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# THE INFLUENCE OF VASOACTIVE SUBSTANCES ON BLOOD FLOW AND CONTRACTILE RESPONSES OF CAT GASTROCNEMIUS

O. HUDLICKA & E. REIT[1]

The Department of Physiology, The Medical School, University of Birmingham, Birmingham B15 2TJ

**1** The effects of several vasoactive substances have been studied on blood flow and acetylochline-induced contraction of the vascularly isolated gastrocnemius of the cat. All substances, including acetylcholine, were administered intra-arterially to the muscle. Blood flow and contractile tension were monitored simultaneously.

**2** All substances which increased blood flow, enhanced the contractile responses to acetylcholine; angiotensin, which decreased blood flow, attenuated them.

**3** Histamine was typical of the vasodilators in doses up to 1 μg (9 nmol), but in a dose tenfold higher it produced only a small and transient increase in blood flow and little or no enhancement of the acetylcholine-induced contractions. These paradoxical effects were brought about by that portion of the high dose of histamine which re-entered the cat's systemic circulation.

**4** The order of potency of the vasodilators was the same for their potentiating effect on the acetylcholine-induced contractile responses as for their enhancing effect on muscle blood flow: bradykinin $>$histamine$\gg$papaverine$\gg NaH_2PO_4$ $>$KCl. Median effective doses ($ED_{50}$s) determined only for bradykinin, histamine and papaverine were, respectively, 20.5, 49.8, and 3,352 pmol as potentiators, and 13.4, 199.8 and 85,000 pmol as vasodilators.

**5** In general, the effect of a given vasoactive substance on the contractile response to acetylcholine was dose-dependent and correlated well with its effect on muscle blood flow at the moment the acetylcholine was injected. Two important exceptions were, firstly, that the highest dose of papaverine was only moderately effective as a potentiator even though highly effective as an increaser of muscle blood flow; and, secondly, that histamine produced its greatest potentiating effect 10 s after it was injected, at which time its effect on muscle blood flow was quite small.

**6** It is suggested that these exceptions and, indeed, the much greater potentiating effectiveness of histamine and bradykinin versus papaverine may be due to the ability of histamine and bradykinin to increase the permeability of the muscle's capillaries as well as increasing blood flow through them, thus facilitating much better than mere vasodilators the access of bloodborne acetylcholine to its receptors on the muscle fibres.

## Introduction

Bowman & Zaimis have established that the catecholamines adrenaline, noradrenaline and isoprenaline can modulate the contractility of skeletal muscle by a mechanism still undefined, but clearly independent of their vasoactive properties (Bowman & Zaimis, 1958; Bowman & Raper, 1966; Sullivan & Zaimis, 1973). However, other investigators (Giarman & Reit, 1967; Cervoni & Reit, 1971; Block & Reit, 1973) have shown that a variety of other substances, apparently solely by virtue of their vasoactive properties, can alter profoundly and predictably the contractile responses of smooth as well as skeletal muscles to injected agonists *in vivo*. For example, in the cat the vasodilator, histamine, potentiates the contractile responses of nictitating membrane to bloodborne noradrenaline and of anterior tibialis to bloodborne acetylcholine, whereas the vasoconstrictor, angiotensin, inhibits them. In contrast, neither histamine nor angiotensin alter the noradrenaline-evoked contractions of the isolated nictitating membrane or the acetylcholine-evoked contractions of the isolated external rectus *in vitro* (Giarman & Reit, 1967; Cervoni & Reit, 1975; Block & Reit, 1973). Based upon this and other indirect evidence, Reit and

[1]Present address:
Department of Pharmacology, The University of Vermont College of Medicine, Burlington, Vermont 05401, U.S.A.

his colleagues have postulated that the modulatory effects they observed *in vivo* were, in fact, vaso-modulatory in nature, being due to either facilitation or restriction of the access of the contractile agonist into the nutritive circulation of the muscles concerned, which in turn affected the ability of that agonist to reach its receptive sites on the muscle cells. The present investigation was undertaken to obtain direct evidence bearing upon these postulated regional circulatory adjustments by the use of techniques for monitoring the muscle's blood flow as well as its contractile tension.

## Methods

The experiments were performed on cats of either sex weighing at least 1.9 kg. They were anaesthetized with a mixture of chloralose (70 mg/kg) and sodium pentobarbitone (4.5 mg/kg) injected intraperitoneally and supplemented as needed with intravenous sodium pentobarbitone. Blood pressure was recorded from either a femoral or common carotid artery with a Statham pressure transducer, and muscle tension with a strain gauge (Dynamometer UF Ether or Grass FT-03), all of which were coupled to a multichannel pen recorder (Devices or Grass). Intravenous injections were made through a polyethylene cannula tied into an external jugular or femoral vein. All surgically exposed tissue was covered with cotton wool soaked in warm 0.9% w/v NaCl solution (saline). After surgical procedures were completed, each cat was given heparin (1000 units/kg i.v.).

The gastrocnemius muscle was prepared so that its venous outflow and contractile tension could be recorded simultaneously. By systematic ligation and judicious use of electrocautery, arterial inflow and venous outflow of the muscle were isolated. Then metal twist drills were inserted into the distal ends of femur and tibia and, with the cat on its back, the leg was firmly anchored by clamping the drills to rigid upright rods attached either to the operating table or to a Brown-Schuster Myographic Apparatus. The tendon of insertion was dissected free, cut and connected by heavy thread to the strain gauge. The sciatic nerve was crushed and tied or cut and in most experiments, electrodes were placed upon the distal portion innervating the gastrocnemius. The nerve was then stimulated supramaximally with 0.5 ms square wave pulses at 5 s intervals whilst the tension on the muscle was adjusted in order to determine that at which the largest contraction was elicited. This optimal tension varied from muscle to muscle (range, 150 to 515 g; mean, 303.2 g) but for each muscle, once determined it was maintained as the resting tension for the entire experiment. In a few of the earlier experiments, before we decided to ascertain and use the optimal tension for each muscle, 10 g was chosen arbitrarily to be the resting tension.

Close intra-arterial injections toward the muscle were made into the femoral artery by means of polyethylene cannulae tied into the central ends of the saphenous and/or gracilis arteries. We measured venous outflow as described by Hilton & Lywood (1954) by inserting into the popliteal vein a polyethylene cannula which delivered the blood dropwise into a sealed, vertically oriented, translucent cylindrical chamber, from which it was conducted through another polyethylene cannula back into the ipsilateral femoral vein. The rate of drop formation was recorded with a photoelectric counter coupled either directly or via an integrator to the pen recorder. Then, from the calibrated volume per drop of blood and the muscle weight, both of which were determined at the end of the experiment, total blood flow was calculated as ml/100 g muscle × min.

### *Drugs*

All substances for intravascular administration were dissolved in saline except for the KCl and $NaH_2PO_4$ which were dissolved in distilled water. The volume for intra-arterial injections was 0.05 ml or less. Other substances used were: acetylcholine chloride, histamine phosphate, histamine dihydrochloride, papaverine sulphate, papaverine hydrochloride, bradykinin, and angiotensin ($Val^5$–angio-tensin II-ß-amide). Doses of these substances refer to the free base or peptide.

## Results

### *Simultaneous recording of muscle blood flow and contractile responses*

The effect of bradykinin on muscle blood flow and on the contractile response of the gastrocnemius muscle to acetylcholine is illustrated by the experiment of Figure 1. On intra-arterial injection toward the muscle in (a), acetylcholine 3 µg, evoked an 80 g contraction after a short latency. Several minutes after the muscle had relaxed again, bradykinin, 0.01 µg (9.4 pmol) injected intra-arterially in (b), did not itself alter the muscle tension but did increase muscle blood flow more than twofold. During this bradykinin-induced hyperaemia, another injection of acetylcholine toward the muscle evoked a 600 g contraction which was almost immediate in onset and slightly shorter in duration than the previous contraction in (a).

In their study of the cat anterior tibialis *in situ*, Block & Reit (1973) found that the degree to which histamine potentiated the contractile effect of acetylcholine was strongly influenced by the time interval between the sequential injections of histamine and acetylcholine as well as by the dose of histamine. Using an intra-arterial dose of about 3 nmol (0.1 µg/kg) of histamine, they ascertained that the optimum injection interval was 15 seconds. In the present in-

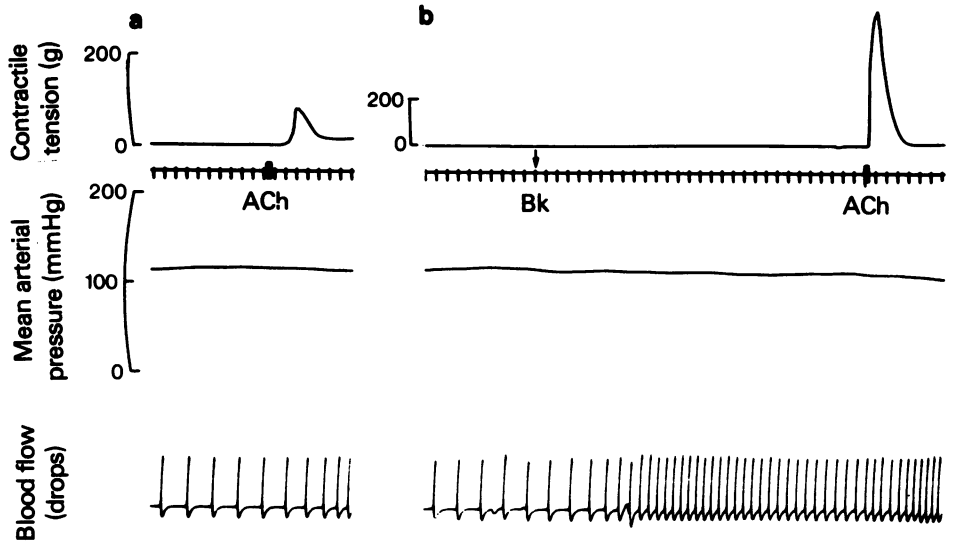


**Figure 1** Record of consecutive responses of gastrocnemius muscle tension (top), systemic blood pressure (middle) and gastrocnemius muscle blood flow (bottom) of a 4.3 kg cat, anaesthetized with a mixture of chloralose and sodium pentobarbitone. Injections intra-arterially toward the muscle of acetylcholine (ACh) 3 μg, at the signals (in a and b) and of bradykinin (Bk) 0.01 μg, at the arrow (in b). Weight of gastrocnemius, 39.2 g. Time marker in seconds.

vestigation on the vascularly isolated cat gastrocnemius, we had intended to use that same interval. However, in preliminary experiments it became apparent that for technical reasons growing out of our desire to monitor blood flow and contractile tension of the same muscle at the same time, we would have to choose a longer interval. The one we settled on and used in all subsequent experiments, unless otherwise noted, was 30 seconds. This injection interval permitted elicitation of an appreciable, if submaximal, degree of potentiation by bradykinin (as illustrated in Figure 1b) as well as by all of the other substances we studied which increased muscle blood flow (i.e. histamine, papaverine, KCl and $NaH_2PO_4$). In contrast, angiotensin, which decreased muscle blood flow, inhibited the contractile effect of acetylcholine injected 30 s afterwards.

*Dose-effect relationships*

These vasomodulatory effects were dose-dependent, as illustrated in Figure 2 for bradykinin, histamine, papaverine and angiotensin. As potentiators, bradykinin and histamine were closely similar in potency and effectiveness, and both were considerably more potent and effective than papaverine. For each of these substances, the median effective dose and maximum degree of potentiation is given in Table 1. Of the two other vasodilators studied, but not included in Figure 2 or Table 1 because of the relative paucity of data, $NaH_2PO_4$ was more potent than KCl, and both were several orders of magnitude less potent than papaverine.

Also shown in Figure 2 is that the dose-response curves for the acetylcholine-evoked contractions

**Table 1** Relative potency and effectiveness of vasodilators in potentiating contractile response of the gastrocnemius to intra-arterial acetylcholine

| *Vasodilator* | n | *$ED_{50}$ (pmol)* | *Maximum increase in tension (g)* |
|---|---|---|---|
| Bradykinin | 6 | 20.5 ± 7.4 | 1,303 ± 87 |
| Histamine | 6 | 49.8 ± 11.2 | 1,305 ± 99 |
| Papaverine | 5 | 3,352 ± 1,527 | 705 ± 144 |

Data are expressed as mean and s.e.; *n* is number of experiments. Significance of differences was determined using Student's unpaired *t* test (Snedecor, 1956). For $ED_{50}$, bradykinin versus histamine, $P < 0.1$ (not significant); either versus papaverine, $P < 0.05$. For maximum tension increase, bradykinin versus histamine $P > 0.99$ (not significant); either versus papaverine, $P < 0.01$.

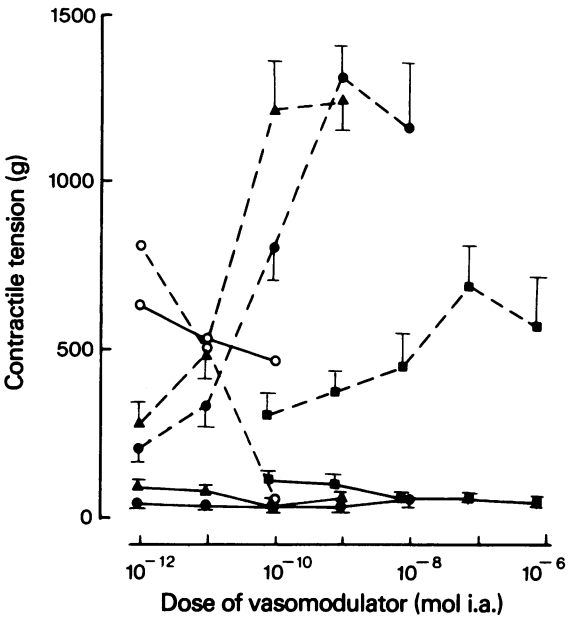


**Figure 2** Effect of intra-arterial injection of histamine (●), bradykinin (▲), papaverine (■) and angiotensin (○) on the contractile response of the gastrocnemius muscle to a fixed dose of intra-arterial acetylcholine. Unbroken lines, control responses to acetylcholine; broken lines, responses to same dose of acetylcholine injected 30 s after the vasoactive substance. Each point represents the mean and the vertical bars, the standard errors of at least five responses, except in the case of angiotensin for which each point is the average of only two responses and therefore no standard errors are shown. Each vasoactive substance was studied in a separate cat anaesthetized with a mixture of chloralose and sodium pentobarbitone, and only one complete dose-response curve was obtained per cat. Although the dose of acetylcholine injected in a given experiment was always the same, it did vary somewhat from experiment to experiment. Doses of acetylcholine used (range among cats) and body weights of the cats were for the experiments on histamine, 1.3 to 6.7 μg and 2.0 to 3.9 kg; on bradykinin, 2 to 6.7 μg and 2.3 to 4.3 kg; on papaverine, 2 to 3 μg and 2.2 to 3.3 kg; and on angiotensin, 6.7 μg and 2.8 and 4.6 kg.

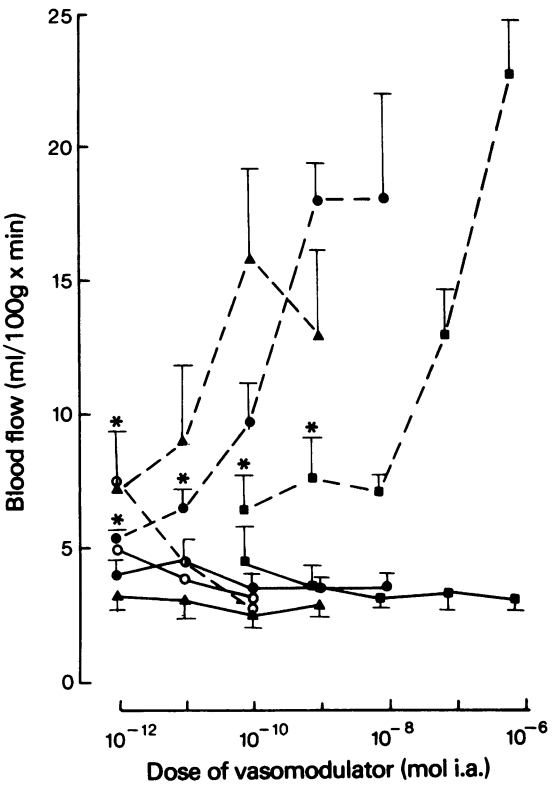


**Figure 3** Effect of intra-arterial injection of histamine (●), bradykinin (▲), papaverine (■) and angiotensin (○) on the rate of blood flow through the same gastrocnemius muscles whose contractile tension responses to a fixed dose of intra-arterial acetylcholine are summarized in Figure 2. Unbroken lines, control blood flows measured in the resting muscles just before the control injections of acetylcholine were made; broken lines, blood flows measured 30 s after the vasoactive substances were injected and just before the test injection of acetylcholine was made. Weights of gastrocnemius muscles (range among cats) were for the experiments on histamine, 14.7 to 40.2 g; on bradykinin, 15.1 to 39.2 g; on papaverine, 13.8 to 27 g; and on angiotensin, 20.9 and 39.3 g. All other conditions and data as for Figure 2. Points designated by asterisks are not significantly different from their controls.

potentiated by bradykinin and histamine were nearly parallel and quite steep, whereas the slope of the corresponding curve for papaverine was clearly different and much more gradual. This suggested that there might be two separate mechanisms of potentiation, one for bradykinin and histamine, and a second for papaverine.

Some additional evidence bearing upon this possibility was obtained when we plotted the corresponding effects of the three substances on muscle blood flow (Figure 3). As in Figure 2, the curves of Figure 3 reflected the differences among the substances in vasodilator potency: the one for bradykinin, the most potent vasodilator, was situated to the left of that for histamine, which was, itself, situated considerably to the left of the one for papaverine (median effective doses are listed in Table 2). However, in contrast to the curves representing effects on acetylcholine-evoked contractions, all three of the curves representing effects on muscle blood flow were

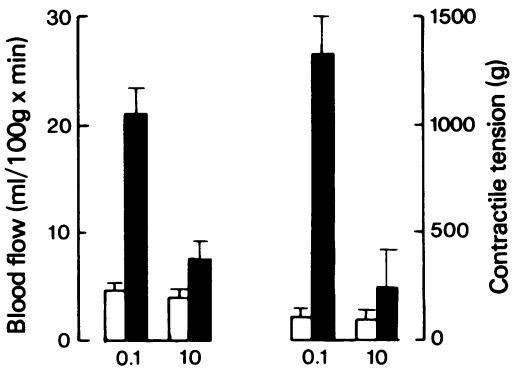


**Figure 4** Comparison of effects of 0.1 μg versus 10 μg of intra-arterial histamine on the rate of blood flow through gastrocnemius muscles and on the contractile responses of those muscles to a fixed dose of intra-arterial acetylcholine. Each column represents the mean of 3 contractile responses (on the right) and 3 muscle blood flow rates (on the left) measured, as described in Figures 2 and 3, in the absence of histamine (open column), and 30 s after it was injected (closed column). Vertical lines show s.e. mean.

parallel. Moreover, the maximum increase in blood flow produced by papaverine was at least as great as that produced by bradykinin and histamine (see Table 2). Relationships identical to those among the blood flow curves were obtained when we plotted the corresponding effects of the three substances on the calculated peripheral resistance of the muscle's vascular bed. Thus these data were consistent with the view that the potentiating effects of all three substances were probably dependent upon their abilities to produce vasodilatation within the muscle leading to an increase in blood flow. However, the data also indicate that change in blood flow, although a good index of potentiating potency, is not a reliable index of potentiating efficacy, since if it were, the maximum potentiation produced by papaverine would have been at least as great as the maximum potentiations produced by bradykinin and histamine.

Another noteworthy difference among the blood flow curves of Figure 3 is that at their upper limits, the ones for bradykinin and histamine flattened out, whereas that for papaverine did not. This meant that unlike the highest dose of papaverine (243 μg, 720 nmol), that of bradykinin and of histamine (1 μg, or 0.94 and 9 nmol, respectively) was unable to increase muscle blood flow any further than the next lower dose. But what if still higher doses were to be injected? Unfortunately, this was feasible only for histamine. In the case of papaverine, we were reluctant to attempt it because in the small (0.03 ml) injection volume required, doses much higher than 243 μg tended to exceed the solubility of the papaverine salts. In the case of bradykinin, economic considerations made us forego studying higher doses. But in the case of histamine, in a few experiments we were able to study a dose tenfold greater than shown in Figures 2 and 3. To our surprise, this dose of histamine (10 μg or 90 nmol) was not merely equieffective to, but instead much less effective than, the optimal potentiating dose (0.1 μg or 0.9 nmol). The results are summarized in Figure 4. In the absence of histamine, the control values for the acetylcholine-evoked contractions were closely comparable, as were the control values for the blood flow rates immediately before the injections of acetylcholine were made. And yet the two doses of histamine differed strikingly in their effects on each of the parameters, the higher dose producing a much smaller increase in blood flow and a correspondingly much reduced potentiation of the acetylcholine's contractile effect.

The question then arose whether this drastic diminution of histamine's effectiveness was due to some additional action exerted by the high dose within the muscle or on structures reached after it had gained access to the systemic circulation. To find out, in two experiments we prevented the intra-arterially injected 10 μg of histamine from entering the systemic circulation by temporarily allowing the venous blood from the gastrocnemius to drain out of the drop chamber into a small siliconized beaker. Results of such an experiment are shown in Figure 5. The control contrac-

**Table 2** Relative potency and effectiveness of vasodilators in increasing blood flow through the gastrocnemius

| *Vasodilator* | n | *$ED_{50}$ (pmol)* | *Maximum increase in blood flow (ml/100g x min)* |
|---|---|---|---|
| Bradykinin | 6 | 13.4 ± 3.3 | 13.4 ± 3.0 |
| Histamine | 6 | 199.8 ± 76.7 | 16.8 ± 2.2 |
| Papaverine | 5 | 85,000 ± 17,128 | 19.6 ± 2.1 |

Data are expressed as mean and s.e.; *n* is number of experiments. Significance of differences was determined using Student's unpaired *t* test (Snedecor, 1956). For $ED_{50}$, bradykinin versus histamine, $P < 0.05$; either versus papaverine, $P < 0.001$. For maximum blood flow increase, bradykinin versus histamine, $P > 0.3$ (not significant); either versus papaverine, $P > 0.1$ (not significant).

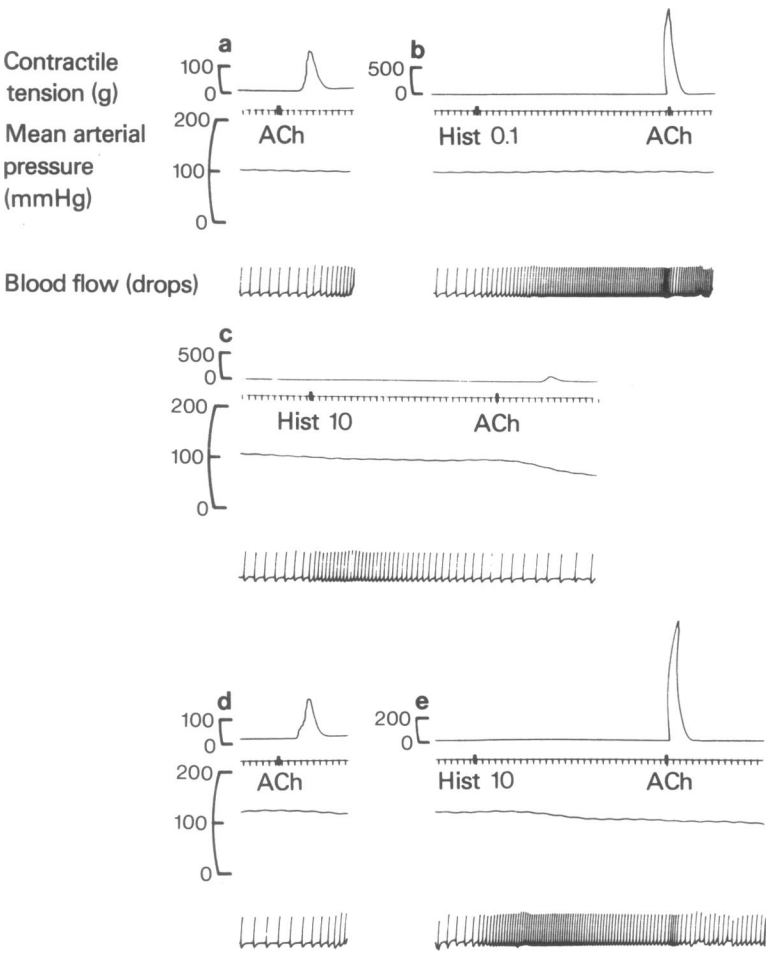


**Figure 5** Record of consecutive responses of gastrocnemius muscle tension (top), systemic blood pressure (middle) and gastrocnemius muscle blood flow (bottom) of a 2.8 kg cat anaesthetized with a mixture of chloralose and sodium pentobarbitone. At the signals, intra-arterial injections toward the muscle of 2 μg of acetylcholine (ACh, in a–e), 0.1 μg of histamine (Hist 0.1, in b) and 10 μg of histamine (Hist 10, in c and e). In (e) return of muscle venous blood from drop chamber to ipsilateral femoral vein was interrupted 10 s before injection of histamine and re-established 15 s after injection of acetylcholine. Weight of gastrocnemius, 28.4 grams. Time marker in seconds.

tions produced by 2 μg of acetylcholine in (a) and (d) were almost identical in size (155 and 158 g, respectively), and there was only a small difference in the blood flow at the moment of acetylcholine injection in (a) and (d) (4.3 versus 3.3 ml/100 g × min, respectively). In (b) 30 s after injecting an optimal dose (0.1 μg) of histamine, the blood flow had increased to 23.6 ml/100 g × min, and 2 μg of acetylcholine injected at that moment produced almost immediately a contraction (1670 g) over 10 times greater than in (a). The effects of 10 μg of histamine in (c) occurred while venous return from the gastrocnemius into the ipsilateral femoral vein was uninterrupted, and in (e), while it was interrupted for just under 1 min starting 10 s before the histamine was injected. Clearly, under the latter conditions, where the large dose of histamine could only have acted within the muscle, its effects on muscle blood flow and on the acetylcholine-evoked contraction were much greater, although still not nearly as great as the corresponding effects of the 0.1 μg dose. The actual values for contractile tension were 936 g (e) versus 100 g (c), and for blood flow were 12.7 (e) versus 4.7 (c) ml/100 g × min. Also noteworthy was the much longer latency of onset of the contraction in (c) than in (e) or, for that matter, in (b), (a) and (d). Thus, doses of histamine greater than 0.1

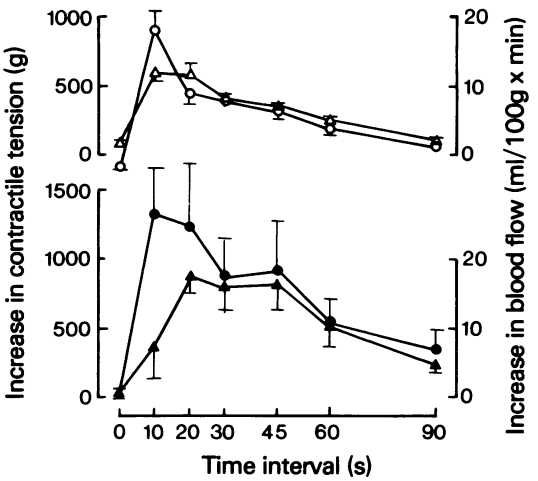


**Figure 6** Effects of the time interval between the intra-arterial injection of an optimal potentiating dose of papaverine or histamine and the subsequent intra-arterial injection of acetylcholine on the degree of enhancement of the contractile responses (g) of the gastrocnemius muscle to acetylcholine and on the degree of increase in the rate of blood flow (ml/100 g x min) through the muscle just before the acetylcholine was injected. Open symbols (○, △) refer to experiments with papaverine, 72 nmol; filled symbols (●, ▲), to experiments with histamine, 0.9 nmol. Circles (○, ●) refer to contractile tension; triangles (△, ▲) to blood flow. Both are expressed as the difference between the values obtained in the presence and in the absence of the vasoactive substances. The time interval(s) were measured from the end of the injection of the vasoactive substance to the end of the injection of acetylcholine. Each point represents the mean and the vertical bars, the standard errors of three measurements. Each vasoactive substance was studied in a separate cat anaesthetized with a mixture of chloralose and sodium pentobarbitone, and only one complete time-response curve was obtained per cat. Although the dose of acetylcholine injected in a given experiment was always the same, it did vary somewhat from experiment to experiment. Doses of acetylcholine used (range among cats) and body weights of the cats were for the experiments with papaverine, 1 to 2 μg and 1.9 to 2.9 kg; and with histamine, 1.5 to 5 μg and 2.5 to 3.2 kg.

μg (0.9 nmol) certainly tend not to be more effective and may even be considerably less effective, but only in small part because of action(s) exerted within the muscle, *per se*. What it is that larger doses of histamine initiate elsewhere in the cat that so adversely affect the muscle's ability to contract in response to intra-arterial acetylcholine has not been determined.

*Time-effect relationships*

For our final series of experiments, acquisition of another piece of equipment resolved our earlier technical difficulty in recording the changes in muscle blood flow and contractile tension simultaneously when intervals much shorter than 30 s were used between injections of vasomodulator substance and acetylcholine (see above). Therefore, in a further attempt to identify possible differences between the mechanisms by which papaverine, on the one hand, and histamine or bradykinin, on the other, potentiate the contractile effect of intra-arterial acetylcholine, we studied the time course of the potentiating effects of papaverine versus histamine in relation to the corresponding time course of the changes they produced in muscle blood flow. For this purpose, we used a fixed dose of vasomodulator substance and of acetylcholine and varied the time interval between injections of the two. The doses of papaverine and histamine were their optimal potentiating doses (72 and 0.9 nmol, respectively) and were used in all of these experiments. For acetylcholine, once a dose was chosen, it was the only one used in a given experiment, but among experiments, the doses were slightly different, depending upon the sensitivity of the different gastrocnemius muscles.

The results of the time course experiments are summarized in Figure 6. Papaverine and histamine each produced their largest potentiating effects quite rapidly, i.e. about 10 s after being injected, and thus even sooner than reported for histamine on the cat anterior tibialis (Block & Reit, 1973). In one of the experiments, we were able to inject the acetylcholine 7 s after injecting the histamine, but the potentiation that occurred was only half that seen with a 10 s injection interval. This solitary observation was not included in the Figure, but serves to point out that the peak potentiating effect of histamine, at least, did not occur much sooner than 10 s after it had been injected. After attaining their maxima, the potentiating effects of both substances gradually declined until at the 90 s interval, they had almost disappeared.

For both substances, the time course of their augmenting effects on muscle blood flow generally followed closely that of their potentiating effects on the acetylcholine-induced contractions. There was, however, one notable exception: histamine produced its greatest augmentation of muscle blood flow, not at the time of its greatest potentiating effect, but 10 s later. In contrast, the peak effects of papaverine on muscle blood flow and on the acetylcholine-induced muscle contraction occurred at the same time, i.e. 10 s after the injection of the papaverine. These results are consistent with the view that an increase in blood flow through the gastrocnemius is at least partly responsible for the potentiating effect of both papaverine and histamine. In addition, however, they indicate that whereas the increase in blood flow may be the sole determinant of the potentiating effect of papaverine, there is another, quantitatively more important mechanism by which the potentiating effect of histamine may be initiated.

## Discussion

In discussing our results, we should first consider the question whether blood flow as measured in the present investigation, by counting the drops of venous blood leaving the muscle, is an accurate reflection of the nutritive blood flow through the muscle's capillary bed. One method that is generally acknowledged to measure this nutritive blood flow is the $^{133}Xe$ clearance technique (see, e.g. Kjellmer, Lindbjerg, Prerovsky & Tønnesen, 1967). According to Tønnesen & Sejrsen (1967) and some unpublished results of our own, blood flow in the vascularly isolated cat gastrocnemius measured by the drop counting method correlates closely with that calculated from the washout curves for intra-arterial $^{133}Xe$ from the muscle. Therefore, we feel confident in interpreting the blood flow data reported in this paper to be valid indices of nutritional blood flow within the muscle.

It follows, then, that the present results provide direct evidence that an alteration in the nutritive blood flow of a muscle by certain vasoactive substances is an important determinant of the modulatory effects they exert on the contractile response of that muscle to a bloodborne agonist. However, contrary to our previously published view (Giarman & Reit, 1967; Cervoni & Reit, 1971; Block & Reit, 1973), this is apparently neither the sole determinant nor even necessarily the most important one for the following reasons: first of all, because papaverine, in doses that increased muscle blood flow as much as histamine and bradykinin did, produced much smaller enhancement than they did of the acetylcholine-induced muscle contractions (compare Figures 2 and 3); secondly, because papaverine produced its greatest effect on muscle blood flow at a dose, not equal to (as was the case for histamine and bradykinin), but tenfold higher than the dose at which it produced its maximum enhancing effect on the acetylcholine-induced contraction (again, compare Figures 2 and 3); thirdly, because as revealed by statistical analysis of the median effective doses ($ED_{50}$s, Tables 1 and 2), papaverine was significantly more potent ($P < 0.005$) as an increaser of muscle blood flow than as a potentiator of the acetylcholine-induced contractile response; fourthly, because at the lowest doses studied papaverine, histamine and bradykinin significantly enhanced the contractile effect of acetylcholine even though none of them produced a significant increase in muscle blood flow (compare Figures 2 and 3); and lastly, because unlike papaverine, histamine produced its greatest enhancement of the acetylcholine-induced contraction at a time when its effect on muscle blood flow was still quite small (see Figure 6).

The haemodynamic effects of histamine and bradykinin are known to differ from those of papaverine in that whereas all three produce vasodilatation and an increase in organ blood flow, histamine and, even more potently, bradykinin also cause an increase in capillary permeability. In this connection, there is some evidence that such an increase in capillary permeability within the muscle could be compatible with our potentiation results. Kjellmer & Odelram (1965) have shown that in the cat gastrocnemius the permeability increase was produced by the same doses of histamine and bradykinin that produced vasodilatation. Moreover, both effects were elicitable repeatedly by successive administrations of the same substance, and both were promptly reversible, following a closely parallel time course in the waning phases. Unfortunately, these investigators did not chart the time course for the onset of the two effects. Obviously, in order to strengthen the contention that an increase in capillary permeability by histamine and bradykinin played the preeminent potentiating role in our studies, it would have to be shown in the case of histamine, for example (see Figure 6), that the permeability begins to increase very soon after injection, reaches its maximum in about 10 s, and does so at a rate roughly three times faster than the vasodilatation.

In marked contrast to doses of histamine one to three log units lower, 10 μg injected intra-arterially to the vascularly isolated gastrocnemius produce only a small and short-lasting increase in blood flow and, instead of potentiating, may actually attenuate the acetylcholine-evoked contraction, unless this relatively large dose of histamine or, more precisely, whatever portion of it survives circulation through the gastrocnemius, is barred from re-entering the cat's systemic circulation (see Figure 5). Although we do not know the mechanism of this paradoxical attenuating effect, there are some possibilities that ought to be considered. One easily ruled out is that the 10 μg dose of histamine, upon re-circulating, was sufficient to trigger reflexly or directly a neural discharge to the muscle which, by the time the acetylcholine was being injected, had exerted an inhibitory influence on the muscle's contractile ability and/or a strong vasoconstrictor influence on its precapillary arterioles. However, any such neural mechanism would be most unlikely since the sciatic nerve supplying the gastrocnemius being studied was crushed at the beginning of these experiments. A second possibility is that a fall in the perfusion pressure could have delayed and, therefore, decreased delivery of the intra-arterial acetylcholine into the capillaries because of its longer exposure to plasma and red blood cell cholinesterases. However, in the experiment of Figure 5, the net histamine-induced decrease in perfusion pressure was 14 mmHg in (e) versus 4 mmHg in (c); i.e., actually a greater fall when the large dose of histamine had been prevented from re-entering the systemic circulation (in e) than when it was permitted to do so (in c), probably because of the mild hypovolemia in the former instance during which about 5 ml of the muscle's venous blood was diverted from the drop chamber into a siliconized beaker. And even though the perfusion

pressure at the moment of the injection of acetylcholine in (c) was 5 mmHg less than in (e), our experience from all of the other experiments indicates that such a small difference would be insignificant. A third possibility is that the recirculating histamine caused the release of a chemical substance which, upon being conveyed to the gastrocnemius in the arterial blood, antagonized the developing potentiating effect of the histamine on the muscle's contractile response to acetylcholine, perhaps by counteracting the effect of the histamine on muscle blood flow and/or capillary permeability. This possibility of a bloodborne histamine-released antagonist substance seems to be the most likely and should be investigated further.

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