (20 μg/kg, intravenously) and acetylcholine (2 μg/kg, intra-arterially), also produced a biphasic contraction-contracture response in the denervated muscle (Brown, 1937; Zaimis,

1951). When adrenaline was injected during the electrical silence following administration of these drugs its contractile effect was abolished.

DISCUSSION

Adrenaline, and other sympathomimetic amines, have previously been shown to cause an increase in the maximal twitches of the fast contracting tibialis anterior muscle, and a decrease in those of the slow contracting soleus muscle (Bowman & Zaimis, 1958; Bowman, Goldberg & Raper, 1962). These changes in the evoked contractions are due to a direct action on the muscle fibres, and, in the directly stimulated chronically denervated muscles, they were observed superimposed on the background changes in muscle tension. While the amines produce opposite effects on the evoked contractions of the two muscles, their effects on the background tension of the two denervated muscles were qualitatively the same. Like their effect on the evoked contractions (Bowman *et al.*, 1962), the effects of the amines on the background tension of the denervated cat muscles correspond, on Ahlquist's (1948) classification, to β-receptor effects, since they are produced most effectively by levisoprenaline, and are selectively blocked by the β-receptor blocking agents dichloroisoprenaline and pronethalol. This is in contrast to their effects on the denervated rat diaphragm, which, according to Paterson (1963), are blocked most effectively by α-receptor blocking agents.

The tension changes produced by the amines appeared to be due to changes in the frequency of the spontaneously occurring fibrillary potentials. When these were decreased in frequency, muscle tone was reduced, and when they were increased in frequency muscle tone was increased, probably because the contractions of the individual fibres became more synchronized and therefore summed. Recording of the demarcation potential showed that, whatever the change in frequency of the fibrillary potentials and in muscle tone, there was a small hyperpolarization of the fibre membranes. When the hyperpolarization was relatively large, spontaneous activity was inhibited and muscle tone fell. This inhibitory effect of the amines on the tone of the denervated muscles therefore resembled their effect on some unitary smooth muscles which exhibit spontaneous myogenic activity, for example, the guinea-pig taenia coli (Axelsson, Bueding & Bülbring, 1961). The increase in the frequency of the propagated fibrillary potentials and in the tone of the denervated muscle, which interrupted or completely masked the inhibitory effect, therefore appeared to occur in spite of the hyperpolarization.

Because the effect of adrenaline on tension could be reduced by tubocurarine, Luco & Sanchez (1959) concluded that it resembled that of acetylcholine. However, the present results indicated that this antagonism was not due to a specific blocking action of tubocurarine, but was the result of the membrane depolarization which this drug may produce in denervated muscles. Other drugs which caused depolarization of the membrane also abolished the contractile responses to the amines. The contractile response to the amines was clearly the result of an increase in the frequency of the fibrillary potentials, and, when the membrane is depolarized, propagated action potentials cannot be initiated. β-Erythroidine and, under certain conditions, tubocurarine were shown not to prevent the response to sympathomimetic amines at a time when the response of the muscle to acetylcholine was completely blocked. Bhoola & Schachter (1961) also found that the contractile response of rat isolated denervated muscle to adrenaline was not abolished by tubocurarine. These results indicate that the effect of adrenaline is unrelated to that of acetylcholine, and the

effects of the two drugs on the demarcation potential confirm this; acetylcholine always caused a powerful depolarization which adrenaline produced a small hyperpolarization.

The effects of the sympathomimetic amines appeared to be due to direct actions on the muscle rather than to the release of some substance from a more distant site, since they were produced equally well by intravenous injections and by local intra-arterial injection of small doses. Furthermore they also occur in isolated muscles (Montague, 1955; Cambridge, 1961; Bhoola & Schachter, 1961; Paterson, 1963). It seems likely that the effect of adrenaline is due to stimulation of whatever process gives rise to the spontaneous activity of the denervated muscle, and elucidation of the mechanism of action of adrenaline may therefore throw light on the origin of the spontaneous fibrillary potentials. There is evidence which indicates that some of the actions of adrenaline on skeletal muscle are secondary to changes in carbohydrate metabolism (Ellis, 1959; Bowman & Raper, 1964b), and in recent experiments we have found that intravenous or intra-arterial injections of hexosephosphates produce an increase in the fibrillary activity and tension of denervated muscle similar to that produced by adrenaline. We have previously suggested (Bowman & Raper, 1964b) that changes in carbohydrate metabolism may alter the distribution of ions within the muscle. The permeability properties of excitable membranes are known to depend on the presence of calcium ions, and it may be that an increased intracellular content of hexosophosphates in some way removes calcium ions from the membrane so that it becomes unstable and propagated action potentials are initiated.

SUMMARY

1. The effects of (−)-adrenaline, (−)-noradrenaline and (−)-isoprenaline (levisoprenaline) on the chronically denervated tibialis anterior and soleus muscles of cats anaesthetized with chloralose have been studied.

2. All three amines may cause an increase or a decrease in the tone of the denervated muscles, and these changes are associated with corresponding changes in the frequency of the spontaneous fibrillary potentials. Demarcation potential records showed that the responses to the amines were always accompanied by a small hyperpolarization.

3. Levisoprenaline was the most potent of the three amines; the effects were abolished by the previous injection of dichloroisoprenaline or pronethalol but were unaffected by phentolamine, tolazoline or phenoxybenzamine.

4. Results obtained with tubocurarine and β-erythroidine showed that the effects of the amines were unrelated to those of acetylcholine. Muscle blood flow recordings showed that the effects were independent of concomitant vascular changes.

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