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EFFECTS OF SYMPATHOMIMETIC AMINES ON NEUROMUSCULAR TRANSMISSION

BY

W. C. BOWMAN AND C. RAPER

From the Department of Pharmacology, School of Pharmacy, University of London, Brunswick Square, London, W.C.1

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Adrenaline, and some other sympathomimetic amines, have been shown to produce two opposing effects on neuromuscular transmission in partially curarized mammalian skeletal muscles, the ability to demonstrate either depending upon the experimental conditions. An anti-curare effect of adrenaline has been demonstrated by numerous workers (for references see Bowman, Goldberg & Raper, 1962), whereas, under different conditions, others (Paton & Zaimis, 1950; Naess & Sirnes, 1953) have shown that it enhances the blocking action of tubocurarine. If tubocurarine is administered in the form of a continuous infusion, both actions of adrenaline are evident, an initial anti-curare effect preceding a protracted enhancement of the transmission failure (Naess & Sirnes, 1953).

The dual action of adrenaline may also be demonstrated if it is administered in conjunction with anticholinesterase or depolarizing drugs. Adrenaline enhances the twitch potentiation and repetitive firing produced by a single injection of an anticholinesterase drug (Bülbring & Burn, 1942a; Bülbring, 1946; Blaber & Bowman, 1963) or a small dose of a depolarizing drug (Paton & Zaimis, 1950). If the anticholinesterase drug is continuously infused, the potentiated contractions are first augmented by adrenaline and then depressed (Naess & Sirnes, 1954), and, if a dose of a depolarizing drug sufficient to produce neuromuscular block is injected with or a short time after adrenaline, its blocking action is reduced (Paton & Zaimis, 1950).

Using isolated skeletal muscles bathed in physiological salt solutions, some workers have been unable to demonstrate some of these effects of adrenaline on neuromuscular transmission (Brown, Bülbring & Burns, 1948; Ellis & Beckett, 1955; Montagu, 1955; Krnjević & Miledi, 1958), suggesting that, in the intact animal, they may be a consequence of vascular changes. This investigation concerns the effects of catecholamines on neuromuscular transmission in relation to muscle blood flow and anti-adrenaline drugs.

Some of the preliminary experiments were described in a communication to the British Pharmacological Society by W. C. Bowman & E. Zaimis in January, 1956.

METHODS

The experiments were performed on cats anaesthetized by intravenous or intraperitoneal injection of a mixture of chloralose (80 mg/kg) and pentobarbitone sodium (5 mg/kg). Contractions of the tibialis anterior, gastrocnemius or soleus muscles were elicited by stimulation of the motor nerves with rectangular

pulses of 50 μsec duration and of about twice the strength necessary to evoke a maximal twitch. The contractions were recorded on smoked paper with flat steel spring myographs or on a cathode ray oscilloscope using mechano-electric transducer valves (RCA 5734). In many experiments gross muscle action potentials were recorded simultaneously with the contractions by means of platinum wires inserted through the belly and tendon of the muscle. Further details of these methods are described in previous reports (Bowman, Callingham & Goldberg, 1961; Bowman *et al.*, 1962).

Venous outflow from the gastrocnemius or the soleus muscle, and the demarcation potential of the tibialis anterior or the soleus muscle, were recorded by methods previously described (Bowman & Zaimis, 1958; Bowman, 1959; Bowman & Raper, 1965). Endplate depolarizations produced by suxamethonium were recorded from gracilis muscles by external wick electrodes. The method used was similar to that described by Burns & Paton (1951). Arterial blood pressure was recorded from a carotid artery with a mercury manometer.

Drugs were injected intravenously through a cannula in a brachial vein, intra-arterially through a cannula tied retrogradely into a branch of the femoral artery (usually the small branch supplying the gracilis muscle), or close-arterially to the soleus or tibialis anterior muscles by the methods described by Brown (1937, 1938). Intravenous infusions were administered from a motorized syringe (C. F. Palmer, Ltd.) into another cannula in a jugular vein.

The drugs used were (−)-adrenaline (British Drug Houses), (−)-noradrenaline bitartrate (L. Light & Co.) (±)-isoprenaline sulphate (Bayer), (−)-isopropylnoradrenaline bitartrate (laevisoprenaline, Wyeth), (+)-tubocurarine chloride (Wellcome), decamethonium iodide (May & Baker), suxamethonium chloride (Allen & Hanbury), neostigmine methylsulphate (Roche), dibenamine and phenoxybenzamine (Smith, Kline & French), phentolamine (Ciba), pronethalol hydrochloride (Imperial Chemical Industries), dichloroisoprenaline hydrochloride (Lilly), acetylcholine chloride (Roche). Drugs were dissolved in 0.9% w/v sodium chloride solution. The doses of sympathomimetic amines and of acetylcholine refer to the base and cation respectively.

RESULTS

The results of the experiments in which isopropylnoradrenaline was used were mainly obtained using the racemic compound (isoprenaline) and quantitative comparisons apply

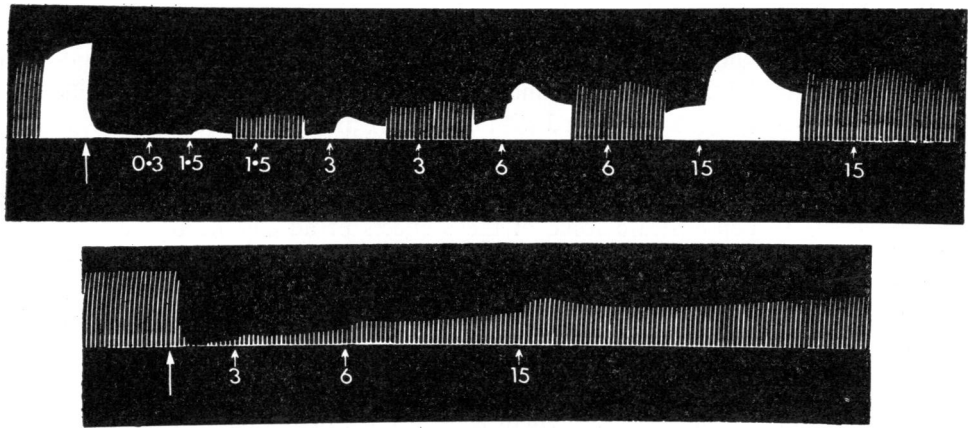

Fig. 1. Maximal twitches of a tibialis anterior muscle elicited by stimulation of the motor nerve once every 10 sec or, for alternate periods in the upper panel, once every second. At the larger arrows, 0.25 mg/kg tubocurarine was injected intravenously. The numbers below the small arrows indicate the doses of adrenaline in μg/kg injected intravenously. In the lower panel, the doses of adrenaline were injected at times when the amplitudes of the twitches corresponded to those in the upper panel when the same doses were injected during stimulation at 1 shock/sec.

to this form. However, most experiments have been repeated using (—)-isopropylnor-adrenaline (laevisoprenaline). The results obtained were qualitatively the same but laevisoprenaline was about twice as potent as isoprenaline.

Tubocurarine

The transient anti-curare effect of adrenaline was dependent upon both dose and frequency of stimulation (Fig. 1). The antagonism was always more pronounced at the

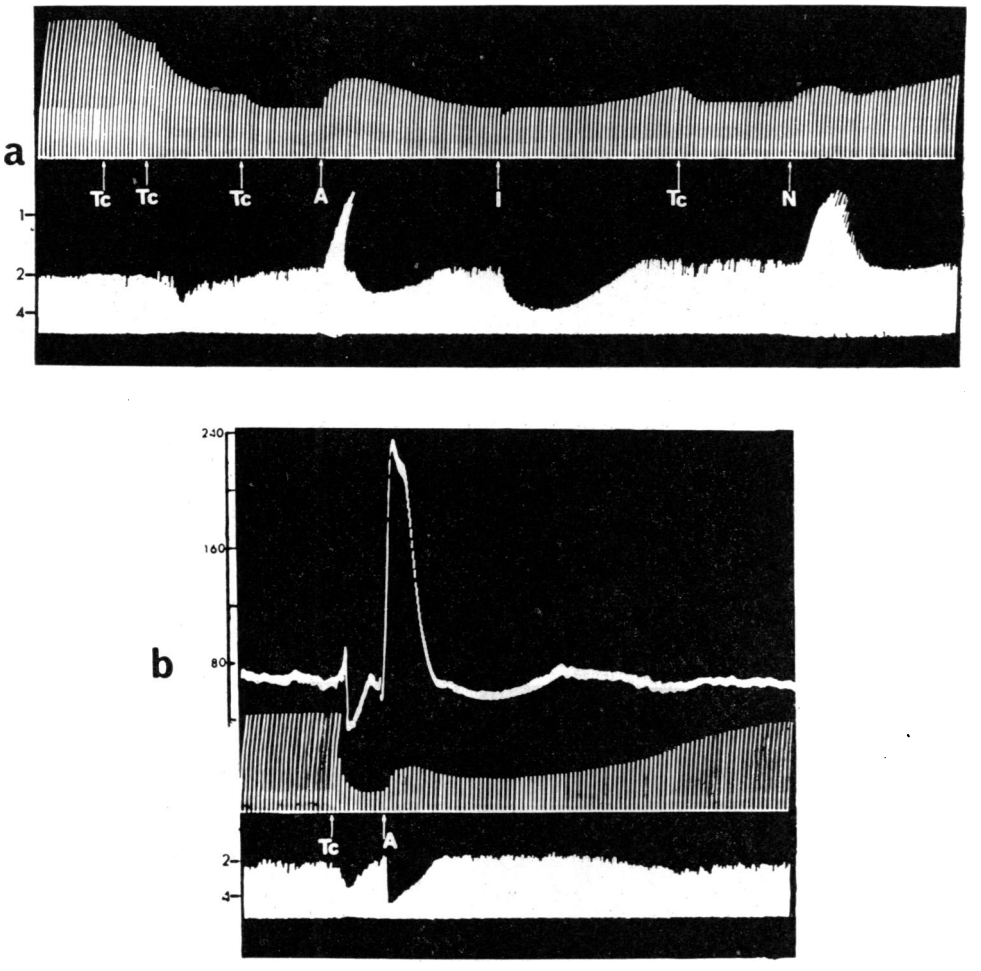

Fig. 2. (*a*) Maximal twitches of a gastrocnemius muscle elicited indirectly once every 10 sec and venous outflow from the same muscle. The venous outflow is calibrated in ml./min. Note that in this, and all other blood flow records, a decrease in venous outflow is recorded as an increase in the height of the trace. At Tc, 0.2 mg/kg tubocurarine was injected intravenously; at A, I and N, 4 μg adrenaline, isoprenaline and noradrenaline respectively were injected intra-arterially. (*b*) As for (*a*) but including a record of general arterial blood pressure. At Tc, 0.4 mg/kg tubocurarine and at A, 5 μg/kg adrenaline injected intravenously. Note that the anti-curare action of adrenaline is accompanied by a decrease in blood flow in (*a*), but by an increase in blood flow in (*b*).

higher stimulation frequency but, as can be seen from the lower record in Fig. 1, this was not solely a consequence of the greater depth of block.

Comparison of the catecholamines showed that adrenaline was always more potent than noradrenaline and that isoprenaline was virtually inactive in antagonizing a partial tubocurarine blockade (Fig. 2,*a*). Simultaneous recording of blood flow responses demonstrated a biphasic response to adrenaline, vasodilatation to isoprenaline and vasoconstriction to noradrenaline, when the amines were injected intra-arterially. Intravenous injections did not always produce the same changes in blood flow but the effects on muscle contractions were always as illustrated in Fig. 2. As shown in Fig. 2,*b*, the usual anti-curare effect coincides with an increase in blood flow, i.e. the pressor effect resulting from intravenous injection of adrenaline has forced more blood through the muscle vessels despite any local vasoconstrictor action. Essentially similar results were obtained for tibialis anterior, gastrocnemius and soleus muscles.

The " curare-potentiating " action of the amines was also evident under these conditions, for when the total duration of a block during which an amine had been injected was com-

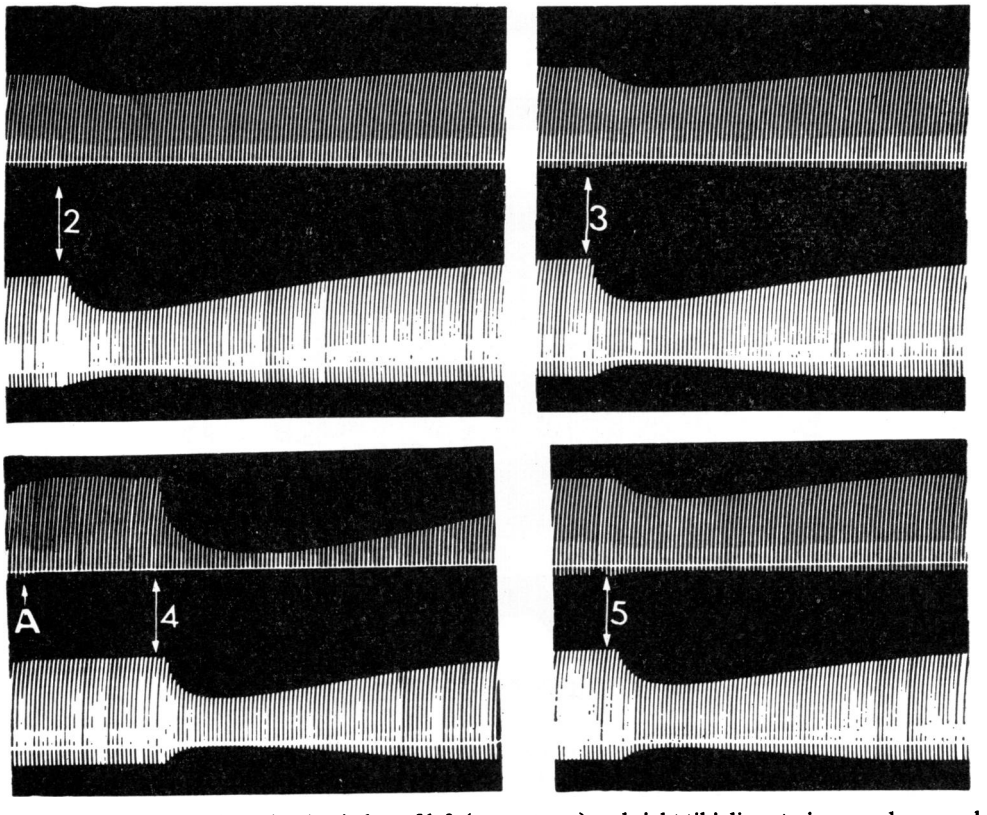

Fig. 3. Indirectly elicited maximal twitches of left (upper trace) and right tibialis anterior muscles recorded simultaneously once every 10 sec. The four panels show responses to the second, third, fourth and fifth of a series of six doses of tubocurarine (0.15 mg/kg) injected intravenously at intervals of 1 hr. At A, 3 μg adrenaline was injected close-arterially to the left tibialis anterior muscle. The block of this muscle produced by the subsequent injection of tubocurarine was enhanced.

pared with that produced by a control dose of tubocurarine alone, either before or afterwards, it was seen to be between 50 and 100% longer-lasting as a result of injection of the amine. Although without an anti-curare action, isoprenaline prolonged the duration of tubocurarine block.

Attempts to quantify the curare-potentiating action of the amines have been successful. Maximal twitches of both right and left tibialis anterior or soleus muscles were repeatedly blocked with intravenous tubocurarine (0.15–0.4 mg/kg). The dose was such that the first block was not more than 30% and the interval between injections (45–60 min) was chosen to reduce cumulative effects. Amines were given close-arterially to one muscle, the doses being too small to exert any effect on the contralateral muscle (Fig. 3).

Parallel lines were obtained when duration or depth of block was plotted against the number of the dose of tubocurarine for both muscles, indicating that cumulation was similar. Estimation of the depth and duration of block that would have been produced had amines not been injected enabled calculation of the amine effect. Qualitatively similar results were obtained in both the tibialis anterior and soleus muscles (Fig. 4).

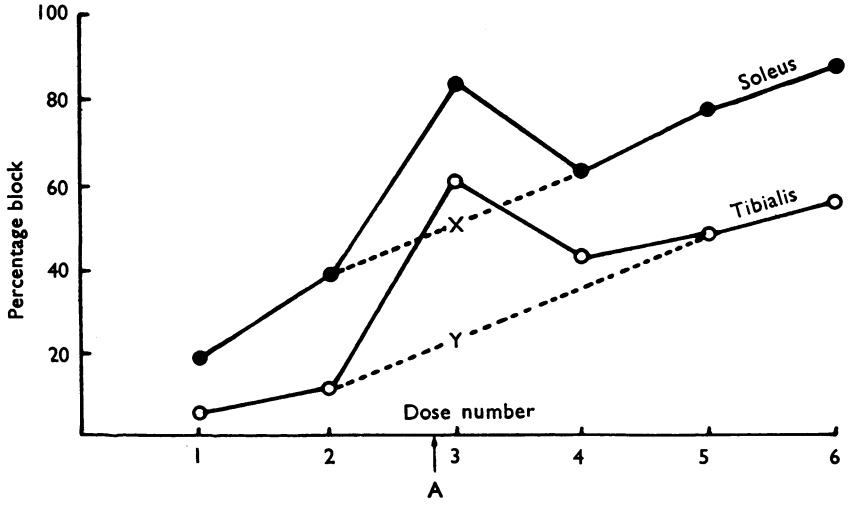

Fig. 4. Graphical representation of the depth of block produced in the soleus and tibialis anterior muscles by six intravenous injections of tubocurarine (0.15 mg/kg) administered at intervals of 40 min. Maximal twitches of both muscles were elicited simultaneously once every 10 sec. A indicates the intravenous injection of adrenaline (10 μg/kg). X and Y indicate the depths of block which probably would have been produced by the third dose of tubocurarine had adrenaline not been previously injected. The block in both muscles was enhanced to a similar degree.

Comparison of the catecholamines showed isoprenaline to be the most potent, adrenaline slightly less and noradrenaline the least active in potentiating tubocurarine paralysis.

It was considered possible that readjustments of the circulation by the amines might have resulted in the skeletal muscles receiving a greater share of intravenously injected tubocurarine, thereby enhancing the paralysis. However, experiments in which muscle blood flow was recorded simultaneously with the maximal twitches showed that this was not the

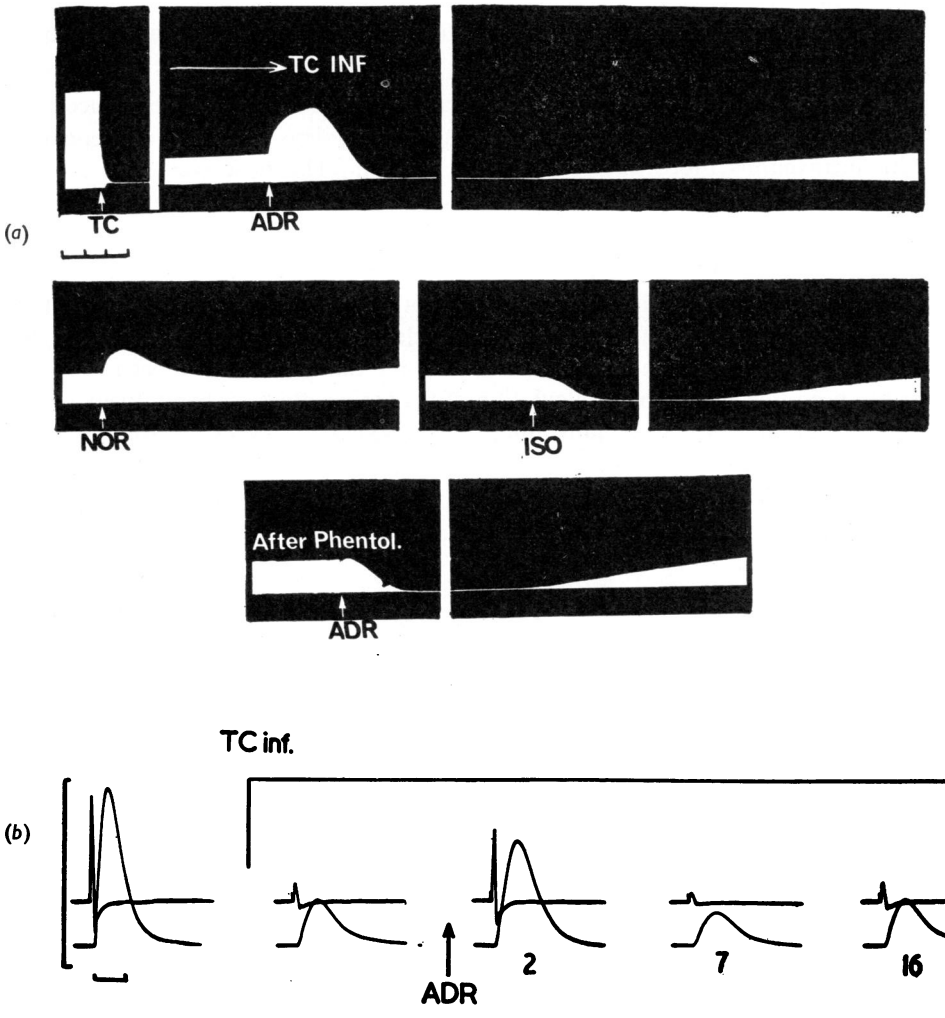

Fig. 5. (*a*) Maximal twitches of a tibialis anterior muscle elicited indirectly once every second. At TC a single intravenous injection of tubocurarine (0.3 mg/kg) was given. During recovery from this dose an intravenous infusion of tubocurarine was started and adjusted to give a constant degree of partial block. The infusion (0.58 mg/kg per hr) was then maintained constant for the remainder of the experiment. At ADR, NOR and ISO, 10 μg/kg of adrenaline, noradrenaline and isoprenaline were injected intravenously. The second response to adrenaline was recorded after the intravenous injection of phentolamine (2 mg/kg). The gaps in the responses to adrenaline and isoprenaline each correspond to 10 min. Time calibration in minutes. (*b*) As for (*a*) but twitches and gross muscle action potentials were recorded on an oscilloscope. In this experiment the dose of adrenaline (ADR) was again 10 μg/kg intravenously. Responses are shown at 2, 7 and 16 min after injection of adrenaline. Note that the changes in twitch tension are accompanied by corresponding changes in the amplitude of the gross action potentials. Calibrations: tension on the left, 1 kg; time below, 30 msec; action potential on the right, 20 mV.

explanation. Tubocurarine paralysis was enhanced by the amines whatever the change in blood flow, and in most experiments the blood flow had returned to normal before the tubocurarine was injected.

When adrenaline (10 μg/kg intravenously) was injected during an intravenous infusion of tubocurarine, both antagonism and potentiation of paralysis could be demonstrated (Fig. 5,*a*). Noradrenaline produced similar but less pronounced effects but isoprenaline only potentiated the blockade. Smaller doses of the amines (0.5–1.0 μg/kg intravenously) augmented tubocurarine paralysis in the tibialis anterior muscle without producing an initial anti-curare effect.

Similar effects to those recorded in the tibialis anterior were produced by the amines in the soleus muscle, except that noradrenaline was about 20 times less potent in augmenting the paralysis in this muscle. As a result it was not possible with noradrenaline to augment the block in the soleus muscle without producing an initial anti-curare effect. Noradrenaline was about two-thirds as potent as adrenaline in its anti-curare action in both muscles, and consequently any dose big enough to augment the paralysis of the soleus muscle also produced an initial antagonism. The stimulation frequency of 1/sec used in these experiments was the optimal, found by Naess & Sirnes (1953) and Dybing (1954) in experiments on unanaesthetized rabbits, for demonstrating potentiation of tubocurarine blockade by sympathomimetic amines.

Fig. 5,*b* illustrates an experiment on the tibialis anterior muscle in which isometric twitch tension and gross muscle action potentials were recorded simultaneously on an oscilloscope. Adrenaline (10 μg/kg intravenously) produced the usual biphasic change in the partially blocked twitches, and this was accompanied by corresponding changes in the amplitude of the gross muscle action potentials. This result indicates that the changes in twitch tension arise mainly from changes in the number of muscle fibres contributing to the contraction, rather than from changes in the contractile force exerted by individual fibres. Similar results were produced in the soleus muscle.

All three sympathomimetic amines also depressed contractions of both normal and curarized muscles elicited by the close-arterial injection of acetylcholine (Fig. 6). In the partially curarized muscle (Fig. 6,*a*), contractions produced by acetylcholine were depressed during the initial anti-curare action of adrenaline. They remained depressed for the duration of the curare-potentiating action, and both twitches and acetylcholine responses returned to control levels at about the same time. In the non-curarized tibialis anterior muscle, the decreased responses to acetylcholine were present throughout the small augmentation of twitch tension produced by adrenaline or isoprenaline (Fig. 6,*b*).

Demarcation potential recordings showed that all three sympathomimetic amines produced a small hyperpolarization of the muscle fibre membranes in both partially curarized and in non-curarized tibialis anterior and soleus muscles. This result confirms that of Brown, Goffart & Vianna Dias (1950), who experimented on the tibialis anterior muscle of the cat. In both muscles the time course of the increase in demarcation potential corresponded to that of the enhanced block produced by the amines. The increase in demarcation potential began during the initial anti-curare action of adrenaline and noradrenaline. Fig. 7 is a graphical representation of results obtained with adrenaline. With isoprenaline, the decrease in the tension of the partially blocked twitches and the increase

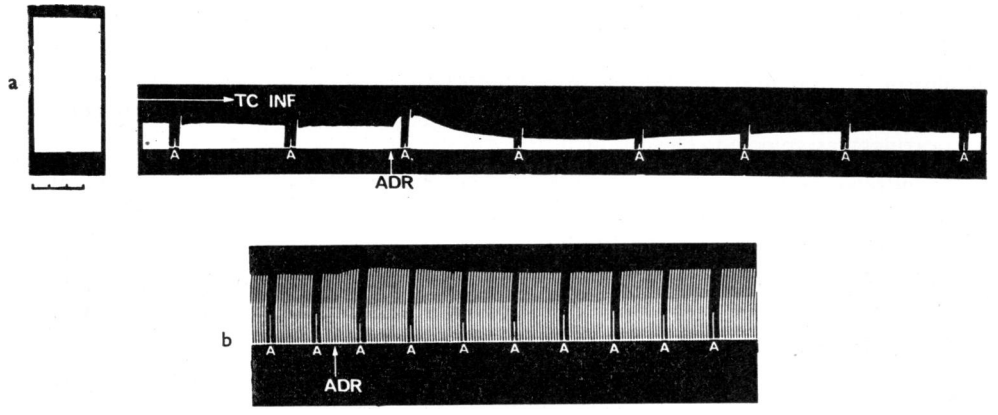

Fig. 6. (*a*) Similar experiment to that of Fig. 5,(*a*). Partial block of the twitches was maintained by an intravenous infusion of 0.52 mg/kg/hr of tubocurarine. Time calibration in minutes. (*b*) Maximal twitches of a non-curarized tibialis anterior muscle elicited once every 10 sec. In both experiments, at A electrical stimulation was temporarily stopped and acetylcholine (5 μg in (*a*) and 1 μg in (*b*)) was injected close-arterially. At ADR, adrenaline was injected intravenously (10 μg/kg in (*a*) and 12.5 μg/kg in (*b*)).

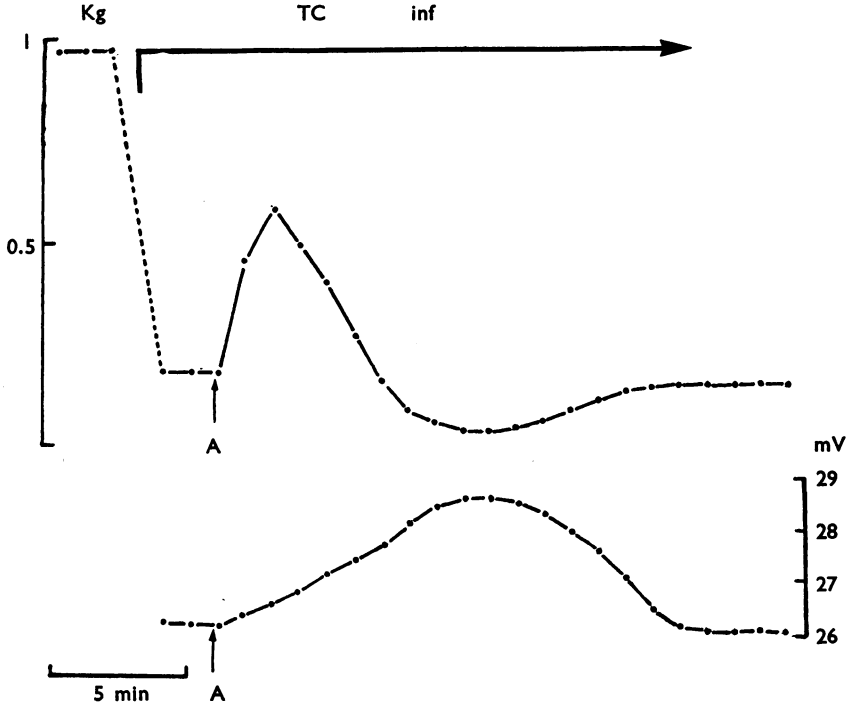

Fig. 7. Graphical representations of the changes in isometric twitch tension of the tibialis anterior muscle (upper graph) and muscle demarcation potential (lower graph) produced by adrenaline during a continuous intravenous infusion of tubocurarine (TC inf). Isometric twitches were elicited indirectly once every second and tubocurarine was infused intravenously (0.52 mg/kg/hr). During the constant partial block of the twitches produced by tubocurarine, adrenaline (10 μg/kg) was injected intravenously at A. The changes in demarcation potential were recorded 45 min later in response to a second dose of adrenaline (10 μg/kg), the tubocurarine infusion being maintained throughout. Muscle twitches were not elicited during the recording of the demarcation potential.

in demarcation potential matched each other exactly. Because of the small changes in demarcation potential produced, accurate comparison of the potencies of the amines was not possible, but isoprenaline was again slightly more potent than adrenaline while noradrenaline was less so. The smallest doses of adrenaline necessary to produce a detectable increase in the demarcation potential were of the order of 1–2 μg/kg intravenously, but with

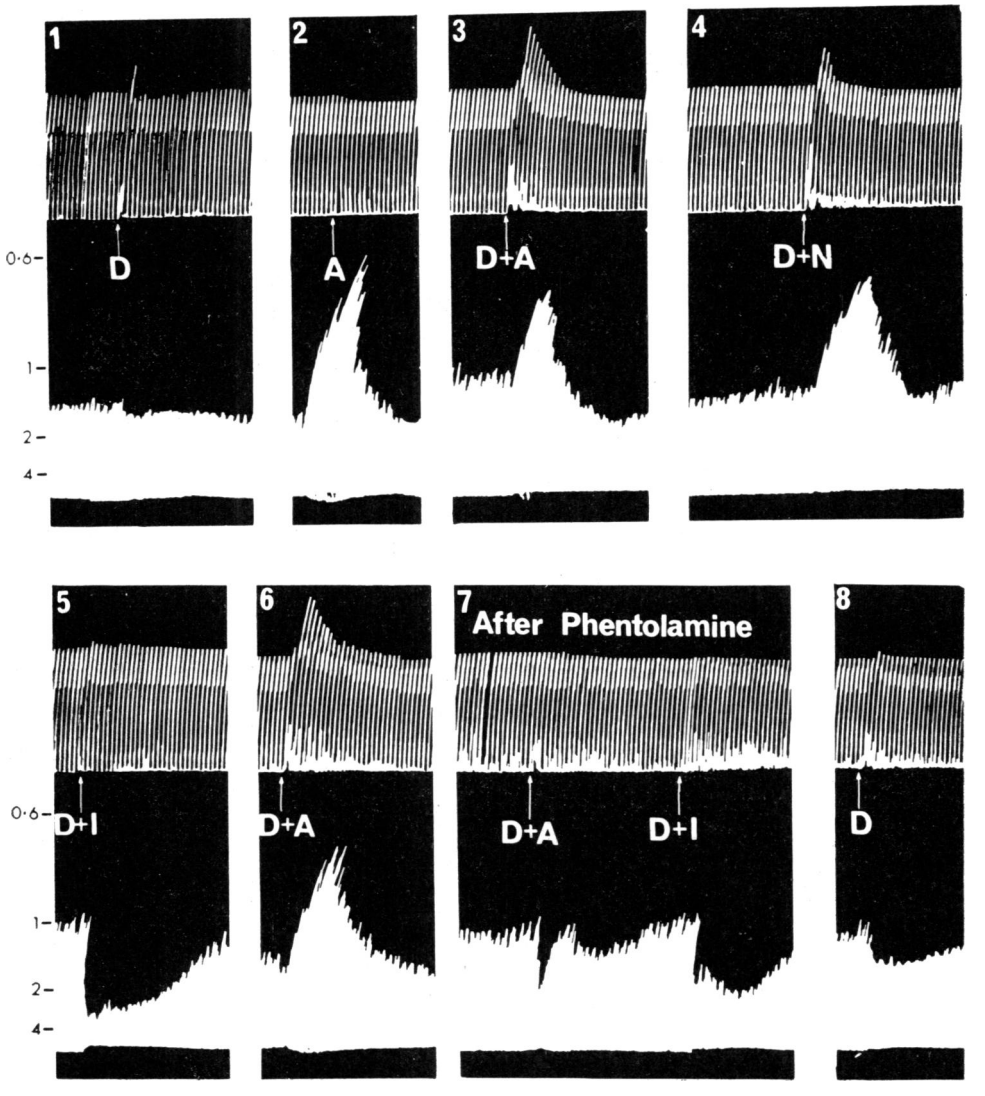

Fig. 8. Maximal twitches of a soleus muscle elicited by stimulation of the motor nerve once every 10 sec, and venous outflow from the same muscle recorded simultaneously. Intra-arterial injections were all administered in a volume of 0.2 ml.; at D, 5 μg decamethonium; at A, 2 μg adrenaline; at N, 2 μg noradrenaline; and at I, 2 μg isoprenaline. When two drugs were injected together (e.g. at D+A), the mixture was prepared beforehand to contain the above doses in 0.2 ml. The responses in panels 7 and 8 were recorded after the intravenous injection of phentolamine (2 mg/kg).

this dose the effects were small and larger doses (5–20 μg/kg) were usually given. In sixteen experiments, the demarcation potential of both tibialis anterior and soleus muscles was within the range 20–28 mV, and adrenaline, in doses of 5–20 μg/kg, increased this by 2.7–16%. No tachyphylaxis was evident when doses of 10 μg/kg were injected at hourly intervals, and there was no definite secondary depolarization. Brown *et al.* (1950), who used large doses of adrenaline injected close-arterially, found a marked tachyphylaxis to successive doses and they believed that a pronounced depolarization followed the initial hyperpolarizing action, although they pointed out that this was difficult to distinguish from instrumental drift and deterioration of the preparation. In five of the present experiments a gradual decrease in the demarcation potential did follow the hyperpolarization but this was probably a reflection of the gradual deterioration of these five preparations, since the effect was irreversible. In the remaining eleven experiments, no secondary depolarization followed the hyperpolarization.

Decamethonium and suxamethonium

Simultaneous administration of decamethonium and adrenaline produced a more pronounced increase in twitch tension and fasciculations in the tibialis anterior, soleus and gastrocnemius muscles than did the same dose of decamethonium injected alone. Recording of muscle blood flow showed that potentiation occurred when drugs were given close-arterially (vasoconstriction) or intravenously (vasodilatation). Noradrenaline was less potent than adrenaline and isoprenaline was without effect (Fig. 8). This result with adrenaline in the tibialis anterior muscle confirms that of Paton & Zaimis (1950).

When, instead of injecting the two drugs together, the sympathomimetic amine was injected 5 or 10 min before the small dose of decamethonium, the twitch potentiation and fasciculations produced by the latter drug were less pronounced than those resulting from a control dose injected alone. Isoprenaline injected in this way also inhibited the stimulant effects of decamethonium, but it was not possible to compare accurately the potency of the amines, as the stimulant effect of decamethonium often tended to decrease with repeated doses.

The effects of the amines on neuromuscular block produced by decamethonium in the tibialis anterior and soleus muscles was tested in experiments of similar design to that illustrated in Fig. 3, the tubocurarine injections being replaced by constant doses of decamethonium. In different experiments on the tibialis anterior muscle the doses of decamethonium used ranged from 20 to 40 μg/kg intravenously. Larger doses (60–150 μg/kg) were necessary in experiments on the soleus muscle. In experiments with decamethonium, it was more important than in experiments with tubocurarine to use the muscle of one limb as control and to inject the amine close-arterially into the contralateral muscle. This was because successive doses of decamethonium at first usually produced a cumulative paralysing effect, but with further repetition of doses tachyphylaxis became evident. Tachyphylaxis was always pronounced in the soleus but was less striking in the tibialis anterior. These changes in sensitivity, which have previously been described by Jewell & Zaimis (1954), are evident in the graphs of Fig. 9. All three amines reduced the blocking potency of decamethonium in both the tibialis anterior and the soleus muscles (Fig. 9), their relative potency being similar to that determined for potentiation of tubocurarine paralysis. Although the effects of the amines on the depth of block were similar in both muscles,

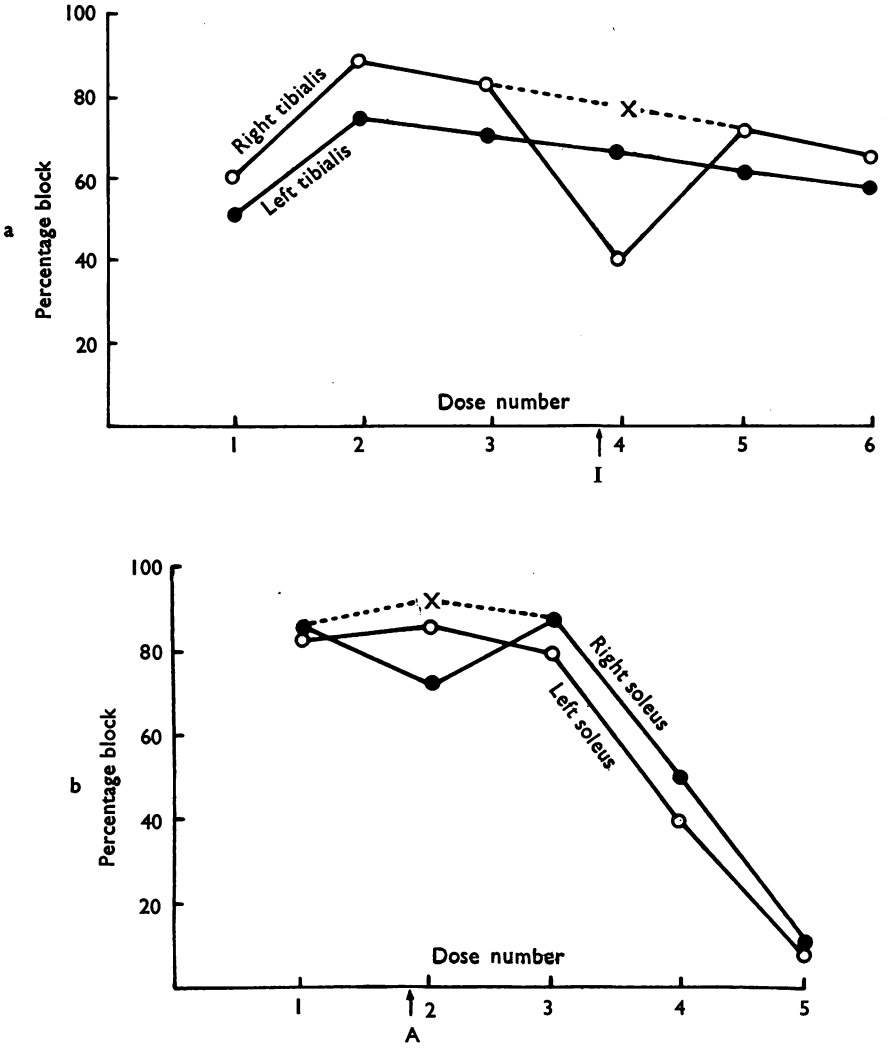

Fig. 9. (*a*) Graphical representation of the depths of block produced in the right and left tibialis anterior muscles by a series of doses of decamethonium (15 μg/kg) injected intravenously at intervals of 50 min. Five min before the fourth dose of decamethonium, 3 μg isoprenaline (I) was injected close arterially to the right tibialis anterior muscle.

(*b*) Graphical representation of the results of a similar experiment in which contractions of the right and left soleus muscles were recorded. In this experiment each dose of decamethonium was 65 μg/kg injected intravenously at intervals of 90 min. Five min before the second dose of decamethonium, 3 μg adrenaline (A) was injected close-arterially to the right soleus muscle.

In both experiments, maximal twitches of the muscles were elicited by stimulation of the motor nerves at a frequency of 1/10 sec. X indicates the probable degree of block which would have been produced by decamethonium had the sympathomimetic amine not been previously injected.

their effects on the durations of the blocks differed. In the tibialis anterior muscle the duration of the block was always reduced by the amines, but in the soleus muscle it was prolonged. This difference in the responses of the two muscles may be explained by the difference in the types of block produced by decamethonium in the two muscles. In the tibialis anterior, decamethonium produces block by depolarization, but in the soleus muscle the same drug produces dual block (Jewell & Zaimis, 1954).

In nine cats, end-plate depolarizations produced by suxamethonium were recorded with surface electrodes on the gracilis muscle. Suxamethonium was used in place of decamethonium in these experiments because of its brief duration of action, which allowed repetition of a greater number of doses, and because tachyphylaxis is less likely to occur. Suxamethonium in doses of 50–150 μg/kg injected intravenously at intervals of 30 min produced submaximal depolarizations of the end-plate region. The amount of depolarization recorded was between 7 and 10 mV in different experiments, the value recorded in seven of the nine experiments being constant with successive equally sized doses. Adrenaline (10–15 μg/kg), injected intravenously 5 min before the third or fourth dose of suxamethonium, did not alter the membrane potential of the end-plate region relative to that of the reference electrode, but reduced by 12–21% the extent of the depolarization produced by suxamethonium (Fig. 10,*a*). Laevisoprenaline (the racemic compound was not used) produced similar effects to adrenaline but with the same doses the extent of the reduction was about twice that produced by adrenaline (Fig. 10,*a*).

Experiments similar to that illustrated in Fig. 5 were attempted using an infusion of decamethonium in place of tubocurarine. Because of the rapid development of tachy-

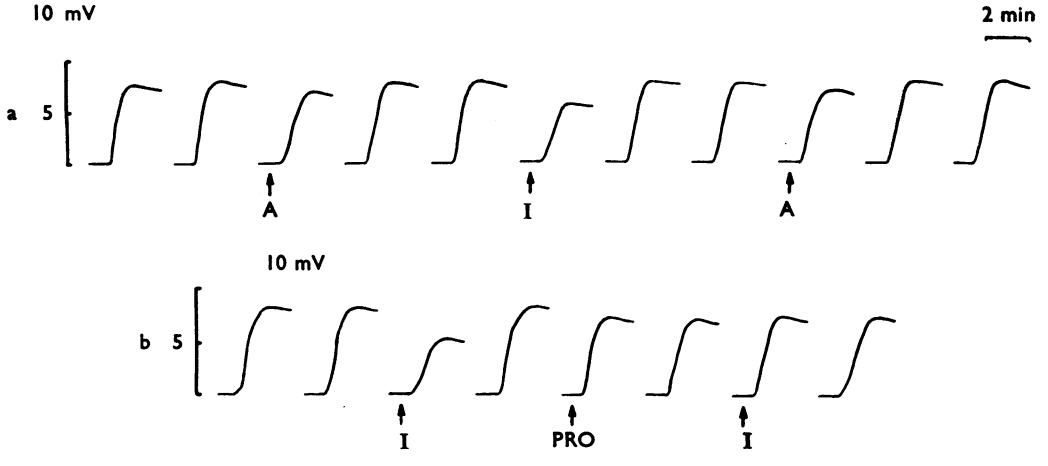

Fig. 10. Graphical representation of depolarizations of the endplate region of the gracilis muscle produced by successive intravenous doses of suxamethonium (100 μg/kg in (*a*), and 150 μg/kg in (*b*)) injected at intervals of 30 min. Five min before the dose of suxamethonium indicated by the arrows, adrenaline (A, 15 μg/kg), laevisoprenaline (I, 15 μg/kg in (*a*) and 10 μg/kg in (*b*)) and pronethalol (PRO, 10 mg/kg) were injected intravenously. (*a*) Laevisoprenaline was more potent than adrenaline in depressing endplate depolarization produced by suxamethonium. (*b*) The effect of laevisoprenaline was prevented by the previous injection of pronethalol. Pronethalol itself slightly depressed the effect of suxamethonium.

phylaxis to decamethonium in the soleus muscle, it was found impossible to maintain a constant degree of partial paralysis and attempts to use this muscle were abandoned. In the tibialis anterior muscle a constant degree of paralysis with decamethonium was more difficult to produce than with tubocurarine, but in five experiments a 60–70% depression of twitch tension was maintained sufficiently constant to study the superimposed effects of the amines. Blood flow was simultaneously recorded in three of these experiments and gross muscle action potentials in the other two. The effect of isoprenaline (10 μg/kg intravenously) was a mirror image of its effects during continued partial tubocurarine paralysis; this amine produced a gradually developing increase in twitch tension which reached its peak (50–100% increase) in about 6 min and then gradually waned until the effect disappeared about 15 min after injection (Fig. 11). The effect of adrenaline was

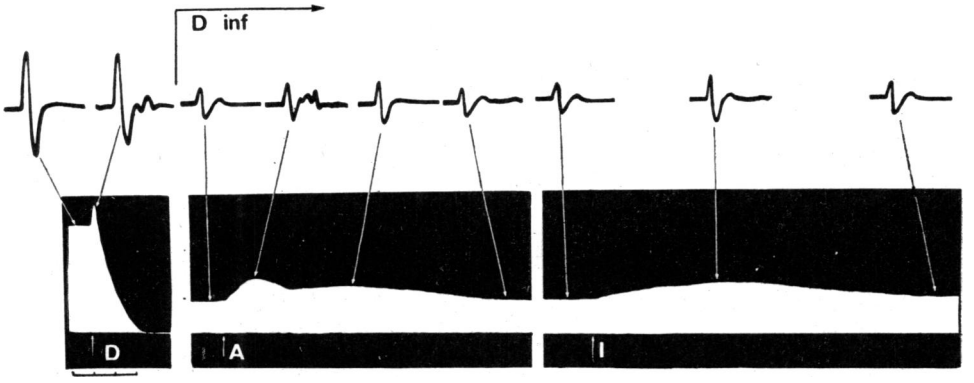

Fig. 11. Maximal twitches and gross action potentials of a tibialis anterior muscle were elicited indirectly once every second and recorded simultaneously on a kymograph and on an oscilloscope respectively. Only representative action potentials are shown and the arrows indicate the twitches with which they were associated. At D, 30 μg/kg decamethonium was injected intravenously. During recovery from this dose, an intravenous infusion of decamethonium (D inf. 154 μg/kg/hr) was started and maintained throughout the rest of the experiment. At A, 10 μg/kg adrenaline and at I, 10 μg/kg isoprenaline were injected intravenously. The initial abrupt increase in the partially blocked twitches produced by adrenaline was associated with repetitive firing in the action potential record, whereas the more sustained secondary increase was associated with an increase in the amplitude of the action potential. Isoprenaline produced only the second of these effects. Time calibration in min.

not a mirror image of its biphasic effect during tubocurarine paralysis. Adrenaline (10 μg/kg intravenously) produced an abrupt increase in the amplitude of the partially blocked twitches which reached its peak in about 1 min and then quickly waned to merge with a more slowly developing increase in twitch tension resembling that produced by isoprenaline (Fig. 11). Although smaller, the initial increase in twitch tension produced by adrenaline superficially appeared to resemble its anti-curare effect. However, it was shown to differ from the anti-curare effect in experiments in which gross muscle action potentials were simultaneously recorded. During partial paralysis produced by depolarizing drugs, some of the muscle fibres which are not blocked may be firing repetitively (Bowman *et al.*, 1961). Slight repetitive firing is detectable in the action potential records from the partially blocked twitches in the experiment of Fig. 11. The abrupt increase in

twitch tension produced by adrenaline was associated with an increase in the amount of repetitive firing and this appeared to be mainly responsible for the effect (Fig. 11). It appeared to be due to the same action as that giving rise to potentiation of the stimulant effect of decamethonium illustrated in Fig. 8. The anti-curare action of adrenaline, on the other hand, is associated with an increase in the amplitude of the action potential (Fig. 5,*b*). In contrast, the secondary, slowly developing increase in the amplitude of the twitches partially blocked by decamethonium, produced both by adrenaline and by isoprenaline, was not associated with increased repetitive firing, but with an increase in the amplitude of the action potential. This effect, therefore, appeared to be due to an increase in the number of muscle fibres contributing to the twitch and may be considered as a true antagonism of the block. Muscle blood flow recording again demonstrated that the effects of the amines were not a consequence of vascular changes, since the effects on the contractions were the same no matter what the blood flow change.

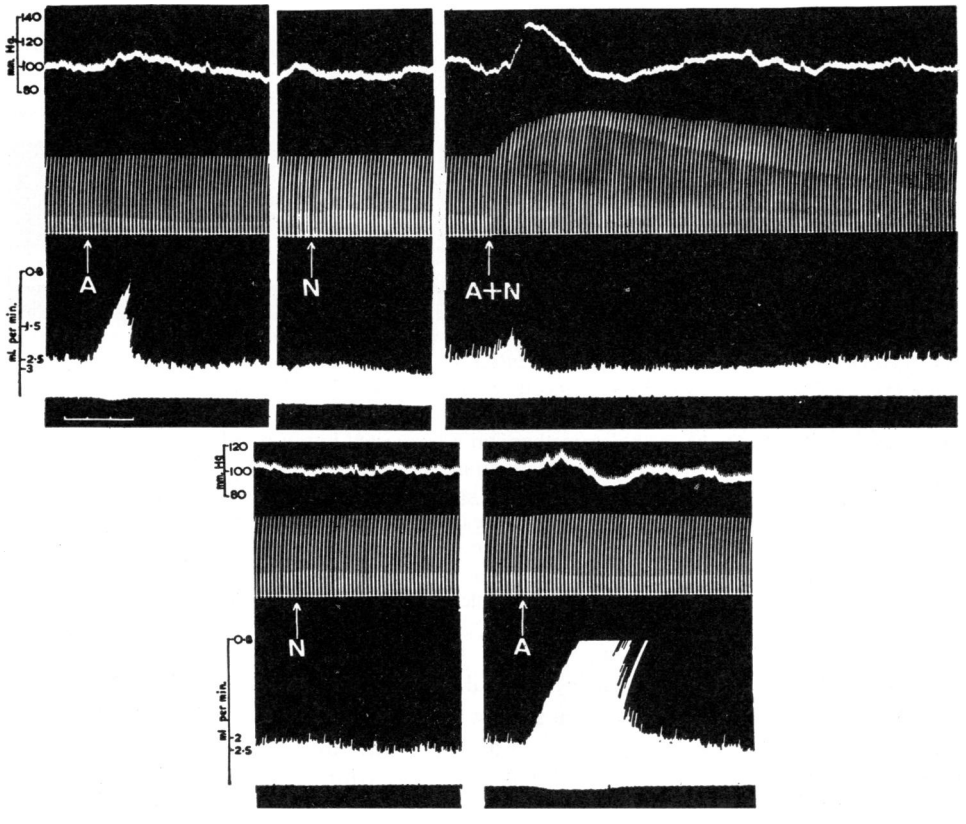

Fig. 12. General arterial blood pressure (uppermost record), maximal twitches of a gastrocnemius muscle elicited indirectly once every 10 sec (middle record) and venous outflow from the same muscle (lowest record) recorded simultaneously. At A, 2 μg adrenaline and at N, 5 μg neostigmine injected intra-arterially, each in a volume of 0.2 ml. When the two drugs were injected together (at A+N) the mixture was previously prepared to contain the above doses in 0.2 ml. Note that the reduced local vasoconstrictor action of the mixture (A+N) is accompanied by an increased pressor response. Time calibration in min.

Neostigmine

Fig. 12 illustrates the action of adrenaline in augmenting the twitch potentiation produced by neostigmine in the gastrocnemius muscle. When injected alone, the doses of adrenaline and neostigmine used had little effect on the twitch tension, but when mixed and injected together a pronounced potentiation of the twitches was produced. Noradrenaline was about two-thirds as active as adrenaline in potentiating the effect of neostigmine, and isoprenaline was without effect. Similar effects were produced in the tibialis anterior and soleus muscles. In the experiment of Fig. 12 the drugs were injected intra-arterially and adrenaline produced vasoconstriction. However, intravenous injection of larger doses (10 μg/kg of adrenaline and 25 μg/kg of neostigmine) produced similarly augmented contractions, but this effect was often accompanied only by an increase in blood flow.

The vascular changes produced as a result of interaction between adrenaline and neostigmine were of interest. When injected first, neostigmine potentiated the vasoconstrictor action of a subsequent dose of adrenaline (last panel of Fig. 12). This effect was also recorded in the perfused hind-leg of the dog by Bülbring & Burn (1942a). However, when the two drugs were injected together (third panel of Fig. 12) the vasoconstrictor action was reduced. The reduced vasoconstrictor effect of the combined injection in Fig. 12 was associated with a small pressor response which was absent when adrenaline was given alone. This suggests that in the presence of neostigmine more of the intra-arterially injected adrenaline survived passage through the muscle to reach the general circulation. Possibly neostigmine briefly reduces the permeability to adrenaline of the endothelium of the blood vessels so that less reaches the smooth muscle in their walls, more remaining in the lumen of the blood vessels to reach the general circulation. The rapid onset of the effect suggests that it is not a consequence of acetylcholine accumulation in the presence of the anticholinesterase. After the initial decrease in permeability the reverse change appears to occur, giving rise to an increased vasoconstrictor action of adrenaline.

Anti-adrenaline drugs

The previous intravenous injection of the α-receptor blocking drugs, dibenamine (10 mg/kg), phenoxybenzamine (5 mg/kg) or phentolamine (2 mg/kg), prevented the anti-curare action (Fig. 5,*a*) and the potentiation of the stimulant actions of decamethonium (Fig. 8, panel 7) and neostigmine produced by adrenaline and noradrenaline, but did not affect any of the other responses produced by the sympathomimetic amines. In the presence of one of these α-receptor blocking drugs, responses to adrenaline closely resembled those to isoprenaline. Abolition of the anti-curare action of adrenaline by dibenamine was first described by Maddock, Rankin & Youmans (1948).

In contrast, the previous injection of the β-receptor blocking drugs, pronethalol (7.5–10 mg/kg) or dichloroisoprenaline (7.5–10 mg/kg), abolished the action of all three amines in potentiating tubocurarine paralysis, depressing responses to close-arterially injected acetylcholine, increasing the muscle demarcation potential, decreasing the end-plate depolarization produced by succinylcholine (Fig. 10,*b*) and antagonizing the neuromuscular block produced by decamethonium, but did not depress responses susceptible to the α-receptor blocking drugs.

DISCUSSION

The results obtained allow the actions of adrenaline on neuromuscular transmission to be divided into two distinct types depending on the ability of isoprenaline to reproduce them, and on their susceptibility to α- and β-receptor blocking drugs. A *facilitatory* action of adrenaline on transmission is reflected by its anti-curare action and by its ability to potentiate the stimulant actions of decamethonium and neostigmine. These effects of adrenaline are rapid in onset, are not reproduced by isoprenaline, and are blocked by α-receptor blocking drugs. An *inhibitory* action of adrenaline on transmission is reflected by its ability to augment tubocurarine paralysis and to depress contractions produced by close-arterially injected acetylcholine. These effects of adrenaline, as well as its actions in increasing the muscle demarcation potential and in reducing the blocking action of decamethonium and the end-plate depolarization produced by succinylcholine, are slower in onset and longer lasting than the initial facilitatory action, are reproduced most powerfully by isoprenaline, and are blocked by β-receptor blocking drugs.

All of the effects of adrenaline could be produced both by intravenous injection and by intra-arterial injection of smaller doses indicating that they are the result of local actions, rather than of the release of other substances (e.g., liver potassium) from more distant sites. Blood flow recordings showed that, with different doses of the amines and in different experiments, the same effects on muscle contractions were accompanied by different changes in muscle blood flow. It is therefore concluded that the effects of adrenaline on neuromuscular transmission are not a consequence of vascular changes.

Facilitatory action. Adrenaline has been shown to increase the amplitude of endplate potentials produced by motor nerve stimulation (Hutter & Loewenstein, 1955; Krnjević & Miledi, 1958) but not to increase the amptitude of endplate potentials produced by iontophoretically applied acetylcholine (Krnjević & Miledi, 1958), indicating that its facilitatory action on transmission is located pre-junctionally. A pre-junctional site of the facilitatory action is supported by the present experiments in which it was found that contractions produced by injected acetylcholine, and endplate depolarizations produced by succinylcholine, were depressed by adrenaline. The anti-curare action and potentiation of the stimulant actions of decamethonium and neostigmine are therefore produced in spite of some decrease in the sensitivity of the post-junctional membrane. An action of adrenaline on the nerve endings, through which the amount of acetylcholine released by a nerve impulse is increased, would account for the anti-curare action and for the enhancement of repetitive firing produced by depolarizing drugs and anticholinesterase drugs.

Indirect evidence is available which suggests that the increase in acetylcholine release produced by adrenaline is the result of a hyperpolarizing action on motor nerve endings. Thus Krnjević & Miledi (1958) found that adrenaline relieved the pre-synaptic failure of transmission which occurs in rapidly stimulated nerve muscle preparations, and in a later paper the same authors (Krnjević & Miledi, 1959) found that hyperpolarization of the nerve endings by anodal currents produced a similar effect. Goffart & Holmes (1962) have shown that adrenaline causes hyperpolarization of mammalian C fibres. Hyperpolarization of motor nerve endings by anodal currents has been shown to increase the amount of acetylcholine released by a nerve impulse (Hubbard & Willis, 1962a). Thus the pre-junctional facilitatory action of adrenaline on neuromuscular transmission appears to

resemble the facilitatory effects of a tetanus which may also be attributed to hyperpolarization of nerve endings as a result of summation of the positive afterpotentials (Hutter, 1952; Liley & North, 1953; Liley, 1956).

Inhibitory action. The ability of adrenaline to increase the demarcation potential of normal muscle, of partially curarized muscle and of chronically denervated muscle (see also Brown *et al.*, 1950; Bowman & Raper, 1965) may largely explain its inhibitory effects on transmission. Hyperpolarization of the muscle fibre membranes, including their motor endplate regions, will be reflected in depressed transmission during circumstances in which the normal safety margin in transmission is decreased. Thus stabilization by hyperpolarization of the fibre membranes will reduce the number of endplate potentials which reach the threshold necessary to trigger propagated spikes, both when contractions are produced by injected acetylcholine and when they are produced by nerve stimulation in a partially curarized muscle; in the latter case this occurs only after the initial pre-junctional facilitatory action has diminished in extent. A post-junctional hyperpolarizing action of adrenaline also explains the reduced endplate depolarization produced by suxamethonium, and the reduced block by depolarization produced by decamethonium.

The post-junctional hyperpolarizing action of adrenaline, which may be the result of changes in muscle carbohydrate metabolism (Bowman & Raper, 1964), gives rise to similar effects in fast-contracting muscles such as tibialis anterior and gastrocnemius, and in slow-contracting muscles such as soleus, in so far as changes in neuromuscular transmission are concerned. However, when the normal safety margin in transmission is in operation, transmission occurs in spite of this action of adrenaline, and under these circumstances its hyperpolarizing action is associated with, or gives rise to, opposite changes in the contractile responses of fast- and slow-contracting muscles (Bowman & Zaimis, 1958).

The actions of adrenaline on transmission in striated muscle may be related to its actions at other sites of cholinergic transmission. In autonomic ganglia small doses of adrenaline may facilitate transmission (Bülbring & Burn, 1942b) and this effect is associated with an increased release of acetylcholine (Birks & MacIntosh, 1961). However, the primary action of the amine at this site appears to be a depressant one (Marrazi, 1939). De Groat & Volle (1965) have recently shown that the inhibitory action of adrenaline on ganglionic transmission is associated with an increase in the demarcation potential of the ganglion cells; this action is not reproduced by isoprenaline and is blocked by α-receptor blocking drugs. Thus the post-synaptic inhibitory effect in ganglia differs from the post-junctional inhibitory effect in skeletal muscle which is produced by isoprenaline and which is blocked by β-receptor blocking drugs. This difference suggests that it is the nature of the tissue, rather than the side of the junction, which determines the response. Hyperpolarization of nervous tissue, whether pre- or post-junctional, may be classified as an α-receptor effect, whereas hyperpolarization of muscle tissue may be classified as a β-receptor effect. This generalization is also borne out by the results of Kosterlitz & Watt (1965), who concluded that the inhibitory effects of adrenaline in guinea-pig small intestine were the result of two actions—an α-receptor effect on intramuscular nervous structures and a β-receptor effect on the smooth muscle cells. The smooth muscle cells of the gut are known to be hyperpolarized by adrenaline (Bülbring, 1954; Burnstock, 1958), and the inhibitory effect on nervous structures might also be the result of hyperpolarization, either of intramuscular ganglion cells or of nerve endings. A weak hyperpolarization of nerve terminals may

enhance transmission by increasing transmitter release, but a strong hyperpolarization will block conduction in the nerve endings.

In their experiments on autonomic ganglia, de Groat & Volle (1965) also observed a secondary depolarizing action of adrenaline which was reproduced by isoprenaline and which was blocked by β-receptor blocking drugs. Other evidence of a depolarizing action of adrenaline on nervous tissue may be derived from the results of Legouix & Minz (1953). If a secondary depolarizing action of adrenaline occurs at motor nerve endings in striated muscle, it would be expected to augment the secondary inhibitory effect on transmission, since depolarization of nerve terminals reduces the release of acetylcholine by nerve impulses (Hubbard & Willis, 1962b).

SUMMARY

1. The effects of adrenaline, noradrenaline and isoprenaline in conjunction with tubocurarine, decamethonium, suxamethonium and neostigmine have been studied in the tibialis anterior, gastrocnemius and soleus muscles of cats under chloralose anaesthesia.

2. Adrenaline and noradrenaline exerted an initial facilitatory action on neuromuscular transmission which was blocked by the α-receptor blocking drugs, dibenamine, phenoxybenzamine and phentolamine, and which appeared to be mainly pre-junctional in origin.

3. All three sympathomimetic amines produced a secondary inhibitory action on neuromuscular transmission which was blocked by the β-receptor blocking drugs, dichloroisoprenaline and pronethalol, and which appeared to be mainly post-junctional in origin and a consequence of hyperpolarization of the muscle fibre membranes.

4. Muscle blood flow recordings indicated that none of the effects of the amines on transmission was a consequence of vascular changes.

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