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ACTIONS OF SYMPATHOMIMETIC AMINES AND THEIR ANTAGONISTS ON SKELETAL MUSCLE

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I. INTRODUCTION

Sympathomimetic amines and the sympathetic nervous system have long been known to affect skeletal muscles in various ways, but some confusion has arisen because it was often not realised that more than one site of action is involved, and there has been a tendency to attribute the different effects to a common although incompletely understood cause. To some extent, the effects differ according to species, age, and the condition of the muscle, and even in the same species, different types of muscle may respond differently.

Much of the early work was done in the belief that the muscle fibers, as well as their blood vessels, received a direct adrenergic innervation. In general, the sympathetic supply to skeletal muscle runs within the main nerve trunk (74). Within the muscle, nonmyelinated sympathetic fibers pass from the main nerve trunks to the blood vessels, around which they form plexuses (337). The literature contains numerous descriptions of an accessory innervation of skeletal muscle with endings terminating within motor endplates. These accessory fibers bear some resemblance to sympathetic fibers and were the basis for a vigorous controversy concerning the existence of a direct sympathetic innervation of the muscle fibers. However, in many cases degeneration studies have shown them to be somatic motor fibers, and, although the possibility has not been entirely ex-

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cluded for all species, for many years it has now been held that skeletal muscle fibers do not receive a direct sympathetic innervation (see for example, reviews 178, 179, 335). Any influence of sympathetic stimulation on skeletal muscle contractions, other than that which is secondary to vascular changes, is therefore considered to be due to adrenergic transmitter from the abundant vascular sympathetics, which diffuses to the muscle fibers. The extraocular muscles may, however, be a special case. These muscles in the cat take up considerable amounts of labelled norepinephrine and this uptake is reduced after chronic denervation of the postganglionic cervical sympathetic trunk (171).

During the late 19th and early 20th centuries a great deal of work was done on the relation between the adrenal glands and the function of skeletal muscle. A number of clinical observations was made (99, 280) and animal experiments were performed (32, 98, 109, 161, 162, 167, 217, 274, 279, 329). Many of the authors used extracts of the whole adrenal glands or based their conclusions on the results of adrenalectomy and, since the functions of the adrenal cortex were not understood at that time, confusion existed for many years because effects now known to be caused by adrenal cortical hormones were attributed to the adrenal medulla. Much of the older literature referring to this was reviewed by Gruber (155, 156).

Depending on the species and the conditions of the experiment, sympathomimetic amines can be shown to influence the muscle fibers themselves, the muscle spindles, the neuromuscular transmitting process, and conduction in the nerve; their effects differ to some extent according to whether the muscle is fatigued or nonfatigued. They also affect the spontaneous activity and tone of chronically denervated muscles. This review is restricted to the direct actions of sympathomimetics and their antagonists on muscle and on the neuromuscular junction. Their actions on muscle spindles have recently been described in detail (278, 314, 315) and there appears to be little information to add. It should be borne in mind that some of these drugs may also influence muscle function by actions within the central nervous system (243, 344).

II. DIRECT ACTION ON NONFATIGUED MUSCLE

A. Mammalian muscles

Mammalian skeletal muscles differ in their speeds of contraction and may be classified as fast- or slow-contracting. The time from the start of the contraction to the tension peak in an isometric twitch of a fast-contracting muscle in the mammal is of the order of 30 msec or less. Slow-contracting mammalian muscles have a contraction time in an isometric twitch of 60 msec or more. Flexor muscles (such as tibialis anterior) and superficial extensor muscles (such as gastrocnemius) are fast-contracting muscles, whereas deep extensors (such as soleus and crureus) are slow-contracting muscles in most species. At birth, all limb muscles in many mammalian species are slow-contracting (9, 70, 89, 107, 210) and differentiation into fast or slow types occurs during the first few weeks after birth.

1. Fast-contracting muscles. Oliver and Schäfer (274) were the first to demonstrate that extracts of the adrenal medulla increase the tension and the duration

of the indirectly elicited twitches of the nonfatigued gastrocnemius muscle of the anaesthetised dog. Epinephrine was isolated from the adrenal medulla in 1901 (1, 328) and in the intervening years up to 1922 much work was done on the effects of epinephrine on fatigued skeletal muscles. However, it was not until 1922 that Gruber (157, 158) tested the effect of epinephrine itself on the contractions of nonfatigued muscles. With huge doses ranging from 50 μg to 5 mg, Gruber found that epinephrine increased by as much as 100% the indirectly elicited twitches of the tibialis anterior muscle of anaesthetised cats. Stimulation of the splanchnic nerves produced a similar, though smaller, change in the muscle contractions. Since that time numerous workers have confirmed that both epinephrine and splanchnic stimulation increase the contractions of nonfatigued fast-contracting mammalian skeletal muscles (35, 38, 40, 42, 43, 45, 46, 60, 144, 146, 149, 191, 266, 353). None of these workers found epinephrine to produce increases in twitch tension as large as those recorded by Gruber, but the doses they used were much smaller. When large doses are used, repeated injections made at intervals of less than about an hour produce diminishing effects (60). In the adult cat, the smallest effective dose of (−)-epinephrine necessary to produce a detectable increase in the maximal isometric twitch tension of the nonfatigued tibialis anterior muscle is of the order of 3 μg/kg intravenously, or 0.2 μg close-arterially (43, 46). After a dose of 10 μg/kg intravenously, the onset of the effect occurs in 20 to 40 sec and reaches its peak in 100 to 180 sec, and the whole effect lasts about 15 min. Other fast-contracting muscles in the cat and in other mammals show a similar sensitivity. In kittens shortly after birth, epinephrine produces effects on the contractions of the tibialis anterior muscle qualitatively similar to those produced in the adult but the effective doses, on a body weight basis, are two or three times larger (38). The adult sensitivity is reached within 1 to 2 months after birth.

Epinephrine also increases the twitches of nonfatigued fast-contracting muscles when the stimulation is applied directly after full curarization or chronic denervation (35, 46, 144, 149). This shows that the effect is independent of neuromuscular transmission and must be exerted on the muscle fibers themselves. Results of experiments on isolated skeletal muscles bathed in physiological salt solutions (40, 141, 146, 149, 266) and of experiments in which muscle blood flow was recorded simultaneously with the contractions (46) have shown that the effects of epinephrine on the contractions are not a consequence of concomitant vascular changes.

The increase in the twitch tension of fast-contracting skeletal muscles produced by epinephrine is associated with an increase in the total duration of the twitch (35, 46, 149). This effect results in an increase in fusion when incomplete tetanic contractions are recorded and the effects of epinephrine on incomplete tetanic contractions are therefore more striking than those on twitches (35, 46). Goffart and Ritchie (149) found that large doses of epinephrine (10 μg) injected close-arterially, depressed the maximal tetanic tension of the tibialis anterior muscle of the cat. Bowman and Zaimis (46) confirmed this finding but showed that it was probably a consequence of the powerful vasoconstriction produced by the large

dose of epinephrine; they concluded that epinephrine did not directly affect the tension of maximal tetani. The increase in twitch tension produced by epinephrine is associated with a delay in the time to peak tension and with a slight slowing in the initial rate of development of tension (46, 149). Since maximal tetanic tension is not increased, Goffart and Ritchie (149) concluded that the effect of epinephrine arose through a prolongation of the plateau of the active state of the stimulated muscle (176). Jurna and his co-workers (28, 201, 202), who made more detailed measurements, obtained similar results but concluded that it is the decaying phase of the active state curve which is prolonged rather than the duration of the plateau.

In addition to epinephrine, several other sympathomimetic amines have been tested for an action on nonfatigued fast-contracting skeletal muscles (35, 40, 42, 45, 46, 60, 144, 145, 191, 353). Most have been shown to be considerably less potent, but racemic isoproterenol is slightly more potent, and (−)-norepinephrine is about two-thirds as potent as (−)-epinephrine. (−)-Isoproterenol is about twice as potent as the racemic compound.

Although norepinephrine is effective, stimulation of the lumbar sympathetic chain at frequencies up to 20/sec is without effect on the contractions of the nonfatigued tibialis anterior muscle of the cat (46, 144), presumably because the quantity of norepinephrine released is insufficient to affect the muscle contractions. From a consideration of the large size of the minimal effective dose of epinephrine (3 μg/kg intravenously) and the high frequency of unilateral splanchnic nerve stimulation (20/sec) necessary to affect the tibialis anterior muscle of the cat, it was concluded (46) that it is unlikely, except possibly in disease, that the effect of catecholamines on the contractions of nonfatigued fast-contracting muscle fibers has any physiological significance, since the effective blood concentrations are probably in excess of any that occur even under conditions of extreme stress.

Catecholamines are much more effective in increasing the contractions of the directly stimulated, fully curarized, fast-contracting, isolated diaphragm muscle of the rat, when the contractions are first depressed by excess KCl (40, 165, 207). The extent of the increase in twitch tension is much greater, and the effective concentrations of the drugs much smaller under these conditions. Since the rank order of potency of a series of amines and the effects of adrenoreceptor blocking drugs are the same in the presence or absence of excess KCl, the effect in its presence could be an exaggeration of the same effect as that occurring in its absence (40). The potassium-depressed diaphragm has therefore proved to be a highly sensitive preparation for the study of catecholamine effects on muscle contractility. Surprisingly, when the amount of excess K^+ in the bath fluid is less than that necessary to depress the twitches, the augmenting action of epinephrine is impaired (146), and K^+ depletion of the inside of the muscle may even reverse the effect (141). Within limits, the augmenting action of catecholamines on the twitches of isolated muscle increases with increase in the concentration of calcium ions in the bathing fluid (144, 266).

2. Slow-contracting muscles. The soleus muscle of the cat, the rabbit, the guinea-

pig, and the rat, the crureus muscle of the cat, and the plantaris muscle of the dog are among those classified as slow-contracting muscles. All of them have been shown to react to sympathomimetic amines and to splanchnic stimulation in the opposite way to fast-contracting muscles (28, 35, 38, 43, 45, 46, 201, 202). Thus, maximal isometric twitch tension, whether evoked by nerve stimulation or by direct stimulation of the fully curarized or chronically denervated muscle, is reduced by up to 20%, and this is associated with decreases of up to 25% in the time to peak tension and up to 35% in the total duration of the twitch. The rate of rise of tension and the tension of maximal tetani are unaffected. The changes in time course suggest that the effect results from a curtailment of the active state (38); according to Jurna and Rummel (201) epinephrine hastens the decline of the active state curve without affecting the duration of the plateau of full activity. Because the overall duration of the twitch is reduced, fusion and tension are markedly reduced by epinephrine during incomplete tetanic contractions. This effect is most pronounced at frequencies of stimulation in the range of 6 to 9 per sec, and a partially fused contraction evoked by stimulation at a frequency of 6/sec may be converted by epinephrine to a series of separate twitches (38, 43, 46). As stimulation frequency is increased, the effect of epinephrine becomes less obvious and is usually undetectable at frequencies above 20/sec. These effects of sympathomimetic amines are independent of concomitant changes in muscle blood flow (46).

When a motor nerve is stimulated with a single shock, the summed action currents associated with the muscle response may re-excite the nerve endings, and the back-response so produced may be recorded antidromically in the ventral root (235). The back-response in the nerve propagates in both directions from the site of its initiation and can be shown to re-excite some of the muscle fibers (63), so that some muscle fibers fire more than once in response to a single synchronous nerve volley. When the motor nerve is stimulated with a pair of shocks with an interval of 1 msec between them, the second of the pair renders the nerve refractory so that no back-response is produced. Since the interval between the two stimuli is within the refractory period of the muscle fibers, the second nerve shock does not elicit a muscle response, and hence a truly single muscle response is evoked by such a closely spaced pair of nerve shocks (63). The resultant twitch is smaller than that evoked by a single nerve shock and superficially its shape resembles a twitch of a slow-contracting muscle which has been depressed by epinephrine. Raper (294) therefore considered the possibility that the effect of epinephrine on a slow-contracting muscle twitch might arise through depression of repetitive responses that may occur in some muscle fibers, as a result either of a back-response in the nerve or of the relatively strong stimuli necessary to stimulate a muscle directly. However, his results showed that epinephrine depressed the cat soleus twitch in response to single-shock stimulation without affecting the back-response, and it also depressed the truly single muscle response evoked by double-shock stimulation, although no back-response was present under these conditions. Furthermore, the depressant effect of epinephrine on the soleus twitch was always much greater than that of double-shock stimulation.

Similar results have been reported with double- and single-shock stimulation of human calf muscles (250). These results therefore show conclusively that the epinephrine effect on slow-contracting muscles is not due to depression of any repetitive firing in muscle fibers.

The soleus muscle of the adult cat is much more sensitive to the action of (−)-epinephrine than is the tibialis anterior muscle (35, 43, 46). The smallest effective dose is of the order of 0.05 μg/kg intravenously or 0.003 μg close-arterially, and the maximal effect obtainable is produced by about 3 μg/kg intravenously. After a dose of 1 μg/kg intravenously the onset of the effect occurs in 20 to 40 sec and reaches its peak in 60 to 100 sec, and the whole effect lasts about 12 min. Other slow-contracting muscles of the cat and of other mammals show a similar sensitivity to epinephrine. Stimulation of the left splanchnic nerve of the cat at a frequency of 10 to 20/sec produces an effect on the cat soleus muscle equivalent to the maximum obtainable with injected epinephrine, and the concentration of catecholamines released from the suprarenal medullae in response to hypoxia produced by occluding the trachea for 10 to 20 sec is sufficient to produce a detectable effect on the soleus muscle. (−)-Isoproterenol is slightly more than twice as potent as (−)-epinephrine, but (−)-norepinephrine is 20 to 30 times less potent than epinephrine on the cat soleus muscle. In fact the sensitivity of the cat soleus to norepinephrine is similar to that of the tibialis anterior muscle, and the lack of sensitivity to this amine probably accounts for the fact that lumbar sympathetic stimulation is without effect on the soleus muscle (46). The marked sensitivity of the soleus muscle to epinephrine and the fact that it is most effective within the physiological range of frequencies for this muscle (106) suggests that the effect on slow-contracting fibers probably does have some physiological significance in relation to stress, although it is not clear what this is. It has been suggested (46) that an action of epinephrine on slow-contracting fibers in postural muscles may be the explanation of the feeling of weakness in the limbs experienced as an effect of fright.

The anaesthetic used may determine the presence of the soleus muscle response to epinephrine. The response is present in the nonanaesthetised decerebrate cat, and under chloralose anaesthesia in all species tested. However, it is small or absent under urethan anaesthesia, at least in the rabbit (35). Urethan is known to cause the discharge of catecholamines from the adrenal glands (121, 182, 319) and under this anaesthetic, the sensitive soleus muscle may already be maximally affected by the circulating hormone so that additional injected epinephrine is without effect. Chloralose does not cause a discharge of epinephrine from the adrenals (132).

In kittens a few days after birth, the effects of epinephrine on soleus muscle contractions are variable and unpronounced and require large doses to elicit them. The sensitivity and type of response characteristic of the adult muscle is gained at about the age of 1 month (38).

By means of nerve cross-union experiments it has been shown (71) that when a nerve formerly innervating a fast-contracting muscle is made to innervate a slow-contracting muscle, the latter is transformed to resemble a fast-contracting

muscle. Slow or tonic motoneurons made to innervate a fast-contracting muscle maintain the slowing initially produced by denervation (120). These changes in the muscle speeds are accompanied by enzymic changes, notably in myosin-ATPase and lactic dehydrogenase (264). These enzymes differ in the two types of muscle, and cross-innervation produces the change appropriate to the opposite type of muscle. In the soleus muscle of the cat that has been cross-innervated with the nerve formerly innervating the fast-contracting flexor digitorum longus muscle, both the muscle speed and the response to catecholamines change to resemble those of a fast-contracting muscle (38). Chronic denervation alone does not produce this change. It therefore appears that the motoneurons innervating fast-contracting muscles exert some influence on the muscle fibers that determines their response to the catecholamines as well as their contraction speed. In the cat flexor digitorum longus muscle that had been cross-innervated with soleus motoneurons, the response to the catecholamines remained that of a normally innervated fast-contracting muscle, and did not therefore differ from that of a chronically denervated fast-contracting muscle (294).

3. Electrical and ionic changes. Epinephrine, norepinephrine, and isoproterenol consistently produce a small increase in the demarcation potential of the fast-contracting tibialis anterior muscle of the cat recorded between an injured and an uninjured part of the muscle surface (42, 60). As with the effect on twitch tension, isoproterenol is slightly more, and norepinephrine slightly less potent than epinephrine in this respect. Similar effects are produced by high frequency (76/sec) stimulation of the ipsilateral lumbar sympathetic chain (60). The increase in twitch tension of the tibialis anterior muscle produced by the catecholamines matches the increase in demarcation potential in its time course. A similar increase in demarcation potential is produced by the catecholamines in the cat soleus muscle (42) although the change in contractions produced in this muscle is opposite to that in the tibialis anterior. Brown *et al.* (60) found that the increase in demarcation potential that they recorded in the tibialis anterior muscle was followed by a depolarization of longer duration and greater magnitude. They also found that a small secondary decrease in twitch tension followed the initial augmentation, although both of these secondary changes were difficult to measure with precision, since they were complicated by instrumental drift and gradual decay of the preparation. Tachyphylaxis was evident both in the electrical response and in the tension response when injections were made at intervals of less than an hour. Any secondary changes in potential and tension were less evident in the experiments of Bowman and Raper (42), who used smaller doses of the catecholamines; nor was tachyphylaxis such a characteristic feature of the responses.

Krnjević and Miledi (214), experimenting on rats, recorded the resting membrane potentials of the isolated diaphragm muscle and of the gracilis muscle *in situ*, with intracellular micro-electrodes. They were unable to demonstrate any significant effect of epinephrine on the resting membrane potential. Since epinephrine has been shown by other workers (142, 144, 146, 149) to have a clear augmenting action on the twitches of the nonfatigued rat diaphragm, Krnjević

and Miledi's (214) results suggest that there may be no causal relationship between effects of epinephrine on membrane potential and effects on tension. However, in view of the variability of the epinephrine response and the fact that its presence may depend on conditions such as the composition of the physiological salt solution used for isolated muscles (146, 266) or the nature of the anaesthetic used in experiments *in situ*, this conclusion would be convincing only if tension responses were measured under identical conditions. Krnjević and Miledi (214) did not measure tension responses in their experiments.

Krnjević and Miledi (214) did detect a small but distinct increase in the stimulation threshold of the muscle fiber produced by epinephrine when stimulating at a low frequency. The literature is contradictory on this point. Gruber (152) claimed to have seen as much as a 62% reduction in threshold, whereas Obré (272), like Krnjević and Miledi, found an increase. Gruber himself found an increase in a later series of experiments on denervated muscle (157). Luco (236) found no change.

Goffart and Perry (148) measured the rate of loss of potassium ions from skeletal muscle, and showed that, in muscles preloaded with ^{42}K, epinephrine, norepinephrine, and isoproterenol produced an initial decrease followed by an increase in the rate of potassium loss. These changes parallelled, both in their time course and in their dual nature, those of the maximal twitch tension and demarcation potential (60).

During a study of active transport of sodium and potassium in rat skeletal muscle, Dockry *et al.* (111) studied the effect of (±)-isoproterenol and (−)-epinephrine on soleus muscles previously rendered sodium rich by soaking them in a cold, potassium-free modified Krebs' solution. When the muscles were denervated just before immersion in a recovery fluid containing both sodium and potassium, Na^+ was excreted and the membrane potential increased to a value greater than the potassium equilibrium potential; this indicates that the sodium pump is electrogenic under these conditions. Isoproterenol stimulated the excretion of Na^+, and this was found to be associated with a decrease in resting membrane potential when measured soon after immersion in recovery fluid. At this stage the membrane potential was still greater than E_K so that the sodium pump was still electrogenic. However, when Na^+ excretion was complete, E_K remained higher in soleus muscles treated with isoproterenol than in control muscles. Assuming that, when Na^+ excretion is complete, $E_m = E_K$, then it follows that treatment with isoproterenol will in the long run cause hyperpolarization of the membrane; and this is compatible with the increase in demarcation potential found in the normal cat soleus muscle *in situ* (42). In the same series of experiments, Dockry *et al.* (111) found epinephrine to be inactive, but this is hardly surprising in view of the fact that the concentration of epinephrine used was one fifth of that of isoproterenol. Racemic isoproterenol is only marginally more potent than (−)-epinephrine in its actions on twitch tension and demarcation potential.

Few detailed studies have been made of the effects of the catecholamines on propagated action potentials in the muscle fiber. Brown *et al.* (57, 58) found that

epinephrine produced an increase in the duration, but not in the amplitude, of the single muscle fiber action potential recorded extracellularly, and they concluded that this effect was due to retardation of the spread of the excitation wave along the fiber. Slowed conduction is the expected effect if the membrane is hyperpolarized. Goffart (143) suggested that the increase in twitch tension might arise because the slowed excitation wave allows more time for the contractile component to stretch the series elastic component. However, Goffart and Ritchie (149) ruled out this explanation by showing that epinephrine was equally effective in augmenting twitch tension when the stimulation was applied at one end of a muscle and when it was applied at many points simultaneously. In the latter case propagation time would be less important.

The decrease in twitch tension of the cat soleus muscle produced by the catecholamines is associated with a slight increase in the amplitude of the gross muscle action potential recorded with belly-tendon leads (35). This effect might be due to hyperpolarization increasing the amplitude of the individual fiber potentials. On the other hand, the increase in twitch tension produced in the cat tibialis muscle is accompanied by a slight diminution in the amplitude of the gross action potential (35, 144); this effect could be explained if the hyperpolarization in some fibers is sufficient to block conduction in them. If this is so, the increase in twitch tension must occur despite fewer fibers contributing to it. However, accurate knowledge of the changes in propagated action potentials in the two types of muscle awaits experiments involving intracellular microelectrode recording.

Epinephrine has also been found to hyperpolarize the membrane of heart muscle fibers, but this is by no means a constant finding and appears to depend on the conditions of the experiment and the part of the heart studied (113, 150, 183, 343). The action potential of artrial muscle stimulated to beat at a constant rate is slightly prolonged (138, 183, 351) and may be slightly increased in amplitude (343). As in skeletal muscle, epinephrine increases the potassium content of atrial muscle fibers, an effect mediated *via* β-receptors (321). The effect differs from that in skeletal muscle in that it occurs only in beating atria and is the result of an increased rate of K^+ uptake. The rate of K^+ loss is unaffected (321).

According to Huidoboro *et al.* (191), the increase in the twitch tension produced by epinephrine in the fast-contracting quadriceps femoris muscle of the cat is associated with repetitive firing of the muscle fibers. However, in the same series of experiments, the authors studied the interaction between epinephrine and neostigmine. Through a completely different mechanism of action (described below), epinephrine augments the repetitive firing produced by neostigmine and is able to restore repetitive firing long after the effect of a previous injection of neostigmine has apparently disappeared. Since this effect of epinephrine was probably not appreciated at the time, it may be that the animals in which epinephrine appeared to produce repetitive responses had previously been treated with neostigmine, and that a subeffective concentration still persisted in the circulation even though repetitive firing was absent from the control records. No evidence of repetitive firing produced by epinephrine alone has been reported by any other workers (35, 57, 58, 144, 312).

4. Adrenoreceptors. The direct effects of the catecholamines on the contractions of both fast- and slow-contracting skeletal muscles, and their effects on demarcation potential are generally agreed to be mediated *via* β-receptors. Isoproterenol is more active than epinephrine, whereas norepinephrine is less so (43, 45, 46, 60, 144, 191, 353). The effects are unchanged, or even enhanced, in the presence of the α-receptor blocking drugs ergotoxine, ergotamine, tolazoline, 933F, dibenamine, phenoxybenzamine, and phentolamine (46, 60, 144), and they are blocked by the β-receptor blocking drugs, dichloroisoprenaline, pronethalol, and propranolol (35, 43). On the basis of experiments on the indirectly stimulated isolated phrenic nerve-diaphragm of the rat, Biver and Goffart (25) have questioned the concept that only β-receptors are involved, since some degree of antagonism was obtained with the α-receptor blocking drug, phentolamine. In unpublished work, Raper has repeated these experiments under identical conditions. He found that propranolol was always effective and phentolamine ineffective when the curarized muscle was stimulated directly. Propranolol was always the more effective when indirect stimulation was used, but in this case phentolamine also occasionally produced a small reduction in the epinephrine effect. In every case, this effect of phentolamine could be demonstrated only when there was an indication that some degree of fatigue was present in the indirectly evoked twitches. The defatiguing action of epinephrine, which is referred to in a later section of this review, differs from the direct action described here and is mediated through both α- and β-receptors. Other workers (40, 124, 127, 129, 221) using the isolated diaphragm preparation of the rat have supported the finding *in vivo* that the direct action of epinephrine on the nonfatigued muscle is mediated through β-receptors.

On the basis of the relative specificity of different agonists and antagonists, it has been proposed (5, 6, 220, 221) that there are two types of β-receptor. These have been designated β_1 which regulate effects on the heart, lipolysis, and intestine, and β_2 which regulate bronchodilatation, vasodilation, effects on the uterus, and muscle glycogenolysis. With this classification, the available evidence indicates that effects of catecholamines on skeletal muscle contractions are mediated *via* β_2 receptors. Lands *et al.* (221) tested the effects of a series of sympathomimetic amines in which methyl or ethyl groups were substituted on the carbon *alpha* to the nitrogen of norepinephrine, or in which various alkyl groups were substituted in the nitrogen center. The ability of the compounds to increase the twitches of the potassium-depressed rat diaphragm were correlated with their ability to inhibit the rat uterus, but not with their ability to stimulate the rabbit heart or relax the rabbit intestine. The apparent difference in receptors is probably not due to species but to a true difference in tissues, since the receptors in the vascular system of the dog correlate with those in the guinea-pig lung (5,220), and adipose tissue in the guinea-pig resembles that in the rat (4). Brittain (51) tested a series of saligenin analogues of isoproterenol on different tissues in the same animal (the cat). These compounds, which included α-[(*t*-butylamino)methyl]-4-hydroxy-*m*-xylene-α',α^2-diol (Salbutamol) described by Hartley and Brittain and co-workers (52, 166), are powerful bronchodilators

and produce vasodilation, but have relatively little cardiac stimulant activity. In bronchodilating doses they also decrease tension and fusion of incomplete tetanic contractions of the soleus muscle. Among the relatively selective antagonists that have been tested on skeletal muscle, methoxamine and its N-isopropyl and N-tertiarybutyl derivatives block isoproterenol-stimulated muscle glycogenolysis but are much less effective in blocking actions on the heart or the intestine (76, 228, 229). Methoxamine and its analogues antagonise isoproterenol effects on skeletal muscle contractions (40, 43). Furthermore, the compound, 4-(2-hydroxy-3-isopropylaminopropoxy) acetanilide (I.C.I. *50, 172*), which shows affinity for β-receptors in the heart and in adipose tissue, but little affinity for β-receptors in blood vessels or the trachea (12, 114), has little effect in blocking isoproterenol effects in skeletal muscle (36). These results with selective antagonists again indicate that the β-receptors in skeletal muscle may be classified as of the β_2 type. However, a true difference in the configuration of different β-receptors should not be accepted without reservation. The apparent difference could be explained if different tissues possess different diffusion barriers or uptake mechanisms that could modify the effects of some drugs.

5. Human muscles. Human muscles are less well differentiated into fast- and slow-contracting types than are those of the cat; they consist of a more even mixture of fast and slow motor units, although the relative proportions of each do vary from muscle to muscle. Since the slow-contracting cat soleus muscle is affected by doses of catecholamines much smaller than those necessary to affect fast-contracting muscles, it might be expected that the slow motor units of human muscles would be selectively affected by catecholamines unless intolerably large doses were used. Bowman and Zaimis (46) therefore suggested that the feeling of weakness in the back and limbs experienced during epinephrine infusions (11) and the tremors experienced by patients with phaeochromocytoma (285) might be due to the action of catecholamines in decreasing fusion in incomplete tetanic contractions of slow motor units. Infusions of epinephrine or isoproterenol enhance physiological tremor (249). Enhancement of tremor is produced by epinephrine even in a totally deafferented limb; hence it can occur independently of any action involving the muscle spindles (251). The tremor produced by infusions of catecholamines in man is blocked by the β-receptor blocking drug (±)-propranolol, but is unchanged by the (+) isomer of propranolol, which has only very weak β-receptor blocking activity (249).

Recently, it has been shown in human muscles (250) that epinephrine and isoproterenol do in fact produce effects closely similar to those produced on the cat soleus, and that the effects are blocked by the β-receptor blocking drug, propranolol. The effects of the catecholamines on human muscles were more pronounced on the triceps surae group (twitch time, 186 msec) than on the tibialis anterior group (twitch time, 135 msec); in the doses used, the catecholamines were without effect on the contractions of the adductor pollicis (twitch time, 123 msec). Thus the extent of the effect of the amines appeared to depend on the proportion of slow motor units in the muscles. Emotional tension increases Parkinsonian tremor, as do infusions of epinephrine (10, 92, 249). This effect of cate-

cholamines in Parkinson's disease is unchanged by the α-receptor blocking drug, phentolamine (92) but is blocked by the β-receptor blocking drugs, pronethalol and propranolol (277). On the other hand, the increase in tremor intensity produced by emotional stress in Parkinsonian patients is not influenced by propranolol; this suggests that it is largely due to a central nervous system mechanism (252). In a controlled clinical trial, oral propranolol relieved spontaneous tremor in a proportion of Parkinsonian patients (277).

The suggestion that bronchodilator effects and skeletal muscle effects are mediated *via* the same type of receptors (β_2) may mean that it is impossible to separate these two actions, and systemic administration of sympathomimetic bronchodilators may therefore carry with it the possibility of inducing skeletal muscle tremor as an unwanted side-effect. Brittain's (51) evidence, referred to above, is suggestive of this.

6. Relation to previous activity. Brown *et al.* (60) first drew attention to the similarity between the effects of epinephrine and the effects of previous activity (a tetanus or treppe) on the twitches of fast-contracting skeletal muscle, and this was extended by Ellis (124). However, although both increase the peak tension, the similarity is limited; the effect of epinephrine is accompanied by a slight initial slowing in the rate of rise of tension (46, 149), whereas the much greater effect of a previous tetanus and the increase in tension that constitutes the positive staircase effect in fast-contracting muscles are associated with a marked increase in the rate of rise of tension (35, 62, 90). During treppe (35) or after short trains of stimuli (90) the augmented twitch tension is accompanied by no change or even by a reduction in time to peak, and by a decrease in half relaxation time. These effects are largely due to an increase in the degree of activation of the contractile mechanism, probably arising because the sarcoplasmic reticulum has insufficient time between stimuli for the re-uptake of freed Ca^{2+}. They are not produced by catecholamines (35, 90). After a prolonged tetanus, however, an additional effect appears to come into play and the augmented twitch tension is now associated with a delay in time to peak and by an increase in half relaxation time (35, 90, 91, 293) presumably as a result of prolongation of the active state (298). In frog muscle at 0°C, a previous tetanus causes an increase in the tension of the post-tetanic twitches that is not associated with a change in the force-velocity curve but only with an increase in the time course of activity (298), so that the effect resembles that of epinephrine on nonfatigued mammalian fast-contracting muscle. Thus it appears that previous activity produces at least two effects contributing to the enhancement of twitch tension in fast-contracting muscles, but only one of these—prolongation of the active state—is shared by catecholamines.

In heart muscle, the effect of epinephrine on contractility is more pronounced than it is in skeletal muscle, and despite the increase in twitch tension, the active state of heart muscle is curtailed (204, 209, 317). The effect of epinephrine on the heart closely resembles the positive staircase effect induced by increasing the frequency of stimulation (209, 295, 313, 318). These effects on the heart also closely resemble the positive staircase effect in fast-contracting skeletal muscle

(35, 295). In all these cases, the increase in amplitude of contraction is accompanied by an increase in the rate of rise of tension, by a decrease in time to peak tension, and by an increase in the rate of relaxation.

In slow-contracting mammalian skeletal muscles, such as soleus or crureus, the effects of previous activity more closely resemble the effects of epinephrine (35). In the cat soleus muscle, a brief, low-frequency tetanus gives rise to a temporary decrease in the the tension of the subsequent twitches (35, 62) and this muscle exhibits a negative staircase effect (35). Though less pronounced, the change in the time course of the depressed twitches closely resembles that produced by epinephrine, and both may be ascribed to a curtailment of the active state (35). Thus, especially in slow-contracting muscles, it appears that a greater insight into the changes produced by previous activity may help to explain the action of epinephrine, and *vice versa*. It is interesting to note that unlike most mammalian hearts, but like the cat soleus muscle, the rat heart responds to an increase in rate by a decrease in contractile force (209).

In cardiac muscle, there are said to be two opposing interval-dependent changes which determine the strength of contractions at a given frequency. One change, designated the negative inotropic effect of activation (NIEA), tends to decrease contractility, whereas the other, the positive inotropic effect of activation (PIEA), is algebraically additive with the NIEA and tends to increase contractility (209). With a change in frequency the two factors cumulate or decay to new steady-state levels. It is interesting to speculate that a similar concept may hold for skeletal muscle. In a fast-contracting muscle, NIEA may be present for only a very short time after a stimulus so that the PIEA accumulates to give a positive staircase unless high subclonic stimulus frequencies are used. In a slow-contracting muscle, on the other hand, it may be that NIEA is present for much longer after a stimulus so that, with increase in frequency, it accumulates and masks any PIEA to give a negative staircase effect. In cardiac muscle, epinephrine produces its positive inotropic effect mainly by increasing the amount of PIEA per beat and therefore has little effect on the strength of contractions separated by long intervals (208, 209, 340). Note however, that this does not appear to be so in the frog atrial trabecula. Brady (48) found that the positive inotropic action of isoproterenol was equally pronounced after 2 min whether the muscle was regularly stimulated or allowed to rest for 2 min after addition of the drug. It may be that in slow-contracting skeletal muscle, epinephrine acts either to prolong or enhance the NIEA or to shorten or reduce the PIEA, whereas the reverse change may occur in fast-contracting muscles. The augmenting action of epinephrine on the twitches of fast-contracting muscles has been shown to be enhanced by an increase in frequency from 0.1 to 1 per sec or after a short-lasting tetanus (35), and this is compatible with an action exerted through an increase in PIEA. However, as far as we know, skeletal muscle has not been studied from this particular standpoint, although such experiments might be profitable.

7. *Mechanism of action.* Gruber (154, 155, 160) first suggested that the effects of epinephrine on muscle contractions may be secondary to changes in muscle carbohydrate metabolism. Epinephrine and other sympathomimetic amines

exert a pronounced stimulant effect on glycogenolysis in tissues, including skeletal muscle (93, 316); the early steps in this process have been recently reviewed (126, 212). Epinephrine stimulates the enzyme system known as adenyl cyclase, and it has been suggested that adenyl cyclase may actually constitute the receptor (300). Adenyl cyclase, in turn, catalyses the conversion of ATP to the cyclic nucleotide 3′,5′-AMP, the levels of which have been shown to be increased by epinephrine in skeletal muscle (287). Cyclic 3′,5′-AMP inhibits the activity of glycogen synthetase (303) and, in the presence of ATP, it converts, probably by way of another enzyme, phosphorylase *b* kinase from an inactive to an active form (102, 286). Activated phosphorylase *b* kinase, in the presence of ATP, catalyses the conversion of inactive phosphorylase *b* to active phosphorylase *a*, the levels of which have been shown to be increased by epinephrine (19, 185, 186, 225). Calcium ions are essential for the activation of phosphorylase by epinephrine, at least in the heart (130). Phosphorylase *a* then catalyses the breakdown of glycogen with the production of hexosephosphates. The cellular content of glucose-6-phosphate in skeletal muscle has been shown to increase in the presence of epinephrine (17). Subsequently the glucose-6-phosphate is catabolised *via* the Embden-Meyerhof and hexosemonophosphate shunt pathways with the production of lactic acid. The pooled evidence from a large number of studies in which a variety of agonists and antagonists were used shows that the effect of sympathomimetic amines on muscle glycogenolysis, like their effects on contractile force, is mediated *via* β-receptors (2, 75, 76, 80, 129, 185, 186, 194, 195, 205, 206, 241, 256, 263, 288, 290) which, as already described, may in both cases be further characterised as of the β-2 type.

With the sensitive potassium-depressed diaphragm, it has been shown (129) that the relative potencies of a series of sympathomimetic amines in increasing the maximal twitches correlated with their relative potencies in stimulating glycogenolysis. Other evidence indicating a relationship between glycogenolysis and effects on contractility may be summarized as follows. Previous activity (a tetanus or treppe), which produces changes in contractility somewhat similar to those produced by epinephrine, also increases the cellular levels of phosphorylase *a* and glucose-6-phosphate (17, 94, 95, 122, 123, 286). In broken-cell preparations, caffeine and theophylline inhibit the phosphodiesterase responsible for destroying cyclic 3′,5′-AMP, and thereby cause an accumulation of the nucleotide (79, 292). In heart muscle this effect leads to increased phosphorylase activation and hexosephosphate formation (16, 139, 173). Although it has not yet been demonstrated, it is not unlikely that a similar effect occurs in intact skeletal muscle. Caffeine and theophylline potentiate the activation of phosphorylase *a* by epinephrine (174). In fast-contracting muscle these xanthines increase the maximal twitch tension (40, 149, 190, 306) and in smaller doses they potentiate the action of epinephrine in producing a similar effect (40). In the slow-contracting cat soleus muscle, however, there is a point of difference between the action of caffeine and that of epinephrine. Epinephrine decreases the twitches of this muscle, whereas caffeine, according to Huidobro and Amenbar (190), increases them. Raper (294) found caffeine to produce variable effects on the cat

soleus muscle. In 2 out of 10 experiments, doses of 2.5 mg intra-arterially or 10 to 20 mg/kg intravenously, caused a reduction in twitch tension and changes in time course closely similar to those produced by epinephrine. In 5 experiments it was without effect, whereas in 3 experiments it produced an increase in twitch tension resembling that recorded by Huidobro and Amenbar (190). Thus, in contrast to the regularly occurring decrease in twitch tension of the soleus muscle produced by epinephrine, the effect of caffeine on this muscle is variable. The concentration of caffeine necessary to inhibit phosphodiesterase *in vitro* is high, and it is not unlikely that equivalent doses given to the intact animal will exert additional effects that may mask the consequences of its action on this enzyme. Calcium ions facilitate the activation of phosphorylase *b* kinase in skeletal muscle fractions (213) and, in the heart, excess calcium ions increase hexosephosphate levels (16). Excess calcium ions also increase the twitches of the potassium-depressed rat diaphragm (40). Calcium ions do of course play important roles in excitation-contraction coupling (306) independently of their action on phosphorylase *b* kinase, and the effect of calcium ions on contractions may therefore be interpreted in a number of ways. Nevertheless, there is ample circumstantial evidence to support the view that the two effects of epinephrine—increased glycogenolysis and changes in contractility, at least in fast-contracting muscles—are in some way related.

However, provided that a sufficient source of metabolic energy is available, epinephrine retains its twitch-potentiating effect on the potassium-depressed rat diaphragm under anaerobic conditions, or in the presence of the glycolytic inhibitor, iodoacetate (40, 124, 127). These results therefore indicate that the action of epinephrine on the muscle contractions is dependent neither on oxidative metabolisms nor on the energy-yielding steps of the Embden-Meyerhof pathway, which is the main pathway for carbohydrate metabolism in this tissue (29). To explain the apparent relationship between effects on glycogenolysis and effects on contractility it may therefore be postulated (40, 124, 126, 127) that the initial step or early steps are common to both effects, but that the pathways diverge before the stage of energy production from glycogenolysis. No intermediate steps are at present known to occur before the stimulation of adenyl cyclase by epinephrine or between this effect and the production of cyclic 3′,5′-AMP, which, at least in cardiac muscle, is a very rapid process (88, 299). It is thus reasonable to suppose that the production of cyclic 3′,5′-AMP is a common intermediate in the action of epinephrine on carbohydrate metabolism and on muscle contractility. However, the question, for which there is as yet no answer, particularly in the case of skeletal muscle, is whether cyclic 3′,5′-AMP production is the final common step in the two processes, or whether phosphorylase activation and increased cellular hexosephosphate levels are also common as originally suggested by Ellis (124). There is evidence that cyclic 3′,5′-AMP production is an intermediary in a number of hormonal actions in addition to those of epinephrine (300, 327). These hormones include ACTH, ADH, LH, insulin, TSH, glucagon, melanocyte-stimulating hormone, and some actions of histamine, serotonin, and acetylcholine (300). If this is so, it is clear that the

nucleotide is capable of triggering a wide variety of otherwise unrelated cellular functions. In the case of the positive inotropic action of epinephrine on the heart, there is a considerable range of evidence that cyclic AMP is a mediator (168, 327), and, although it is not universally accepted (see for example 168, 184, 204) there is also circumstantial evidence that this is the final step in common with the simultaneously occurring stimulation of glycogenolysis. Several series of experiments appear to rule out activation of phosphorylase and hexosephosphate production as causal events in the positive inotropic effect of epinephrine on the heart. Briefly these are based on dose-response (112, 254, 255) and time-response studies (88, 299, 355), which have shown first that the effect on contractility occurs with doses too small to increase phosphorylase activation and accumulation of hexosephosphates, and second that when doses big enough to affect both are used, phosphorylase activation and increase in hexosephosphate levels lag behind the effect on contractility. Furthermore, acetylcholine inhibits the glycogenolytic effect of epinephrine in the heart, including phosphorylase activation, but not its effect on contractility (30, 345). Thus, if the mechanical and metabolic effects of catecholamines in the heart are causally related, an increased level of cyclic 3′,5′-AMP appears to be the final common step, and according to Levine (226, 227) injection of cyclic 3′,5′-AMP itself produces increases in heart rate and cardiac output resembling those produced by epinephrine. It is therefore tempting to assume that the production of cyclic AMP is also the final common intermediary in epinephrine-stimulated skeletal muscle glycogenolysis and changed contractility. However, analogous experiments to exclude phosphorylase activation in this tissue have yet to be performed. Although the appropriate measurements have not yet been made, it seems unlikely that phosphorylase activation can be excluded on the basis of time relationships, since the latent period of the effect of epinephrine on contractility of skeletal muscle is of the order of 20 to 40 sec, which is presumably ample time for phosphorylase activation to occur. Results supporting Ellis' original postulate (124) that increased hexosephosphate formation is the common intermediary in both cardiac and skeletal muscle have yet to be explained away. Thus, hexosephosphates themselves, but not glycero-β-phosphate, increase the contractions of the potassium-depressed diaphragm (40, 123) and produce a positive inotropic effect on the heart (124). A previous tetanus produces effects on contractility bearing some resemblance to those of epinephrine, and although a tetanus gives rise to increased cellular levels of hexosephosphates the effect is thought not to involve the mediation of 3′,5′-AMP (257, 287). Insulin and glucagon are in some ways like epinephrine in affecting contractility of isolated skeletal muscle and heart (40, 123, 124, 135). Insulin increases the cellular levels of hexosephosphates (31, 86, 169) by facilitating the passage of extracellular glucose to the intracellular sites of hexokinase action, and not *via* the adenyl cyclase-3′,5′-AMP system. The action of glucagon in these peripheral tissues is also not easily explained on the basis of 3′,5′-AMP production, since it is believed to be without effect in stimulating the formation of this nucleotide in skeletal muscle (291, 324). Glucagon stimulates adenyl cyclase in the liver

(248, 326) and in adipose tissue (78). Glucagon increases the peripheral utilization of glucose (131, 323, 342) and it increases the phosphorylation of glucose in the heart (281). Thus, the actions of a tetanus, insulin, and glucagon might be more readily explained on the basis of an increase in the cellular levels of hexosephosphates than of cyclic 3′,5′-AMP. In the potassium-depressed diaphragm, the action of glucagon further resembles that of insulin in that the effects of both were blocked by phloridzin (40). Possibly both facilitate glucose transport across the cell membrane and it is possible that this action of both may involve a different adenyl cyclase-3′,5′-AMP system. The action of glucagon also resembles that of the catecholamines in that, in the potassium-depressed diaphragm, it is blocked by β-receptor blocking drugs (40). This effect is difficult to explain; it seems unlikely that it could be due to catecholamine release from the diaphragm, since this tissue is known to contain very little stored amine. The positive inotropic action of glucagon on the heart was also reported to be blocked by β-receptor blocking drugs (135), but recent evidence contradicts this (140a, 235a).

In summary it may be said that at the present stage there is some evidence to suggest that the effects of epinephrine on skeletal muscle contractility may involve the production of cyclic 3′,5′-AMP as an intermediate, but there is little or no evidence to exclude the possibility that phosphorylase activation and hexosephosphate production are additional intermediates in the effects on this tissue.

Increase in demarcation potential and effects on contractility produced in skeletal muscle by sympathomimetic amines exhibit the same characteristics with regard to order of potency of the drugs and sensitivity to antagonists. This is compatible with the possibility that both effects are triggered by a common mechanism, but whether they are causally related or merely occur in parallel is not known. The hyperpolarization may be directly attributed to increased retention of K^+ within the muscle cells (148) but the events leading to this change in ionic balance are not known, although it may well be the result of the increase in the cellular levels of indiffusible anions (hexosephosphates) as suggested by Shanes (311). According to Bueding and Bülbring (64), epinephrine-induced hyperpolarization of smooth muscle cells of the taenia coli arises from generation of energy-rich phosphate compounds (ATP and CP), possibly mediated by cyclic AMP, but whether an analogous process occurs in skeletal muscle is not known.

The question arises as to whether the membrane hyperpolarization can account for the changes in twitch tension. The magnitude of the muscle action potential depends on the pre-existing membrane potential (311), and, although the appropriate measurements with intracellular electrodes do not appear to have been made, the amplitude of the muscle fiber action potential is presumably increased during the hyperpolarization produced by epinephrine. From a study of potassium depolarization contractures in single muscle fibers (181 and see also 134 and 305), it has been deduced that depolarization of the membrane by the spike potential does not trigger contraction until the membrane potential falls from its normal resting value of about −90 mV to about −50 mV, which is thus the

threshold of mechanical activation. Mechanical activation then occurs throughout the period that the membrane potential is shifted beyond the mechanical threshold. However, mechanical saturation occurs at −20 mV so that depolarization beyond this value, including the overshoot of the spike potential, does not cause any additional tension output, and this part of the action potential may, therefore, be considered as a safety factor. Consequently, any action of epinephrine that results in small changes in the amplitude of the action potential is unlikely to contribute to its effects on twitch tension, although changes induced in the duration of that part of the action potential above the mechanical threshold may well do so. Epinephrine also hyperpolarizes the membrane in the slow-concontracting soleus muscle and it is difficult to see how the same change in membrane potential could induce opposite changes in contractility in this muscle. Furthermore, epinephrine does not directly affect the contractility of the nonfatigued gastrocnemius muscle of the fowl, yet it produces an increase in the muscle demarcation potential similar to that occurring in cat muscles (296). We are, therefore, inclined to the view that membrane hyperpolarization is not the factor responsible for the changes in contractility of normal muscles. Others have reached the same conclusion (144, 214).

The situation may be different, however, in the potassium-depressed diaphragm preparation. Since the depressant effect is the result of depolarization of the muscle fibers by the excess K^+, the enhanced effect on twitch tension produced by catecholamines under these circumstances may well be mainly due to their ability to produce hyperpolarization. This constitutes a possible criticism of conclusions based on the use of this preparation, since it may be that the variety of drugs found to mimic the effects of epinephrine, do so, not because they share its action on contractility in normal muscle, but because they produce hyperpolarization. Insulin, for example, has been shown to hyperpolarize rat excised extensor digitorium longus muscle (358) and this effect may account for its ability to increase the twitches of the potassium-depressed diaphragm. Calcium ions probably also produce this effect but it is not known whether hyperpolarization is produced by the other compounds (hexosephosphates, xanthines, glucagon) with a twitch-augmenting action.

Sandow (305, 306) has reviewed the action of potentiating agents on skeletal muscle contractions, and among the diverse substances that produce this effect, perhaps least is known about the action of epinephrine. Nevertheless, some speculation on its action is permissible by analogy with the actions of other agents. In general, agents that affect the twitch by altering the duration of the active state are believed to do so, not by acting directly on the contractile material, but by affecting one or another process in the excitation-contraction coupling sequence (306). Briefly summarised, the process of E-C coupling is believed to comprise at least the following steps. The action potential in the plasma membrane gives rise to an electrical signal which is conducted into the interior of the fiber by the T tubules. This signal then releases bound calcium ions from the lateral sacs of the sarcoplasmic reticulum in close proximity to the myofilaments. The released Ca^{2+} then activates contraction by a direct action on the myofila-

ments. Relaxation occurs because the sarcoplasmic reticulum has the property of sequestering calcium ions which are taken up again, and thus removed from the contractile mechanism, by an active pumping process involving hydrolysis of ATP. It is not unlikely, therefore, that effects of epinephrine on the contractility of both fast- and slow-contracting muscles involve changes (opposite in direction for the two types of muscle) in the amounts of Ca^{2+} released from or in the rates of its re-uptake by the sarcoplasmic reticulum. The evidence previously quoted (28, 201, 202) that it is the decaying phases of the activity curves that are affected, rather than the durations of the plateaux, may mean that epinephrine alters the rates of re-uptake of Ca^{2+} by the sarcoplasmic reticulum without altering the times at which the relaxation process is switched on.

Some potentiating agents, notably certain lyotropic anions such as NO_3 (133, 181, 305), lower the mechanical threshold. Presumably this means that release of Ca^{2+} from the sarcoplasmic reticulum occurs at a smaller than normal level of membrane depolarization by the spike potential and, therefore, earlier, and that release continues for the rest of the duration of the spike above the lowered threshold so that there is a release that is greater than normal. This type of twitch augmentation is associated with an increase in the rate of development of tension, especially in the early part of the twitch, and it is thought to involve an action on the T tubules (306). Since, in the isolated diaphragm of the rat and the tibialis anterior muscle of the cat (46, 149) the potentiating action of epinephrine is associated with a slight diminution in the early rate of development of tension, a lowered mechanical threshold cannot be responsible for its effect on contractility (the situation may be different in fatigued skeletal muscle as described below). The hyperpolarizing action of epinephrine (42, 60) which must shift the membrane potential further away from the mechanical threshold, may account for the early diminution in the rate of tension development.

Other potentiating agents, including divalent metal ions such as Zn^{2+} and uranyl (196, 305, 307), do not change the mechanical threshold but they prolong the repolarization phase of the spike, apparently by an action on the plasma membrane, and they thereby increase the duration of the mechanicly effective period. Epinephrine increases the duration of the spike in fast-contracting muscle (57, 58, 144) and this effect may well contribute to its potentiating action on twitches. Caffeine and quinine act in both ways, that is by lowering mechanical threshold and by prolonging the spike (305).

Some of the lyotropic anions (118) and caffeine (172) cause Ca^{2+} release from, or inhibition of Ca^{2+} uptake by, isolated sarcoplasmic reticulum, or both. In the same concentrations as those causing potentiation of contractions, caffeine also inhibits the relaxing activity of crude muscle extracts of the sarcoplasmic reticulum relaxing factor (270). Thus, inhibition of relaxation by this means is a further possible mechanism of prolonging the active state. Caffeine is especially interesting in relation to epinephrine, since both drugs increase the twitch, both increase the tension in denervated muscle, both exert an anticurare action (described below), and both may cause accumulation of cyclic 3′,5′-AMP. It is, therefore, tempting to suppose that at least some aspect of the mechanisms re-

sponsible for their twitch-potentiating actions is similar, and that for the reasons already discussed, it involves cyclic 3′,5′-AMP production.

With rabbit skeletal muscle homogenates, Rabinowitz and co-workers (289) have shown that adenyl cyclase is located only in the mitochondrial and microsomal fractions, and that there is an apparent parallelism between the distribution of this enzyme and that of the particles which show relaxing activity. The authors do not state which skeletal muscles were used but presumably they were of the white fast-contracting type, since Butcher and Robison (77) have confirmed these observations for the white fast-contracting cat tibialis anterior muscle, but have also shown, as described below, that the situation is different in the red slow-contracting cat soleus muscle. There is suggestive evidence (320) that relaxing activity in red muscle (rabbit semitendinosus) may be regulated by a different mechanism from that in white muscle (rabbit vastus lateralis), since Ca^{2+} uptake per milligram of protein in all fractions of red muscle homogenates is much less than that in the corresponding white muscle fractions. In attempting to relate these observations, it may be tentatively postulated that in white fast-contracting muscle the adenyl cyclase-3′,5′-AMP system either is involved in the release of Ca^{2+} from the sarcoplasmic reticulum or acts as a brake on re-uptake and that, by increasing the levels of the nucleotide, epinephrine increases the levels of free Ca^{2+} in the region of the myofilaments, and thereby prolongs the active state. Uchida and Mommaerts (339) found that the contraction of actomyosin systems by ATP was strongly enhanced when cyclic 3′,5′-AMP was added in the presence of Ca^{2+} ions and they suggested the alternative possibility that changes in the levels of cyclic AMP are responsible for sensitising actomyosin towards Ca^{2+}.

The relative insensitivity of fast-contracting muscle to epinephrine may be related to the sarcoplasmic reticululum site of the adenyl cyclase and it is possible that the greatly increased sensitivity of the potassium-depressed diaphragm is the result of the membrane depolarization allowing epinephrine easier access to its site of action. In contrast to the situation in fast-contracting muscle, adenyl cyclase in the slow-contracting soleus muscle is located in the membrane fraction (77) and this may account for the greater sensitivity of the soleus to epinephrine, when compared with fast-contracting muscles.

Uchida and Mommaerts (339) found that cyclic AMP caused relaxation of actomyosin when the Ca^{2+} level was kept low by the addition of oxalate. Their results suggested that under these conditions, cyclic AMP behaved like relaxing factor. In a later paper (265) with more carefully controlled conditions, these authors were unable to repeat this part of their earlier observations and consequently recanted their views, although they pointed out that a relaxing effect of cyclic AMP was not necessarily excluded by their negative findings. On the whole, the collected circumstantial evidence suggests that the nucleotide may be involved in both contraction and relaxation under different conditions, and it may be that in the cat soleus the nucleotide is involved with relaxing activity, and that its increased production resulting from the interaction of epinephrine with membrane adenyl cyclase causes a more rapid re-uptake of Ca^{2+} by the

sarcoplasmic reticulum and a consequent increase in the rate of decay of active state in this muscle.

It is of interest that in both the heart (204, 317, 318, 354) and the soleus muscle, the active state is curtailed by epinephrine. When the cat heart papillary muscle is bathed in a medium rich in calcium and poor in sodium so that no further tension increment can be produced by epinephrine, the effect of the drug is then to decrease twitch tension and time to peak and to reduce the total duration of the twitch (see, for example, fig. 4 of 204), the effect being strikingly similar to that on the soleus twitch. Kavaler and Morad (204) are of the opinion that the increased rate of relaxation produced by epinephrine in the heart is the result of a different mechanism from that causing the increase in tension. Whatever the mechanism may be, it is possible that it is the same in slow-contracting skeletal muscles such as the soleus.

8. *Extraocular muscles.* The superior rectus muscle of the eye, which contains numerous fibers with multiple innervation (110, 175), is unusual in that epinephrine or stimulation of the cervical sympathetic produces a small contractile response (116, 117, 308). This effect does not appear to be an artifact arising from contraction of smooth muscles in the oribit (116, 117, 308), although it is difficult to exclude the possibility entirely. Tubocurarine abolishes the response of this muscle to acetylcholine, but has only a very small depressant effect on responses to epinephrine or sympathetic stimulation; hence the latter responses are probably not due to release of acetylcholine from cholinergic nerve endings and therefore appear to be the result of a direct action on the muscle fibers (117, 203). Unlike the effect on limb muscles and on the diaphragm, this direct action on the extraocular muscle shows the characteristics of an effect mediated *via* α-receptors (117). Surprisingly, in view of the contractile response, epinephrine increases the demarcation potential of the cat's extraocular muscle (56) as it does in other muscles. This combination of contractile response accompanied by an increase in demarcation potential is reminiscent of the response of chronically denervated muscles (described below). It is not known whether the increase in demarcation potential is mediated through β-receptors as in other muscles.

B. Amphibian and avian muscles

In nonfatigued muscles of the frog, it is generally agreed that epinephrine or sympathetic stimulation are without effect on the maximal twitches and on the demarcation potential (60, 97, 161, 193, 217). Thus in frog muscle epinephrine lacks the direct action on the muscle fibers that it produces in the mammal. Neuromuscular transmission in frog muscle is facilitated by epinephrine, as it is in mammalian muscle (see below), but in the normal nonfatigued frog muscle this effect is not reflected by an increase in maximal twitch tension.

According to Brown and Harvey (61) supramaximal shocks applied infrequently to the motor nerve innervating the gastrocnemius muscle of the domestic fowl excite only about 75% of the muscle fibers, transmission to the remaining 25% being subeffective unless facilitation of transmission is produced by applying closely-spaced double shocks. In the nonfatigued fowl gastrocnemius muscle,

epinephrine produces a slight augmentation of the twitches evoked by motor nerve stimulation, and this is accompanied by a corresponding increase in the amplitude of the gross muscle action potential (296). The effect appears to be due to a facilitatory action on neuromuscular transmission causing recruitment of some of the previously dormant fibers. Like the anticurare effect of epinephrine (described below) the effect on the indirectly evoked twitches of the fowl muscle is blocked by α-receptor blocking drugs but not by β-receptor blocking drugs (296). At the same time, *via* β-receptors, epinephrine increases the demarcation potential of the fowl muscle (296), as it does in mammalian muscle. Like the cat extraocular muscles, the fowl gastrocnemius contains many fibers with multiple innervation (140), but no increase in the resting tension of the fowl muscle, like that occurring in the extraocular muscles, was produced by epinephrine (296).

C. Chronically denervated muscles

Among the many changes that occur when a muscle is chronically deprived of its motor nerve supply (163) is the onset of spontaneous fibrillations in many of its muscle fibers (222, 223, 310). The membrane potential of a denervated muscle fiber undergoes spontaneous oscillations which trigger propagated spikes whenever the depolarizations reach a critical level (55, 230, 239, 240, 261). The propagated spikes resemble those of a normally innervated fiber in amplitude, but the rising phase is steeper and the declining phase is longer than normal, and the spike is followed by a pronounced positive after potential; conduction velocity is somewhat reduced (198, 333). The propagated spikes originate not only from the original endplate zone but also from elsewhere on the fiber membrane (333). Changes in the passive electrical properties of the membrane after denervation, which may underly the occurrence of fibrillary potentials, include an increase in its specific resistance, together with a decrease in the critical depolarization necessary to trigger a propagated spike (271, 333). Chronaxie is prolonged in mammalian muscle (164) but, according to Nicholls (271), membrane capacity, at least in frog muscle, is unchanged after denervation. Changes in potassium conductance may underlie many of the electrophysiological changes after denervation (333).

The propagated spikes trigger asynchronous contractions (fibrillations) of the fibers and the denervated muscle exhibits a background tone, arising from the contractions of its component fibers and therefore dependent on the frequency of the fibrillations. In general, the spontaneous activity of the denervated cat soleus muscle is more vigorous than that of the tibialis anterior muscle (39, 41). The denervated muscle is supersensitive to acetylcholine (55) and this is associated, at least in mammalian muscles, with a spread of the acetylcholine-sensitive area to include the entire surface of the fiber membrane (7). Small doses of acetylcholine augment the fibrillation frequency (55, 137), although larger doses abolish all propagated electrical activity as a result of membrane depolarization (55, 302). It has been suggested that the increased sensitivity to acetylcholine is the main factor giving rise to spontaneous fibrillations (22, 108, 246). However, there is convincing evidence against this possibility (39, 41, 177, 258, 302, 331–333).

Chronically denervated muscles *in vivo* respond to small doses of isoproterenol, epinephrine, or norepinephrine by an increase in the frequency of the fibrillary potentials and by a corresponding increase in the tone of the muscle (41, 47, 66, 237, 238, 246). There is probably also an increase in the number of fibers undergoing fibrillation. The effect is qualitatively the same in the denervated soleus and tibialis anterior muscles of the cat, although the former gives a more pronounced response and is sensitive to smaller doses (41). The denervated gastrocnemius muscle of the domestic fowl also responds in a similar way (296). After injection, there is usually a latent period of 30 to 50 sec before the onset of the response, which then lasts for several minutes. This response also occurs in denervated human muscles and has been used as a diagnostic aid for the detection of small amounts of denervation (304).

Surprisingly, the increase in fibrillation frequency and tone produced by catecholamines is accompanied by a small increase in the muscle demarcation potential (41, 60, *cf.* the superior rectus muscle of the cat's eye described above). However, a marked increase in demarcation potential, as produced by large doses of the catecholamines, may be accompanied by inhibition of spikes and a fall in tone (41). Doses of intermediate size may produce a biphasic change in spike frequency and tone. Inhibition of spike frequency and tone produced by catecholamines is more pronounced at low muscle temperatures. Tachyphylaxis to all the effects of the amines is evident when large doses are repeatedly injected.

According to some authors (240, 333, 348), the membrane potential of a denervated muscle fiber is lower than normal, although others (230, 271) found no change. If the membrane potential, after denervation, is lower than normal, it is possible that a weak hyperpolarizing action of the catecholamines raises it in some fibers into the range of increased excitability, and that this contributes to their effect in increasing fibrillations.

Like the effects on evoked contractions, the effects of catecholamines on fibrillary potentials and tone of denervated muscle *in vivo* exhibit the characteristics of effects mediated *via* β-receptors. Isoproterenol is more potent than epinephrine, whereas norepinephrine is less potent. The effects are blocked by the β-receptor blocking drugs, dichloroisoproterenol, pronethalol, propranolol, MJ 1999 and methoxamine and its derivatives, but are not reduced by the α-receptor blocking drugs, ergotoxine, phenoxybenzamine and phentolamine (41, 43, 47, 66, 297, 304).

Sympathomimetic amines also cause contraction of the isolated chronically denervated rat diaphragm bathed in a physiological salt solution (23, 81, 266, 282). This type of response appears to differ from that recorded *in vivo*, since no fibrillary potentials are evident and it is blocked most effectively by α-receptor blocking drugs (282). In this respect the response resembles that of the superior rectus muscle of the cat's eye (117).

Muscle blood flow recording has shown that the effects of catecholamines on the fibrillary potentials and tone of chronically denervated muscles *in vivo* are not a consequence of concomitant vascular changes (41). However, if the vasoconstrictor effect of the amine is large, it may result in temporary inhibition of the in-

crease in frequency and tone (41). Fibrillation is very sensitive to a decrease in blood supply (223, 338). Inhibition of fibrillation and tone resulting from vasoconstriction may be distinguished from that due to hyperpolarization, since the former exhibits the characteristics of an effect mediated *via* α-receptors, whereas the latter is due to β-receptor stimulation (41).

It has been suggested (238) that the contractile response of denervated muscle to epinephrine arises because the cholinoceptive receptors are sufficiently nonspecific to respond to epinephrine as they do to acetylcholine. This suggestion was based mainly on the finding that it is possible to abolish contractile responses to epinephrine with tubocurarine. However, tubocurarine produces a powerful depolarization of denervated muscle fibers and a consequent abolition of propagated spikes, which outlasts the contractile response (39, 242). Abolition of the contractile response to epinephrine by tubocurarine is a nonspecific effect in the sense that any substance that depolarizes the membrane and thus blocks propagated activity, will produce the same effect (41). Depolarization by tubocurarine occurs only with the first dose. Subsequent doses are without effect on fibrillation and on the membrane potential. Responses to acetylcholine continue to be blocked by subsequent doses of tubocurarine or of β-erythroidine, yet at this stage, responses to epinephrine are not depressed (41). Responses to epinephrine in isolated denervated muscles are also unaffected by tubocurarine (23). Furthermore, epinephrine increases muscle demarcation potential, whereas acetylcholine decreases it (41). These results show that responses to catecholamines are not due to reaction with cholinoceptive receptors in denervated muscle. For the same reasons, a mechanism involving release of acetylcholine by catecholamines can be excluded. Nevertheless, physostigmine enhances the contractile responses of denervated muscles to epinephrine (44). This appears to be because physostigmine causes a small, but sustained, increase in the background fibrillation frequency (39, 302), presumably as a result of preservation of small circulating amounts of acetylcholine. Any procedure that increases background fibrillation, such as increase in temperature, or within limits, increase in resting tension (39), will enhance the responses to epinephrine (41), and the effect of physostigmine cannot, therefore, be taken as evidence that the normal response to epinephrine is mediated by acetylcholine.

The facts that the rank order of potency of catecholamines in their effects on fibrillation and tone of denervated muscle, and on contractility of innervated muscle, is the same, and that the effects are susceptible to the same blocking agents, suggests that a common basic mechanism may give rise to them. It has been suggested that catecholamines may stimulate whatever process gives rise to the spontaneous fibrillations in the first place (41), and a knowledge of this process may, therefore, aid in the understanding of the trophic influence of the nervous system on skeletal muscle.

Fibrillary activity of denervated muscle disappears within 2.5 to 15 min, according to different authors, on occlusion of the muscle blood supply (180, 197, 223, 338) yet direct electrical excitability persists for several hours (180). This suggests that fibrillation is dependent on substances carried to the muscle by the

blood. When arterial occlusion is performed at a temperature of 27°C, the disappearance of fibrillation takes 2.8 times longer than at 37°C (*i.e.* $Q_{10} = 2.8$). This indicates that metabolic processes are involved (180). Relaxing-factor activity, as determined by the amount of Ca^{2+} binding to microsomes, rises significantly after denervation (53). This suggests that the biochemical mechanism for coupling excitation to contraction and relaxation is facilitated in denervated muscle. Possibly the Ca^{2+}-sequestering ability of the sarcoplasmic reticulum is enhanced to the extent that membrane-bound Ca^{2+} is removed, with a consequent instability and the production of fibrillary potentials. The possible involvement of cyclic 3′,5′-AMP in excitation-contraction coupling and relaxation has been discussed in a previous section of this review. It might, therefore, be tentatively suggested that stimulation of adenyl cyclase by catecholamines enhances the coupling mechanism and that this then mobilizes more Ca^{2+} from the membrane with a consequent increase in fibrillary potential frequency. The mobilized membrane Ca^{++} may become temporarily available for the contractile proteins and the increased fibrillary potentials will trigger further contractions so that the one effect reinforces the other in a repetitive positive feed-back mechanism. Blood-borne calcium may, therefore, be the essential constituent to replace that lost from the membrane. It has been shown that epinephrine does not produce contractions of denervated muscle in the absence of calcium (23). A difficulty in this suggested interpretation might be that, although the responses of denervated cat tibialis anterior and soleus muscles to epinephrine are qualitatively the same with regard to fibrillation frequency and tone, they are opposite, whether the muscle is innervated or denervated, with regard to evoked contractions. However, excitation-contraction coupling and relaxation are closely linked events and, in a rhythmically oscillating process such as that giving rise to fibrillations, a facilitating action on either the contraction or the relaxation components of the cycle may result in the same end effect. If cyclic AMP is an important mediator of the spontaneous activity of denervated muscle, it may be that the trophic influence of the motor nerve in inhibiting this activity is the result of a control exerted on the production of the nucleotide. The chemical transmitter, acetylcholine, inhibits the formation of cyclic AMP in some tissues (327) and a similar effect in skeletal muscle, quite apart from its membrane depolarizing action, may be the factor responsible. However, prostaglandin is another possible candidate for this role. Prostaglandins are present in nervous tissue (22a) and there is evidence to suggest that they inhibit the formation of cyclic AMP in some tissues (322a).

Use has been made of chronically denervated muscles to distinguish the adrenoreceptor blocking activity of β-receptor blocking drugs from the membrane stabilising or local anaesthetic activity that many of them possess (43, 297). Compounds possessing only β-receptor blocking activity abolish the responses to catecholamines without reducing the tone or the frequency of the fibrillary potentials below the background control level, whereas those possessing membrane stabilising activity in addition, lower the background tone and fibrillary frequency as well as blocking responses to catecholamines.

The increase in background tension produced by catecholamines in a dener-

vated muscle is usually small in comparison with the maximal twitch tension evoked by direct electrical stimulation. Catecholamine-induced changes in tension and time course of the twitches can, therefore, easily be detected superimposed on the relatively weak background effects.

III. ACTIONS ON NEUROMUSCULAR TRANSMISSION

Much of the evidence that catecholamines affect neuromuscular transmission is based on their interaction with other drugs active at this site, including tubocurarine, neostigmine, decamethonium, and succinylcholine. Epinephrine has in fact been shown to influence the transmission process in two opposing ways, an initial facilitatory action being followed by a more protracted depressant action.

1. Facilitatory action. An anticurare action of epinephrine in the frog was first demonstrated by Panella (279); it has since been confirmed by many other workers in amphibian, mammalian, and avian muscle after both intravenous and local intra-arterial injections (42, 45, 49, 60, 98, 153, 191, 244, 268, 273, 296, 301, 353, 356). In the cat, the smallest effective dose of epinephrine is 0.2 μg intra-arterially or 1 μg/kg intravenously. The effect is more pronounced at a stimulation frequency of 1/sec than at one of 0.1/sec (42). The onset of the anticurare effect is more rapid than the onset of the direct action on the nonfatigued, noncurarized muscle, and the extent of the increase in twitch tension obtainable is greater. Recording of gross muscle action potentials shows that the anticurare effect is a true antagonism of the neuromuscular block, in the sense that it is the result of recruitment of formerly blocked muscle fibers (42, 43, 296). Muscle blood flow recording has shown that the effect is independent of concomitant vascular changes produced by the amine (42).

Some workers (15, 58, 128) have been unable to demonstrate any relief of tubocurarine paralysis in the isolated diaphragm muscle of the rat, and it does appear that the effect is less obvious and more unpredictable in isolated muscle. However, others have succeeded in demonstrating a facilitatory action on transmission under these circumstances (49, 214, 266) and its presence or absence appears to be strongly dependent on the composition of the physiological salt solution used (266).

Several observations indicate that the anticurare effect of epinephrine is the result of an entirely different mechanism of action from that underlying its direct effect on nonfatigued, noncurarized muscles. Thus, 1) the anticurare action occurs in the frog and the fowl as well as in mammals, whereas the direct action is not demonstrable in the frog and the fowl. 2) The anticurare effect of epinephrine occurs similarly in fast-contracting muscles and in the slow-contracting cat soleus muscle, and the two types of muscle exhibit the same sensitivity to the amine (42); on the other hand, the direct action gives rise to opposite effects in the two types of muscle, and the soleus muscle is many times more sensitive to epinephrine than are fast-contracting muscles. 3) The characteristics of the anticurare action suggest that it is mediated through α-adrenoreceptors. It is produced by epinephrine and to a slightly less extent by norepinephrine, but isoproterenol is ineffective; the effect is blocked by Dibenamine (60, 244) and other α-receptor

blocking drugs, including phenoxybenzamine and phentolamine, but not by β-receptor blocking drugs (42, 43, 296). In contrast, the direct action on the muscle fibers exhibits the characteristics of an effect mediated through β-adrenoreceptors: isoproterenol is more potent than epinephrine in producing the effect, and it is blocked by β-receptor blocking drugs but not by a α-receptor blocking drugs.

Epinephrine and norepinephrine increase the amplitude of endplate potentials produced by motor nerve stimulation in both frog and rat muscle when neuromuscular transmission is depressed by tubocurarine or excess Mg^{++} (193, 199, 214). At the same time, the amplitudes of the miniature endplate potentials and of the potentials produced by iontophoretically applied acetylcholine are not increased, although there is some increase in the frequency of the miniature endplate potentials. These results strongly suggest that epinephrine and norepinephrine facilitate neuromuscular transmission by a prejunctional action through which the amount of acetylcholine released by a nerve impulse is increased. In unpublished experiments, Jenkinson (quoted in 199) has shown that the increase in endplate potentials produced by norepinephrine in frog muscle is blocked by phentolamine but not by pronethalol; this confirms that the anticurare effect is mediated through α-receptors. Other phenolic compounds, including phenol and catechol, also exert an anticurare action and increase endplate potentials by enhancing the release of acetylcholine by the nerve impulse (262, 276), but this effect appears to differ from that of epinephrine and norepinephrine since it is not modified by adrenoreceptor blocking drugs.

Epinephrine has been shown to increase acetylcholine release by nerve impulses at another site of cholinergic transmission. Birks and MacIntosh (24) demonstrated an increase in the amount of acetylcholine present in the venous outflow from the plasma-perfused cat superior cervical ganglion during preganglionic stimulation, when epinephrine was present in the perfusion fluid. According to Kulkarni *et al.* (216) epinephrine and norepinephrine cause the release of infused acetylcholine from some temporary binding site, possibly the plasma proteins. This effect is not altered by the β-receptor blocking drug, dichloroisoproterenol, and may therefore also be mediated through α-receptors. However, its relevance, if any, to the effect of epinephrine on cholinergic nerve endings is not clear. A prejunctional action of epinephrine being responsible for the anticurare effect in the cat is supported by the observations that during the epinephrine-induced increase in the partially blocked twitches evoked by motor nerve stimulation, contractions produced by close-arterially injected acetylcholine are slightly depressed, and at a time corresponding to the anticurare action, endplate depolarizations produced by succinylcholine are also slightly inhibited (42). These results indicate that facilitation of transmission produced by epinephrine can occur in spite of a small decrease in the sensitivity of the postjunctional membrane.

Although epinephrine and norepinephrine do not augment endplate potentials produced by iontophoretically applied acetylcholine, they increase the endplate depolarization elicited by acetylcholine added to the fluid bathing isolated frog muscle, reduce the rate at which endplate potentials decline during prolonged

application of acetylcholine, and increase the amplitude of contractures produced by acetylcholine both in the frog rectus abdominis muscle and in chronically denervated mammalian muscle (15, 72, 100, 193, 199). The effect on chronically denervated mammalian muscle might be a reflection of the direct action of the amines on contractility described in the preceding section, but this cannot explain the action on acetylcholine-induced endplate depolarization or on contractures in frog muscle, and these results therefore suggest that epinephrine and norepinephrine also possess a postjunctional facilitatory effect through which sensitivity to acetylcholine is increased. However, the time course of this effect makes it unlikely that it contributes to the anticurare action; it is slow in onset, and at a time corresponding to the anticurare action postjunctional sensitivity is unaffected or depressed. It may be that the postjunctional increase in sensitivity to acetylcholine corresponds to the delayed secondary depolarization evidenced by the fall in demarcation potential recorded by Brown *et al.* (60).

Indirect evidence suggests that the increase in acetylcholine release produced by epinephrine is the result of a hyperpolarizing action on motor nerve endings. Thus, Krnjević and Miledi (214) found that epinephrine relieves the presynaptic failure of transmission that occurs in rapidly stimulated nerve-muscle preparations, and in a later paper the same authors (215) reported that hyperpolarization of the nerve endings by anodal currents produced a similar effect. Epinephrine also causes hyperpolarization of isolated mammalian C fibers (147). Other evidence that epinephrine may in general cause hyperpolarization of nervous tissue comes from the experiments of de Groat and Volle (103) who showed that epinephrine increased the demarcation potential of ganglion cells. This postsynaptic hyperpolarizing action depresses ganglionic transmission, but, like the anticurare action of epinephrine, it exhibits the characteristics of an effect mediated through α-receptors. Hyperpolarization of motor nerve endings by anodal currents increases the amount of acetylcholine released by a nerve impulse (104, 189). It is of interest that here again, the actions of epinephrine bear some resemblance to the action of an indirect tetanus. A tetanus, like epinephrine, exerts a facilitatory action on neuromuscular transmission in both fast- and slow-contracting skeletal muscle. This facilitatory effect of a tetanus, which accounts for its anticurare action, differs from the simultaneously occurring effect on the contractility of the muscle fibers described above. It is a prejunctional effect attributed to hyperpolarization of nerve endings arising from summation of the positive after potentials (187, 347), and it results in an increase in the amount of acetylcholine liberated by each post-tetanic nerve impulse (192, 232, 234).

An observation that may argue against the possibility that epinephrine and norepinephrine hyperpolarize motor nerve endings is that they cause a small increase in the frequency of the miniature endplate potentials (199), an effect characteristic of terminal membrane depolarization rather than of hyperpolarization (233). The same argument might be applied to the facilitatory effects of a tetanus, since a preceeding tetanus also increases the frequency of the miniature endplate potentials (54, 231, 232), yet there is good evidence that a tetanus does hyperpolarize the terminal membrane (187, 347). It may therefore be that the

site of the terminal membrane hyperpolarization produced by a tetanus or by epinephrine is more localized than that produced by anodal currents, or that some factor in addition to a change in terminal membrane potential may contribute to their facilitatory action. It is of interest that in the experiments of Hubbard *et al.* (188) evidence was obtained that acetylcholine depolarized the motor nerve endings and yet the frequency of the miniature endplate potentials was not increased. The authors concluded that the site of action of acetylcholine was more central than the fine nonmyelinated terminals and suggested a site at the first node of Ranvier. Possibly epinephrine exerts the opposite action at the same site.

A facilitatory action of epinephrine on neuromuscular transmission is also reflected by its ability to potentiate the twitch augmentation and the repetitive firing in both nerve and muscle produced by anticholinesterase drugs or by depolarizing drugs such as decamethonium and succinylcholine (27, 42, 65, 69, 73, 191, 284, 330). Like the anticurare action these effects occur in both fast- and slow-contracting mammalian muscles, and in isolated muscles, and they are independent of concomitant vascular changes. Burn (72) discussed the possibility that potentiation of neostigmine by epinephrine might be the result of an anticholinesterase action of epinephrine. However, this possibility was excluded when it was shown (20, 21) that the anticholinesterase activity of epinephrine is much too weak to account for this action. Bowman and Raper (42) found that potentiation of neostigmine, decamethonium or succinylcholine by epinephrine also exhibited the characteristics of actions mediated through α-receptors, and they attributed these effects to the same basic mechanism as that underlying the anticurare action; that is, to a prejunctional effect through which transmitter release is augmented. In contrast to the results of Bowman and Raper (42), Breckenridge *et al.* (50) found that potentiation of neostigmine by epinephrine was blocked by a β-receptor blocking drug, and concluded that the effect was mediated through β-receptors. However, the β-receptor blocking drug used by these authors was propranolol, which is known to possess a powerful local anaesthetic action (267). In the same dose range, propranolol exerts both β-receptor blocking action and an independent depressant action on nerve function (322). Standaert and Roberts (322) have shown that propranolol abolishes post-tetanic repetitive firing in the cat soleus muscle and nerve by an action unrelated to blockade of β-adrenoreceptors, and a similar effect may account for the observation that it abolished twitch augmentation produced by a combination of neostigmine and epinephrine in the experiments of Breckenridge *et al.* (50). It would therefore be of value to repeat the experiments of these authors with β-receptor blocking drugs such as MJ 1999 or MJ 1998, which possess very little local anaesthetic activity, and with the (+)-isomer of propranolol which possesses local anaesthetic activity but relatively little β-receptor blocking activity. Bowman and Raper (42) found that, in β-receptor blocking doses, dichloroisoproterenol and pronethalol did not block the interaction between epinephrine and neostigmine whereas phentolamine did, and all other evidence also points to the facilitatory action of epinephrine on transmission being mediated through α-receptors.

Caffeine and theophylline, like epinephrine, produce an increase in maximal

twitch tension by a direct action on the muscle fibers (190), as well as a facilitatory action on neuromuscular transmission (50, 190). Since the best known action of the xanthines is to inhibit the enzyme phosphodiesterase and thus cause an accumulation of cyclic 3′,5′-AMP (79, 292, 327), these observations raise the possibility that the α-receptor mediated facilitatory action of epinephrine on transmission, as well as the β-receptor mediated direct muscle action, involves the cyclic nucleotide. Although it is β-receptor effects that are generally considered to be mediated by cyclic 3′,5′-AMP (3, 18, 325, 341), there is suggestive evidence that some α-receptor effects may be similarly mediated (13, 14, 300). It is tempting to suppose that whatever action epinephrine exerts on excitation-contraction coupling in fast-contracting muscles, it exerts a related action on excitation-release coupling in nerve, the former being mediated *via* β-receptors and the latter *via* α-receptors. Excitation-release coupling is also known to be strongly dependent on calcium ions (105). Although stimulation of adenyl cyclase and accumulation of cyclic 3′,5′-AMP in muscle is clearly mediated by β-receptors, it may be that in nerve this effect involves α-receptors.

2. Inhibitory action. Under conditions in which neuromuscular transmission is already partially depressed by tubocurarine or by Ca^{++} deficiency or Mg^{++} excess, epinephrine may be shown to possess an inhibitory action which is slower in onset than the initial facilitatory action. Thus, epinephrine enhances the depth of paralysis produced by tubocurarine injected about 5 min later (42, 128, 284). When tubocurarine is administered in the form of a continuous infusion so that a steady state of partial block is maintained, both actions of epinephrine are evident. Under these conditions the initial anticurare action precedes a more protracted enhancement of the transmission failure (42, 268); the effects are especially pronounced at stimulation frequencies of 1/sec. Krnjević and Miledi (214) were unable to detect an inhibitory effect in their experiments on isolated muscle and suggested that it might be a consequence of vascular changes produced *in vivo*. However, other workers have recorded the effect in isolated muscle (128, 266) and muscle blood flow recording *in vivo* has shown the effect to be independent of vascular changes (42). The inhibitory effect occurs both in fast- and slow-contracting mammalian muscles (42, 43), and in avian muscle (296), but has not been described for amphibian muscle. It matches, in its time course, the increase in demarcation potential produced by epinephrine and, like the latter, shows the characteristics of an effect mediated through β-adrenoreceptors (42, 43, 115). Bowman and Raper (42) concluded that when the normal safety margin in transmission (283) is in operation, transmission occurs in spite of the inhibitory action of catecholamines. However, when transmission is impaired, catecholamine-induced hyperpolarization of the muscle fiber membranes, including their endplate regions, causes a further depression of transmission by reducing the number of endplate potentials which reach the threshold necessary to trigger propagated spikes.

The postjunctional inhibitory action of catecholamines is also reflected in a number of other drug interactions. Thus, a previous injection of a catecholamine 1) depresses contractions of mammalian muscle evoked by close-arterially in-

jected acetylcholine (42), 2) depresses endplate depolarizations produced by succinylcholine (42), 3) depresses contractures of avian muscle produced by depolarizing drugs (245, 296), 4) depresses repetitive firing and twitch augmentation produced by anticholinesterase drugs (269) or depolarizing drugs (42) and 5) reduces the depth of block produced by decamethonium in muscles that respond to this drug by depolarization block (42, 284). In muscles that respond to decamethonium by dual block (200), the effect of epinephrine on the block resembles that produced when tubocurarine is the blocking agent used (42). All of the depressant effects of catecholamines on neuromuscular transmission are explicable in terms of a postjunctional hyperpolarizing action. A similar depressant action on transmission has been recorded in the cat's extraocular muscles (116, 117, 308) and here, too, epinephrine increases the muscle demarcation potential (56).

The β-receptor blocking drugs pronethalol and propranolol depress slightly the endplate depolarizing action of succinylcholine (42) and its ability to produce contractures of extraocular muscles (116). These effects of the β-receptor blocking drugs are probably a reflection of their local anaesthetic action.

IV. FATIGUED MUSCLE CONTRACTIONS

Dessy and Grandis (109) first showed that epinephrine temporarily restored the contractions of frog skeletal muscle depressed by prolonged and rapid stimulation of its motor nerve. Similar results on fast-contracting mammalian muscles were subsequently obtained by Cannon and Nice (84, 85), who studied the fatigued tibialis anterior muscle of cats, dogs, and rabbits, and since that time numerous workers have confirmed that epinephrine and, to a lesser extent, norepinephrine markedly increase both the twitch and the tetanic tensions of fatigued frog and fast-contracting mammalian muscles, both when isolated and when *in situ* (33, 57, 58, 65, 68, 82, 83, 98, 151–155, 157–162, 218, 236, 273, 301, 312). The effect on fatigued muscles is much greater than that in nonfatigued muscles, especially in the frog, in which epinephrine is without effect on nonfatigued muscles. In the mammal, stimulation of the splanchnic nerves produces similar effects to those of epinephrine on the fatigued muscle contractions (84, 85).

In 1923, Orbeli (275) showed that when the gastrocnemius muscle of the frog was fatigued by prolonged stimulation of the motor roots, simultaneous stimulation of the lumbar sympathetic chain then partially restored the contractions in most preparations. This effect of sympathetic stimulation, which is known as the Orbeli effect, has subsequently been confirmed in both fatigued frog and fast-contracting mammalian muscle (8, 67, 87, 97, 219, 247, 334, 352, 357). Mammalian muscle is more susceptible to deficient circulation than is frog muscle, and if the frequency of motor nerve stimulation is high and vasoconstriction pronounced, a common effect of epinephrine, norepinephrine, or sympathetic stimulation is a depression of the fatigued contractions in the mammal. Nevertheless, the enhancement of contractions can occur accompanied by a moderate degree of vasoconstriction, and since the effect also occurs in isolated muscles and in muscles perfused at a constant rate, it is clear that it is not simply a consequence of vascular changes, although it may be modified by them.

Orbeli's observations were made in the belief that skeletal muscle fibers received a direct adrenergic innervation, but this is now considered not to be so. The effect of lumbar sympathetic stimulation is therefore presumably due to adrenergic transmitter from the vascular sympathetics diffusing to the muscle fibers. That adrenergic transmitter is released into the blood stream on sympathetic stimulation was demonstrated in experiments in which the venous outflow from the muscle was perfused through the isolated frog heart (224, 334).

The earlier literature is controversial concerning the site of the defatiguing effect of epinephrine or sympathetic stimulation—whether it is the neuromuscular junction or the muscle fibers (for a review see 335). In the light of more recent knowledge it seems clear that the site of action depends partly on the method of producing fatigue (and therefore the site of the fatigue) and partly on the animal used (frog or mammal). In the frog, the site of action is undoubtedly the neuromuscular junction. The endplate potential diminishes as fatigue progresses (309), and epinephrine increases the amplitude of the endplate potential in frog muscle (193). On alternate direct and indirect stimulation, epinephrine and sympathetic stimulation augmented only the indirectly evoked contractions, and in chronically denervated or fully curarized frog muscles stimulated to fatigue by direct stimulation, epinephrine was without effect (97). The defatiguing action in frog muscle resembles the anticurare action in that it is blocked by the α-receptor blocking drug ergotoxine (97), and this provides further evidence that the site of action is the neuromuscular junction.

In mammalian fast-contracting muscles the situation is more complex, but here again it is clear that at least part of the effect is at the neuromuscular junction (26, 68), although in this case additional actions on the muscle fibers are superimposed (58, 153, 236).

Indirectly stimulated fatigued mammalian fast-contracting muscles again resemble indirectly stimulated partially curarized muscles in their responses to catecholamines. Epinephrine is more potent than norepinephrine, and isoproterenol is the least active of the three (33). The effect of epinephrine or sympathetic stimulation is greater, the higher the frequency of stimulation up to about 1/sec, after which vasoconstrictor effects may convert the response to a decrease in twitch tension (8, 67, 236, 349, 350).

The change in time course of the fatigued twitch produced by epinephrine resembles the change produced in the indirectly stimulated partially curarized muscle. The increase in twitch tension is associated with a faster rate of rise of tension and there is little change in the overall duration of the twitch (33). The effect is accompanied by an increase in the amplitude of the gross muscle action potential (312); this suggests that it is partly the result of recruitment of previously dormant fibers, or of improved synchronization. According to Shapiro (312), there is also a reduction in the latency of contraction as measured by the time between the initiation of the muscle action potential and the onset of contraction.

The defatiguing effect of epinephrine in the mammal is much reduced but not abolished after administration of one of the α-receptor blocking drugs, ergotoxine (68, 236), phenoxybenzamine, and phentolamine (33). The weaker effect of

isoproterenol remains unchanged in the presence of α-receptor blocking drugs. In the presence of an α-receptor blocking drug, isoproterenol is slightly more potent than epinephrine and, under these conditions, the effects of both amines are abolished by β-receptor blocking drugs. The effects of the amines in the presence of an α-receptor blocking drug resemble those in the fatigued, directly stimulated, fully-curarized muscle and are therefore the result of a direct action on the muscle fibers. They are more pronounced than the effects in nonfatigued muscles, but not markedly so.

In experimental conditions it seems clear that fatigue of the type induced by rapid motor nerve stimulation can be at least partly due to failure of neuromuscular transmission. Krnjević and Miledi's (214, 215) experiments on presynaptic failure of transmission are illustrative of this. The prejunctional action of epinephrine in restoring transmission under these circumstances (214) has already been referred to, and probably accounts for that part of the defatiguing action that is sensitive to α-receptor blockade, especially when the rapid stimulation is maintained during the action of epinephrine. Presynaptic failure is more pronounced in rapidly stimulated isolated muscles or in perfused muscles when the circulation is inadequate, and under these conditions the prejunctional defatiguing action of epinephrine is also more pronounced (67). However, fatigue induced by rapid motor nerve stimulation does not always involve or is not always confined to the neuromuscular junction (59, 119, 253, 259, 260). It may be due to a failure in excitation-contraction coupling in which the undiminished muscle fiber action potential fails to trigger contraction. The variation in the site of fatigue probably accounts for the variation in the response to catecholamines, which is a common feature in experiments on fatigued mammalian muscle. It seems unlikely that presynaptic failure plays a part in physiological fatigue, and the more pronounced junctional part of the action of catecholamines would therefore probably not be involved in any action on fatigued voluntary movements, where the site of the fatigue seems to be entirely postjunctional in origin (260).

When skeletal muscle is stimulated to contract, a rise in its phosphorylase *a* content occurs after a short latent period (101). When the muscle is fatigued, the conversion of phosphorylase *b* to phosphorylase *a* is strikingly reduced so that the phosphorylase *a* content falls to low levels (96, 170). Epinephrine facilitates the conversion of phosphorylase *b* to phosphorylase *a* and thus increases the levels of phosphorylase *a* in the fatigued muscle (96, 170). It has therefore been suggested that the effect of epinephrine in delaying or reversing muscle fatigue may be related to its ability to maintain phosphorylase *a* levels (170). These experiments certainly suggest a relationship between the metabolic and postjunctional defatiguing actions of epinephrine, but, since the increased phosphorylase *a* levels are mediated by a primary increase in cyclic 3′,5′-AMP levels, they do not exclude the possibility that the nucleotide is the main intermediate in the defatiguing action. Phosphorylase *a* levels are increased by epinephrine in both fatigued frog (170) and rat (96) muscles, yet the postjunctional defatiguing action is absent in the frog. It may therefore be that the mechanism for linking the action

potential to the contractile mechanism is different in frog muscle and does not involve the same metabolic intermediate.

The slow-contracting soleus muscle of the cat is difficult to fatigue, excessively prolonged high frequency stimulation either of the nerve or of the muscle being necessary before any depression of twitch tension becomes evident. Although the anticurare effect of epinephrine in the cat soleus muscle is pronounced, epinephrine has only very weak and variable effects on the tension of fatigued soleus twitches (33). This suggests that when fatigue of the soleus is produced by prolonged high-frequency stimulation of the nerve, it does not involve a component of neuromuscular transmission failure. This resistance of the soleus neuromuscular junctions to fatigue may be related to a high content of stored, preformed transmitter, since the soleus is more resistent than the tibialis anterior to transmission failure produced by hemicholinium, which acts by impairing the synthesis of acetylcholine (37). The absence of a postjunctional defatiguing effect of epinephrine in the soleus muscle is not surprising, since catecholamines depress the maximal twitches of this muscle.

When prolonged tetanic stimulation is applied to a muscle or its nerve, mechanical compression of its blood vessels occurs. The initial high tension is maintained for only a short period, possibly because of the impaired blood flow, and the muscle soon relaxes to a lower tension plateau which allows its blood flow to increase (34, 211). Epinephrine may markedly increase the plateau tetanic tension of the soleus muscle, but this occurs only when there is a concomitant increase in the muscle blood flow. When epinephrine produces vasoconstriction, the plateau tension decreases. The extent and duration of the tension changes are directly related to the extent and duration of the changes in blood flow, and the one is clearly a consequence of the other (33). Other vasoactive substances also give rise to the appropriate changes in tension. In fast-contracting muscles, on the other hand, the depressed plateau tetanic tension may be increased by epinephrine despite a weak concomitant vasoconstrictor effect (34, 67).

Although, under experimental conditions, epinephrine or sympathetic stimulation may produce pronounced effects on fatigued contractions of fast-contracting muscles, it seems doubtful whether the sympathetic nervous system plays any role in delaying or reversing fatigue of voluntary movements. In dogs with one sympathectomised leg there was no detectable difference in the onset and severity of the fatigue in the sympathectomised leg compared with the normal legs, when the dogs were made to run in a treadmill (336), and the frequencies of lumbar sympathetic stimulation necessary to elicit the Orbeli effect are probably considerably higher than that of any sympathetic discharge occurring under physiological conditions. Although fatigued fast-contracting muscles are somewhat more sensitive to epinephrine than are nonfatigued muscles, it remains doubtful whether stress-induced release from the adrenal medullae would ever give rise to an effective blood concentration. The smallest effective dose of epinephrine necessary to affect the contractions of the fatigued tibialis anterior muscle of the cat was found to be of the order of 1 μg/kg intravenously (33), which, according to Folkow (136), would produce a blood concentration in excess of any that occurs naturally. Effective blood concentrations are thus likely to occur only in

diseases such as phaeochromocytoma, and it therefore seems unlikely that the sympathetic nervous system plays any part in Cannon's defence reaction in the normal animal, in so far as fatigued skeletal muscle is concerned.

V. CONCLUSION

Epinephrine exerts a direct action on mammalian muscle fibers which is mediated *via* β-receptors. Activation of these receptors results in prolongation of the active state of white, fast-contracting muscles and curtailment of the active state of red, slow-contracting muscles. In chronically denervated mammalian muscles of both types, and in chronically denervated avian muscles, activation of the β-receptors increases the fibrillary discharge and the tone exerted by the muscles. It is suggested that these effects on muscle contractility may arise through activation of the adenyl cyclase/cyclic 3′,5′-AMP system, which in turn leads to changes in the rates of uptake of Ca^{2+} by the sarcoplasmic reticulum. Activation of the β-receptors also increases the muscle demarcation potential, presumably as a result of hyperpolarization of the muscle fiber membranes. Under sensitive conditions, this effect can be shown to lead to a depressant effect on neuromuscular transmission.

Epinephrine also exerts an initial facilitatory action on neuromuscular transmission in all types of muscle, and this is mediated *via* α-receptors, probably located in the motor nerve endings, activation of which appears to cause an inincrease in the amount of acetylcholine released by the nerve impulse. Hyperpolarization of motor nerve endings increases release of transmitter by nerve impulses, and epinephrine may act by hyperpolarizing some part of the terminal membrane. It may be that, although mediated by different types of receptor, epinephrine exerts an effect on the coupling of excitation and transmitter release in nerve which is analogous to its action on excitation-contraction coupling in muscle, both effects involving changes in the levels of free calcium ions. Cyclic 3′,5′-AMP may also be a mediator in the facilitatory effect on neuromuscular transmission, and this fact implies the unusual situation that, in nerve, increased levels of the nucleotide may be evoked through α-receptors.

The defatiguing action of epinephrine and sympathetic stimulation in mammalian fast-contracting muscles mainly involves the prejunctional α-receptors, but the postjunctional β-receptors also contribute. In fatigued frog muscle, only prejunctional α-receptors are involved. Apart from an indirect action arising from circulatory changes, epinephrine is without a defatiguing effect in red, slow-contracting mammalian muscles.

A consideration of the effective doses of catecholamines suggests that only the action of epinephrine on the sensitive slow-contracting red muscle fibers has any physiological significance. This action is probably responsible for the enhancement of physiological tremor and of Parkinsonian tremor produced by catecholamines in man, and for the tremor often associated with phaeochromocytoma.

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