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The influence of histamine and other vasoactive substances on contractile responses of cat skeletal muscle to acetylcholine

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Summary

1. The effect of vasoactive substances, chiefly histamine, on contractions of cat skeletal muscle evoked by acetylcholine has been studied. For this purpose all of these agents were administered intra-arterially to anterior tibialis, chiefly, and also to soleus and external rectus muscles *in vivo*.

2. Under these conditions, histamine itself never altered the resting tension of the muscle, but always enhanced the increase in tension produced by acetylcholine injected shortly afterward.

3. On the anterior tibialis muscle, it was determined that the threshold potentiating dose of histamine was 0·001 μg/kg, and that maximal enhancement occurred when 0·1 μg/kg was injected 15 s before fixed, submaximal doses of acetylcholine. Injected 15 s before graded doses of acetylcholine, histamine 0·1 μg/kg shifted the acetylcholine dose-response curves approximately 1·5 log units to the left.

4. Under similar experimental conditions the vasodilator, bradykinin, 0·001–1 μg/kg, and procedures which produce a local increase in nutritive capillary blood flow also enhanced acetylcholine-evoked contractile responses of the anterior tibialis, whereas the vasoconstrictor, angiotensin, 0·001–1 μg/kg, inhibited them.

5. The potentiating effect of histamine was antagonized by mepyramine, but was undiminished by denervation of the muscle either acutely or for 4 days.

6. Histamine failed to enhance responses of the muscle to electrical stimulation of either the muscle, directly, or its motor nerve.

7. Histamine also had no effect upon acetylcholine-evoked responses of the isolated external rectus muscle *in vitro*.

8. It is suggested that the potentiating effect of histamine *in vivo* is due to its vasodilator action within the muscles which, by relaxing arterioles and precapillary sphincters, could allow more of the blood-borne acetylcholine to reach more of its receptors on the muscle fibres in a given time.

Introduction

Histamine enhances responses of the cat nictitating membrane to bloodborne agonists, ostensibly by causing vasodilatation within its smooth muscle, since

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other vasodilator substances and procedures also exert an enhancing effect whereas vasoconstrictors exert an inhibitory one (Giarman & Reit, 1967; Cervoni & Reit, 1971). That responses to bloodborne agonists of cat skeletal muscle also may be influenced by substances which alter the state of its nutritional blood supply receives support from earlier experiments on denervated (Dale & Gaddum, 1930) and innervated (Macmillan, 1956) skeletal muscle. However, the characteristics of such vascular influences upon skeletal muscle contraction have never been rigorously defined. Therefore, in the present investigation we have attempted to analyse systematically the modulatory relationship between certain vasoactive substances and contractile responses of cat skeletal muscles to injected acetylcholine. Some of the results described here have been reported to the American Society for Pharmacology and Experimental Therapeutics (Block & Reit, 1971).

Methods

The experiments were performed on 2·0 to 6·0 kg cats of either sex anaesthetized with sodium pentobarbitone (35 mg/kg i.p.) and, in most instances, rendered spinal as described by Burn (1952). The adrenal glands of some cats were excluded from the circulation by ligation, as specified in the figure legends. Blood pressure was recorded from either a femoral or common carotid artery with a Statham P23Dc pressure transducer, and muscle tension with a Grass FT-03 force-displacement transducer, coupled to a Grass model 5 polygraph. Intravenous injections were made through a polyethylene cannula tied usually into a femoral or an external jugular vein. Rectal temperature was maintained at approximately 37° C, and all exposed tissue was covered with gauze soaked in 0·9% NaCl solution. After surgical procedures were completed, each cat received i.v. injections of heparin, 300 U.S.P. units/kg, and, to attenuate the muscarinic effects of acetylcholine, atropine, 30 or 100 μg/kg.

Anterior tibialis muscle

Most experiments were done on the anterior tibialis, a predominantly fast-twitch muscle, which was prepared for recording its contractile tension as described by Liddell & Sherrington (1929). The severed tendon of insertion was connected by heavy thread to the force-displacement transducer, and a resting tension of 10 g was applied to the muscle.

Close i.a. injections towards the muscle were made into the popliteal artery by means of a polyethylene cannula tied into the central end of the sural artery. For indirect stimulation of the muscle, the peroneal nerve was transected at its origin from the sciatic nerve, and its distal end was placed on bipolar platinum electrodes. For direct electrical stimulation of the muscle, a sufficient portion of it was exposed to permit placement of two electrodes, one on its dorsal surface and one on the ventral. The exposed tissue near the electrodes was covered with paraffin oil to prevent short circuiting. Square wave shocks of 10 ms duration and appropriate frequency and voltage were applied with a Grass S4B Stimulator.

In one group of cats, the anterior tibialis muscle was denervated either acutely during the experiment or in a prior operation. For the latter, each cat was anaesthetized with sodium pentobarbitone as above and a 2 cm length of one peroneal nerve was removed aseptically close to the muscle. Four days after

recovering from this surgery, each cat was anaesthetized with alpha chloralose (80 mg/kg, i.v.) following induction with ether. In these cats, both anterior tibialis muscles were prepared for recording their contractile tension, the innervated muscle serving as a control for the denervated one.

Other muscles

Some experiments were done on the soleus, a slow-twitch muscle, and the external rectus, an extraocular muscle.

Soleus. After immobilizing the hind leg, as for studying the anterior tibialis, all muscle insertions onto the calcaneus, except that of the soleus, were severed. The part of the calcaneus with the soleus tendon attached was then excised, connected by heavy thread to the force-displacement transducer, and resting tension of 10 g was applied to the muscle.

Intra-arterial injections towards the soleus muscle also were made *via* the centrally-cannulated sural artery simply by ligating the popliteal artery distal to the origin of the posterior tibial artery, the main supply to the soleus.

External rectus. The tendon of insertion of the external rectus was exposed through a lateral canthotomy and cut away from the eyeball which then was enucleated. The cat was placed on its back and its jaws were tied tightly around a rigid horizontal bar. The tendon was connected by heavy thread to the force-displacement transducer, and resting tension of 5 g was applied to the muscle. Thereafter, the cat's head was covered by a box similar to that described by Brown & Harvey (1941) for keeping it warm and moist.

Intra-arterial injections towards the external rectus muscle were made into the external carotid artery by means of a polyethylene cannula tied into the central end of the lingual artery.

In two experiments, the tendon of insertion of the external rectus was exposed and cut as above, and then the muscle was removed from the orbit in order to study its contractile responses *in vitro.* It was mounted with a resting tension of 5 g in a 30 ml organ bath containing Krebs solution maintained at 37° C and constantly bubbled with 95% oxygen and 5% carbon dioxide. The Krebs solution was composed of (g/l) NaCl 6·9, KCl 0·35, $CaCl_2$ 0·28, $MgCl_2$ 0·29, $NaHCO_3$ 1·0, NaH_2PO_4 0·16 and dextrose 1·0. Drugs were injected into the bath in a volume of 0·1 ml and remained in contact with the muscle for at least on minute, after which the bath was flushed out twice with fresh Krebs solution.

Drugs

All substances for intravascular administration, except KCl, were prepared as concentrated stock solutions in 0·9% NaCl and stored frozen. As required, they were thawed out and diluted appropriately with 0·9% NaCl solution. A concentrated solution of KCl was freshly prepared in distilled water and it, too, was diluted in 0·9% NaCl solution as needed. Other substances used were acetylcholine chloride and mepyramine maleate (Merck & Co., Inc., Rahway, New Jersey); histamine dihydrochloride and atropine sulphate (Nutritional Biochemical Corpora-

tion, Cleveland, Ohio); angiotensin (Val^5-angiotensin II-β-amide, Ciba Pharmaceutical Company, Summit, New Jersey); bradykinin (Schwarz Bioresearch, Inc., Orangeburg, New York). Doses refer to the free base or peptide. The volume for intra-arterial injections was 0·1 ml or less.

Results

Anterior tibialis muscle

Effect of histamine on responses to i.a. acetylcholine

The effect of histamine on the contractile response of the anterior tibialis muscle to acetylcholine is illustrated by the experiment of Figure 1. On injection i.a. toward the muscle in Fig. 1a, acetylcholine, 30 μg/kg, evoked, after a short latency, a small response consisting of a fused series of smaller, brief contractions. Several minutes after the muscle had relaxed again, histamine, 0·1 μg/kg, injected i.a. in Fig. 1b did not itself alter the muscle tension, but greatly enhanced the tension increase evoked by the acetylcholine injected again 15 s later. This enhanced response was immediate in onset and exhibited a steeper rate of rise than the preceding response in Figure 1a.

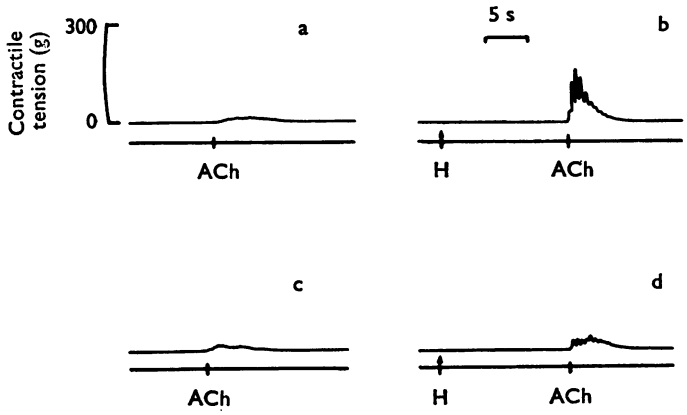

FIG. 1. Record of consecutive responses of the anterior tibialis muscle of a 2·5 kg cat anaesthetized with sodium pentobarbitone and rendered spinal. Injections i.a. toward the muscle of acetylcholine (ACh), 30 μg/kg, at the signals (in a, b, c and d) and of histamine, 0·1 μg/kg, at the arrows (in b and d). After b and 20 min before c, i.v. injection of mepyramine, 0·5 mg/kg.

To analyse this apparent potentiating effect of histamine, the histamine-enhanced responses to acetylcholine were always preceded by and compared with control responses to acetylcholine. For example, in the experiment of Fig. 1 the antihistamine, mepyramine, 0·5 mg/kg, injected i.v. 20 min before (c) did not alter the control response to acetylcholine (Fig. 1c), but strongly inhibited the histamine-induced enhancement of the response to acetylcholine (Figure 1d). Similar results in each of four experiments attested to the specific nature of the histamine-induced potentiating effect.

On further investigation histamine's potentiating effect was found to vary in degree depending upon the time interval between the sequential injections of histamine and acetylcholine and upon the dose of each substance.

Effect of interval between injections of histamine and acetylcholine

Figure 2 summarizes the results of seven experiments in which the interval between an injection of histamine, 0·1 μg/kg, and the subsequent injection of acetylcholine was varied randomly from 0 to 120 seconds. For each experiment a dose of acetylcholine was selected which produced a contractile response of approximately 10 g. This dose was as low as 3 μg/kg in the most sensitive preparation and as high as 100 μg/kg in the least sensitive one. But in each experiment, once selected the same dose was used throughout. When the acetylcholine was injected immediately after the histamine (time interval=0 seconds), no significant enhancement was produced. However, as the injection interval was increased, enhancement occurred, became maximal with an interval of 15 s, and then gradually declined until at intervals of 60 s or greater it was no longer evident. Based upon these results, we employed an interval of 15 s between the injections of histamine and acetylcholine in all subsequent experiments.

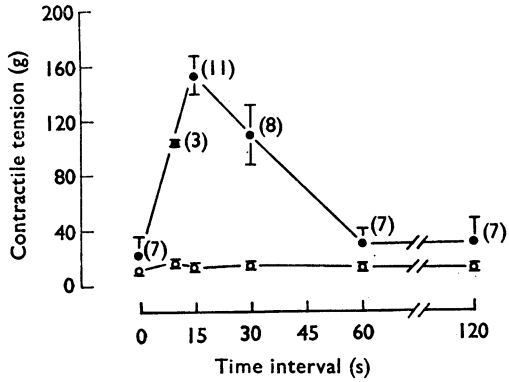

FIG. 2. Effect of the time interval between the i.a. injection of histamine and the subsequent i.a. injection of acetylcholine on the degree of enhancement of the responses of the anterior tibialis muscle to acetylcholine. The intervals, in seconds, were measured from the end of the injection of histamine, 0·1 μg/kg, to the end of the injection of acetylcholine. Each point represents the mean, and the vertical bars, the standard errors. In parentheses are numbers of observations made at each time interval. A total of seven pentobarbitone-anaesthetized cats (2·4 to 4·1 kg) were used, most of which were spinal or had their adrenal glands ligated. Open circles (○), control responses to acetylcholine; closed circles (●), responses to same doses of acetylcholine elicited after injection of histamine.

Effect of dose of histamine

Figure 3 shows the relation between the dose of histamine and the degree of histamine-induced enhancement. Injected 15 s before a fixed dose of acetylcholine, histamine, 0·001 μg/kg, produced threshold, and, 0·1 μg/kg maximal enhancement of the response of the anterior tibialis muscle to acetycholine. The degree of enhancement produced by the higher doses of histamine studied did not differ significantly from that produced by 0·1 μg/kg. Therefore, in order to minimize histamine-induced systemic vasodepression we used a dose of 0·1 μg/kg to produce maximal enhancement in all subsequent experiments.

Effect of dose of acetylcholine

When histamine (0·1 μg/kg) was injected i.a. toward the anterior tibialis muscle 15 s before graded i.a. doses of acetylcholine, it caused an apparently parallel,

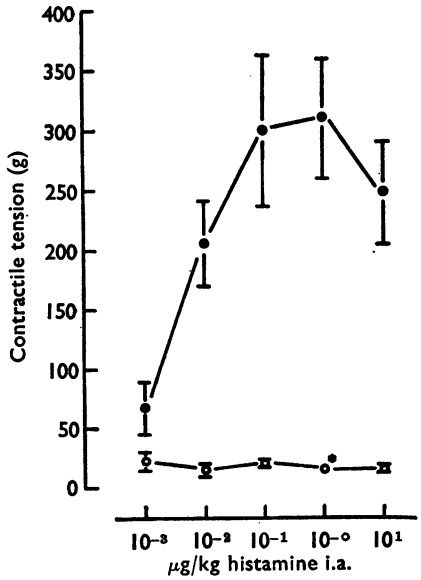

FIG. 3. Effect of the dose of i.a. histamine on the response of the anterior tibialis muscle to i.a. acetylcholine. Each point represents the mean and the vertical bars, the standard errors, of three responses to acetylcholine, 10 μg/kg, one from each of three cats (4·0 to 4·1 kg) anaesthetized with sodium pentobarbitone and having both adrenal glands ligated. The point designated by an asterisk was the mean of three responses, each of 15 g, and therefore the S.E.=0. Open circles (○), control responses to acetylcholine; closed circles (●), responses to same dose of acetylcholine injected 15 s after histamine.

shift to the left of the dose-response curve for acetylcholine of approximately 1·5 log units. The degree of enhancement of the response to a given submaximal dose of acetylcholine was influenced by the size of the cat and the height of its resting systemic arterial pressure. In general, the larger the cat and the higher the blood pressure, the greater was the enhancement.

Effect of other vasoactive substances on responses to i.a. acetylcholine

The experiment of Fig. 4 illustrates that the vasodilator, bradykinin (Fig. 4d), like histamine (Fig. 4b), enhanced responses evoked by acetylcholine injected i.a. 15 s later. Again, like histamine, bradykinin also shortened the rate of rise and the latency of the enhanced response (Fig. 4d), which began even before the injection of acetylcholine had been completed.

In 4 additional experiments, on cats whose adrenal glands were ligated, we studied how responses to a fixed dose of acetylcholine were influenced by graded doses of bradykinin. Bradykinin, 0·001 to 0·01 μg/kg, produced threshold, and, 0·1 μg/kg, maximal enhancement of the approximately 10 g contractile response evoked by the acetylcholine. Maximal enhancement was also produced by 1 μg/kg, but a tenfold higher dose produced no enhancement at all. Reminiscent of the observations with histamine, for bradykinin there was a direct correlation between the greatest degree of enhancement it produced and both the body weight and resting blood pressure of the cat.

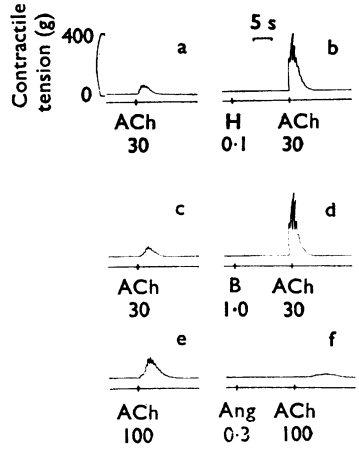

FIG. 4. Record of responses of the anterior tibialis muscle of a 2·9 kg cat anaesthetized with sodium pentobarbitone and rendered spinal. At the signals, i.a. injections toward the muscle of acetylcholine (ACh), histamine (H), bradykinin (B), and angiotensin (Ang). Numbers refer to doses in μg/kg.

In contrast to the two vasodilators, angiotensin never potentiated responses of the anterior tibialis muscle to acetylcholine. Figure 4(f) illustrates that on injection i.a. toward the muscle, angiotensin, 0·3 μg/kg, decreased markedly the size of the response to acetylcholine and also delayed the onset of the response by about 5 seconds. The inhibitory action of angiotensin on response size was dose-related. In 3 experiments on cats whose adrenal glands were ligated, the threshold dose of angiotensin for inhibiting contractile responses to i.a. acetylcholine of approximately 100 g of tension was just over 0·001 μg/kg, whereas a dose of 0·1 μg/kg inhibited such responses completely.

Compared to the relatively brief duration of histamine's potentiating action, which usually disappeared completely in less than one minute (Fig. 2), the duration of angiotensin's inhibitory action was much longer. In each of three experiments, exemplified by that of Fig. 5, after an i.a. injection of angiotensin 0·1 μg/kg

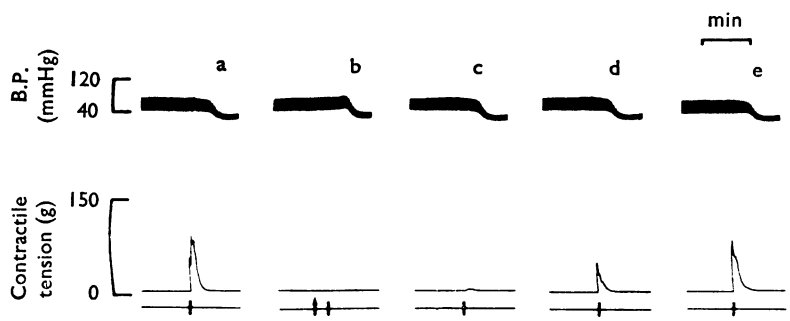

FIG. 5. Record of consecutive responses of the systemic blood pressure (top) and anterior tibialis muscle (bottom) of a 3·6 kg cat anaesthetized with sodium pentobarbitone, rendered spinal, and having both adrenal glands ligated. Injections i.a. toward the muscle of acetylcholine, 100 μg/kg, at the signals (in a–e) and of angiotensin, 0·1 μg/kg, at the arrow (in b). Injections of acetylcholine in c, d and e were made 4, 8 and 15 min, respectively, after injection of angiotensin in b.

toward the anterior tibialis muscle, it took nearly 15 min for the muscle to fully recover its responsiveness to acetylcholine. This relatively prolonged inhibitory effect was a local one, not reflected by any comparably prolonged alteration of the systemic blood pressure, which rose only slightly about 30 s after the angiotensin was injected (in b) and had returned to the control level at a time when the responsiveness of the muscle to acetylcholine was still more than 90% inhibited (in c). Throughout this time, the systemic vasodepressor effect of the acetylcholine remained undiminished.

Effect of histamine on responses to indirect and direct electrical stimulation

In four experiments, histamine, 0·1 to 10 μg/kg, was injected i.a. towards the anterior tibialis muscle while the peroneal nerve innervating it was being stimulated at 0·1 to 2 Hz with sub- or supramaximal voltages. In two other experiments, histamine (0·1 μg/kg) was injected while the nerve and the muscle, itself, were being stimulated in alternate fashion at 0·1 Hz with supramaximal voltages. The histamine consistently failed to enhance any of the electrically-evoked contractile responses, but at the higher doses studied (1 to 10 μg/kg) depressed the responses elicited by the higher frequencies (1 to 2 Hz) of nerve stimulation. This inhibitory effect is consistent with the findings of previous investigators (Macmillan, 1956; Hirvonen, Korobkin, Sonnenschein & Wright, 1964; Sonnenschein, Wright & Mellander, 1967).

Effect of procedures that increase nutritive blood flow

Hyperaemia, accompanied by an increased functional capillary (nutritive) blood flow, occurs in skeletal muscle immediately following exercise (i.e. serial or sustained contractions) of that muscle or occlusion of its main arterial blood supply. To ascertain whether such non-drug-induced vasodilatation also could affect acetylcholine-evoked contractile responses of the anterior tibialis muscle, we injected the acetylcholine i.a. toward the muscle immediately upon unclamping its popliteal artery which had been clamped for one minute (seven experiments), and also 3 to 10 s after a one-minute series of intermittent, neurally-evoked con-

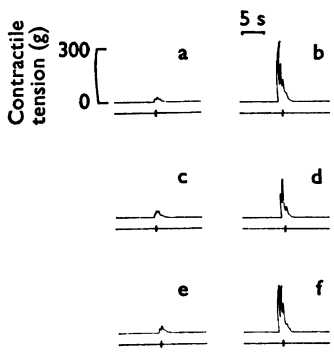

FIG. 6. Record of responses of the anterior tibialis muscle of a 4·4 kg cat anaesthetized with sodium pentobarbitone and having both adrenal glands ligated. At the signals, injections i.a. toward the muscle of acetylcholine, 10 μg/kg. Injection in b made 1 s after unclamping the popliteal artery which had been clamped for one minute. Injection in d made 15 s after injecting histamine, 0·1 μg/kg, *via* the same i.a. route. Injection in f made 10 s after discontinuing stimulation of the peroneal nerve, which had been stimulated for 1 min supramaximally with 10 ms square wave shocks at 2 Hz.

tractions of the muscle (five experiments). In every instance, the contractile response to acetylcholine was enhanced, usually to a degree approximately equal to that produced in the same experiment by the maximally effective potentiating dose of i.a. histamine, 0·1 μg/kg. This is illustrated by the experiment of Fig. 6 which also shows that the enhanced responses following arterial occlusion (in b) and 'exercise' (in f) further resembled those enhanced by histamine (in d) in having a short latency of onset and a steeper rate of rise than their respective controls.

Effect of KCl

In two experiments we studied the possibility that histamine's potentiating effect on injected acetylcholine might be mediated indirectly *via* potassium ions which histamine is known to be capable of releasing into the bloodstream of the cat (Macmillan, 1956; Macmillan & Vane, 1956). In each experiment, KCl, 0·01 μg to 10 mg, injected i.a. toward the anterior tibialis muscle 10 to 15 s before acetylcholine failed to cause any significant enhancement of the acetylcholine-evoked responses. On the contrary, 0·1 and 10 mg of KCl appeared to inhibit them.

Effect of denervation

Most of the animals used in our investigation were rendered spinal in order to preclude any influence of medullary vasomotor reflexes upon the contractile responses of the skeletal muscles. However, this procedure did not eliminate the possibility that other neuronal structures (e.g., the spinal cord or peripheral motor nerve terminals), when acted upon by histamine, somehow might have modulated the acetylcholine-evoked contractions. Therefore, experiments were done on anterior tibialis muscles that had been denervated either acutely during the experiment (3 cats) or in a separate operation 4 days before it (3 cats). Acute denervation had no significant effect upon either the contractile effect of i.a. acetylcholine or the enhancement of that effect by histamine. Four day denervation increased the sensitivity of the muscle to acetylcholine slightly and rendered the histamine somewhat more effective than usual as a potentiator. Thus it appears that the presence of a fully functional peripheral innervation of the muscle did not contribute to and may have detracted from the potentiating effect of the histamine.

Other muscles

Consistent with our findings on the anterior tibialis muscle, prior i.a. administration of histamine also enhanced the responses to i.a. acetylcholine of the soleus (3 out of 4 experiments) and external rectus (7 out of 8 experiments) muscles, *in vivo*. *In vitro*, however, histamine (1 to 100 μg/ml) had no effect upon acetylcholine-evoked responses of the external rectus muscle in each of two experiments.

Discussion

Macmillan (1956) was the first to demonstrate that, when injected i.a. toward an innervated skeletal muscle in the cat, histamine potentiated the contractile effect of exogenous acetylcholine injected shortly afterwards but inhibited that of neurally released acetylcholine. He ascribed both of these actions to a sudden

release by the histamine of pharmacologically active amounts of potassium ions into the extracellular fluid. Sonnenschein and his colleagues (Hirvonen *et al.*, 1964 ; Sonnenschein *et al.*, 1967) confirmed that histamine exerted an inhibitory effect on the force of neurally-evoked skeletal muscle contractions. However, they attributed the inhibition not to the release of potassium, although such release does in fact take place (Macmillan, 1956 ; Macmillan & Vane, 1956), but rather to the histamine-induced vasodilatation within the actively contracting muscle that also takes place and which, under such circumstances, apparently causes blood to be diverted from nutritive to non-nutritive channels. In the present investigation, our interest has centred upon the effect of histamine on responses of cat skeletal muscle to exogenous acetylcholine, and we have been able to confirm the other part of Macmillan's original finding ; namely, that in this case the effect of histamine is a potentiating one. However, our evidence forces us, also to conclude that the potentiation, rather than being causally related to potassium release, is due chiefly if not solely to histamine's vasodilator action within the resting muscle.

In resting skeletal muscle, normally about 75% of the capillaries are closed and therefore excluded from participation in exchange processes (Krogh, 1919). However, a variety of conditions, such as relative hypoxia or increased metabolic activity, cause many more capillaries to open up as indicated by an increase in the muscle's capillary filtration co-efficient (C.F.C.) (Cobbold, Folkow, Kjellmer & Mellander, 1963 ; Mellander & Johansson, 1968). With the muscle in a resting as opposed to a contracted state (see above), the microvascular segments affected by the vasodilator action of histamine apparently include the precapillary sphincters of nutritive capillaries, since Kjellmer & Odelram (1965) have shown that on i.a. injection toward such a resting muscle, histamine causes not only a decrease in arteriolar resistance but also an increase in C.F.C. Under these conditions, an i.a. dose of acetylcholine should be carried to more capillaries more rapidly than usual. This, in turn, would permit more of the acetylcholine molecules to reach more of their receptors on the muscle fibres in a given time, thereby generating a faster, larger and perhaps more synchronous contraction. This we postulate to be the mechanism of the histamine-induced potentiation.

There are several pieces of evidence in support of this view. First of all, the potentiation was clearly not a neural phenomenon triggered by the histamine either reflexly or in some other way, since it could be demonstrated in spinal cats and also on muscles *in situ* whose peripheral innervation had been functionally impaired by denervation. Secondly, under conditions identical to those necessary for histamine to best produce potentiation, bradykinin, another vasodilator, also produced potentiation whereas angiotensin, a vasoconstrictor, produced inhibition. Thirdly, two procedures known to cause hyperaemia plus an increase in nutritive capillary blood flow within muscle tissue, namely exercise of that muscle (Hilton, 1953 ; Cobbold *et al.*, 1963 ; Kjellmer, 1964) and occlusion of its main arterial blood supply (Folkow, Haeger & Kahlson, 1948 ; Thulesius, 1962 ; Cobbold *et al.*, 1963) also caused histamine-like potentiation of the contractile effect of acetylcholine injected i.a. toward the muscle when the two vascular adjustments were at their peaks. Fourthly, histamine never enhanced responses of the muscle to direct electrical or to nerve stimulation, the latter being a situation in which the acetylcholine evoking the contractions did not have to traverse the muscle's capillary bed but was instead deposited by the nerve terminals in close proximity to the

motor endplates. And finally, histamine failed to enhance responses of a skeletal muscle to exogenous acetylcholine *in vitro*, a situation in which the acetylcholine again circumvented the muscle's capillary bed, this time diffusing directly from the bath fluid through the extracellular space to the motor endplates. This last piece of evidence also argues against the possibility that the potentiation observed *in vivo* was contributed to by a direct effect of histamine upon the muscle's contractile process.

The present results show that in the cat, discrete circulatory adjustments produced by drugs within a skeletal muscle, although without effect upon its resting tension, can modulate its contractile responses to a subsequently injected agonist in a predictable manner, vasodilatation augmenting the responses and vasoconstriction reducing them. In this respect, these results are in exact correspondence with those obtained previously (Giarman & Reit, 1967; Cervoni & Reit, 1971) from studies of the effects of various vasoactive substances and procedures upon the contractile responses to bloodborne agonists of the cat nictitating membrane, a smooth muscle effector organ. Thus, they provide a firm basis for suggesting that such interactions between drugs that affect vascular muscle and drugs that affect non-vascular muscle are not merely peculiarities of a particular muscle or muscle type but instead are probably phenomena of general occurrence in muscle tissue of cats. Whether such phenomena occur in other species also is now being investigated.

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