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Role of Vasoactive Substances in Active Hyperemia in Skeletal Muscle[1, 2] (38520)

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(Introduced by J. B. Scott)

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The mechanism of exercise hyperemia is not completely understood. Current evidence suggests that the vascular smooth muscle relaxes because of multiple metabolic factors, among which are stable substances such as oxygen (acting directly or indirectly), hydrogen, potassium, and osmolality and possibly more labile substances such as adenosine and the adenine nucleotides (1, 2). The relaxation may also result in part from a myogenic response to the decrease in vascular transmural pressure which occurs subsequent to the skeletal muscle contraction (3). There is evidence which suggests that some of these factors are more involved in the initiation of the hyperemia while others are more concerned with its maintenance (2, 4–9).

In this study, two techniques have been brought to bear on the mechanism of exercise hyperemia. These are chemical analysis of the venous effluent during prolonged exercise (4) and bioassay of the venous effluent during graded exercise (10). The first technique allows a temporal correlation between the venous concentration of certain stable, easily measured chemical factors and the magnitude of the hyperemia while the second separates the contribution of the substances which are stable from the possible contribution of the labile, more difficultly measured substances and the myogenic response.

Methods. Mongrel dogs ranging in weight from 20 to 25 kg were anesthetized with sodium pentobarbital (30 mg/kg) and ventilated artificially. Following the surgical procedures described below, heparin sodium (5 mg/kg) was administered intravenously to prevent coagulation.

The first preparation has been described previously (4). In brief, the right gracilis muscle of seven animals was exposed and freed from connective tissue. All blood vessels communicating with the gracilis, except the major artery and vein, were ligated. A heavy cord ligature was placed around the insertion of the muscle and attached to a Grass force transducer in order to record changes in muscular contractile force. The gracilis nerve was severed and the gracilis vein was cannulated and its outflow diverted into a reservoir. Blood flow was measured with graduated cylinder and stopwatch. Reservoir blood was continuously returned to the animal via the jugular vein with a pump that maintained reservoir volume constant. Abdominal aortic pressure was continuously recorded. When gracilis blood flow and aortic pressure became stable, venous and arterial blood samples were taken and a 2-hr exercise period was simulated by faradic stimulation of the gracilis nerve (3 V, 1.6 msec, 6/sec) with the electrode fixed in position. Venous blood flow measurements and samples (2–4 ml) were taken at 1, 5, 10, 30, 34, 60, 75, 90, 105, and 120 min of stimulation. The last flow and venous sample were obtained just prior to stopping exercise. An arterial blood sample was also taken at this time. Blood pressure, gracilis muscle blood flow and venous blood samples were obtained 1 min after stopping stimulation.

Plasma magnesium and potassium ion concentrations were determined by atomic absorption spectrophotometry and flame photometry, respectively and plasma osmolality by the freezing point depression method. Blood pH was measured with an Astrup pH meter.

In eight other dogs both gracili were prepared as described above and the cannulations shown in Fig. 1 were made. Blood was taken from the right femoral artery and

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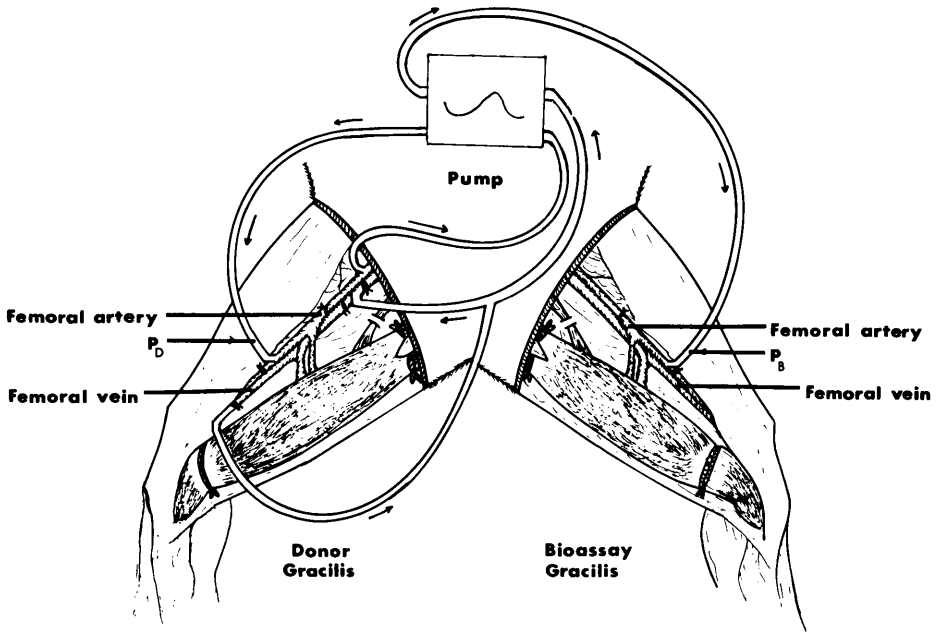

FIG. 1. Method used to bioassay the vasoactive properties of gracilis venous blood. P_D = perfusion pressure of the donor gracilis. P_B = perfusion pressure of the bioassay gracilis.

pumped at a constant rate into the right gracilis muscle (donor). Most of the venous blood from this muscle was then pumped into the downstream or bioassay muscle while a small amount ($\leq$ 1 ml) was diverted into the right femoral vein. Perfusion pressures were continuously monitored and when they became stable the severed nerve of the donor muscle was electrically stimulated (3 V, 0.2 msec). Graded levels of active dilation were produced by increasing the frequency (range = 0.2–6.0/sec) of nerve stimulation. Each level was maintained for at least two minutes. In six of the eight experiments the reactivity of the preparation and the circuit lag time were tested by injecting acetylcholine or norepinephrine into the inflow of the upstream (donor) muscle before and/or after the stimulation sequence.

Vascular resistances in both series of experiments were computed by dividing perfusion pressure by gracilis muscle flow. In the first series of experiments, the first arterial sample was utilized to calculate arteriovenous differences of Mg^{2+}, K^+, pH, and osmolality both during the control period and at the first minute of exercise and the second arterial sample was used to calculate them at both the 120th min of exercise and one minute following termination of exercise. Statistical evaluation was by the Student's t test modified for paired replicates. Differences at the $P \leq 0.05$ level were considered to be significant.

Results. Figure 2 shows that gracilis contractile force waned during the 2-hr period of nerve stimulation such that at 120 min, it was significantly below the maximal value but still well above the control value. Perfusion (aortic) pressure did not change but blood flow increased and remained elevated; consequently vascular resistance remained depressed throughout the entire period of exercise. Vascular resistance and blood flow had not yet recovered completely 60 sec after terminating exercise.

Figure 3 shows that the venous plasma concentration of magnesium did not change significantly but that venous plasma potassium concentration, venous blood hydrogen ion concentration and venous plasma osmolality were increased significantly in the first minute of exercise. The increase in potassium concentration waned somewhat during the next 10 min while pH and osmolality remained approximately at their 1

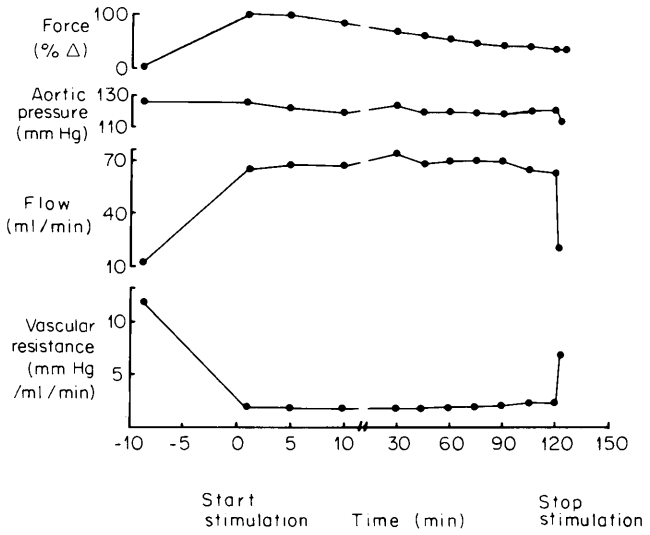

FIG. 2. Average effects of a 2-hr period of electrical stimulation of the gracilis nerve on gracilis contractile force, aortic pressure, gracilis venous outflow, and gracilis vascular resistance. $n = 7$

min levels. Table I presents the arteriovenous differences at the beginning and end of the exercise period in six of the seven animals. It is apparent that the difference for hydrogen ion increased and that differences for potassium and osmolality appeared in the first minute of exercise. It is also apparent that the difference for hydrogen decreased, that for potassium became almost negligible, and that for osmolality disappeared by the 120th min of exercise. One minute following exercise the arteriovenous differences for Mg^+, K^+, pH, and osmolality were $+0.01$, -0.2, -0.02, and -2.0 respectively (not shown in Table I).

Figure 4 presents tracings from a representative bioassay experiment. Slight dilation in the donor muscle produced at $f = 0.4$, 0.8, 1.2, and 1.7 was not followed by comparable dilation in the bioassay muscle. However, when the donor muscle was maximally dilated there was also appreciable dilation in the bioassay muscle. In fact resistance levels are essentially the same in this experiment at the highest frequency. In this and five other experiments (Table II), onset of dilation in the bioassay muscle followed onset of appreciable dilation in the donor muscle by a time only slightly longer than the circuit lag time while, on stopping exercise, dilation in the bioassay muscle began to wane soon after that in the donor muscle. Furthermore, the time courses of the response in the two muscles were similar.

Figure 5 shows vascular resistance in the bioassay muscle as a function of the vascular resistance in the donor muscle for each of eight experiments, expressed as percent of initial resistance. Mild exercise produced a much greater decrease in vascular resistance in the donor than in the bioassay muscle (note that the slope is less than one down to a vascular resistance in donor of approximately 60%). However, in six of the eight experiments there was an abrupt change in the slope of the curve such that maximal or near maximal dilation in the donor muscle was followed by appreciable dilation in the bioassay muscle.

Discussion. The hyperemia seen in the gracilis, a mixed red and white muscle, at the 120th min of exercise is apparently independent of potassium and osmolality; blood flow is still well elevated while the arteriovenous differences for potassium and osmolality are negligible.

In a previous study of exercise hyperemia in this preparation, it was shown that venous plasma potassium concentration reaches its maximum value within 10 sec of starting exercise and then gradually decreases from this maximum to a lesser elevated value over the next 5 min (4). Furthermore, ouabain, which blocks the vasodilator effect of potas-

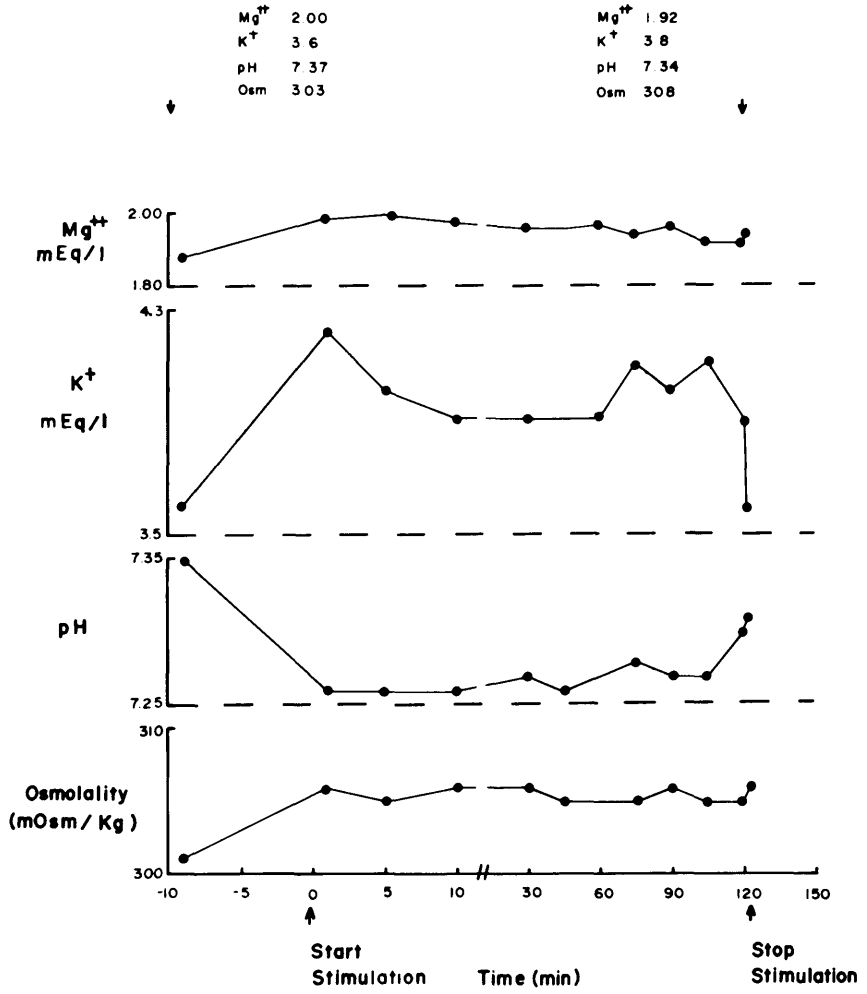

FIG. 3. Venous plasma magnesium concentration, potassium concentration, and osmolality and venous blood pH in the same experiments shown in Fig. 2. Figures above present values in arterial blood prior to stimulation and at 120 min of stimulation.

TABLE I. ARTERIOVENOUS DIFFERENCES AT THE BEGINNING AND END OF A 120-MIN PERIOD OF EXERCISE.

	Control			1 min			120 min		
	Arterial	Venous	A–V Diff.	Arterial	Venous	A–V Diff.	Arterial	Venous	A–V Diff.
Mg^{2+} mEq/liter	2.00	1.89	−0.11	2.00	2.03	+0.03	1.92	1.94	+0.02
K^+ mEq/liter	3.6	3.6	0.05	3.6	4.2	+0.5[a]	3.8	4.0	+0.1[a]
pH	7.37	7.35	−0.02[a]	7.37	7.26	−0.11[a]	7.34	7.30	−0.04[a]
Osm mOsm/kg	303	304	+0.5	303	308	+5.0[a]	308	308	−0.3

[a] $P \leq 0.05$.

sium, delays the onset of exercise hyperemia and time to maximum hyperemia but has no effect on the magnitude of the hyperemia (5). Potassium concentration has been observed to increase in the effluent from the tibialis cranialis and extensor digitorum muscles following a one second period of tetanus (6). Total potassium content of

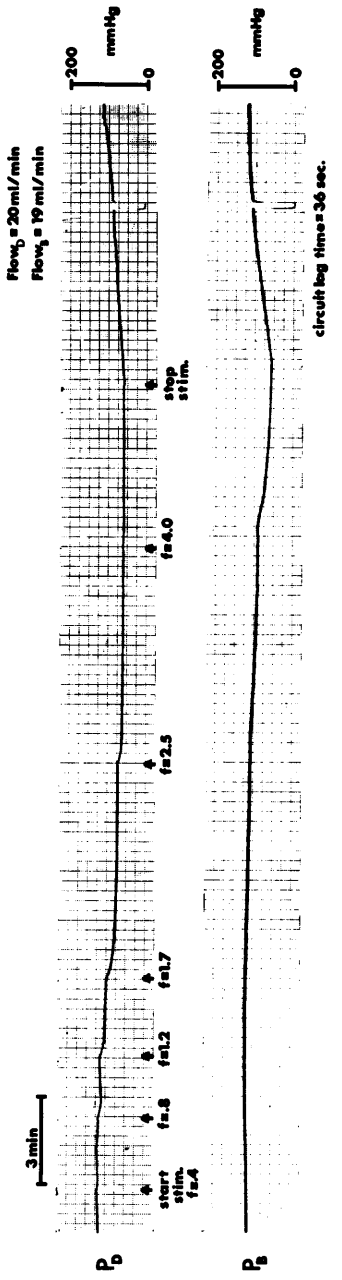

FIG. 4. Representative tracing from the bioassay series of experiments. P_D refers to perfusion pressure at constant flow of donor (exercising) gracilis. P_B refers to perfusion pressure at constant flow (with venous blood from donor muscle) of bioassay (resting) muscle.

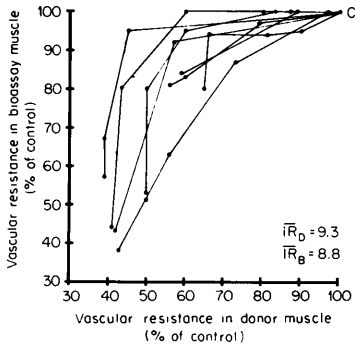

FIG. 5. Relationship between the resistance changes in the donor (exercising) and bioassay (resting) muscles in each of eight experiments. $\overline{IR}_D$ = average initial resistance in the donor muscles in mmHg/ml/min; $\overline{IR}_B$ = same for the bioassay mucsles; C = control or initial value.

TABLE II. BIOASSAY RESPONSE TIMES FOLLOWING ONSET AND OFFSET OF DILATION IN THE DONOR MUSCLE.

Experiment No.	Onset (sec)	Offset (sec)	Circuit lag time (sec)
1	72	58	47
2	84	108	76
3	100	88	80
4	88	68	68
5	48	48	36
6	32	44	32
	71	69	57

gastrocnemius skeletal muscle decreases rapidly in the first few minutes of exercise but then much more slowly over the next hour (9). The vasodilation seen in dog forelimb during intraarterial infusion of potassium tends to wane with time (11). Furthermore, the degree of dilation produced by potassium in the gracilis does not approach that seen during exercise and reducing the inflow potassium has little effect on exercise hyperemia (12). All of these studies suggest that the potassium released from skeletal muscle during depolarization is more involved in the initiation of hyperemia than in its maintenance.

In the previous study in gracilis (4) mentioned above, it was shown that the osmolality in venous plasma is not significantly changed by the 10th sec of exercise but is maximally elevated by the end of the first minute. It then falls to the control value over the next 5 min of exercise while the blood flow does not. Comparable increases in osmolality produced by infusion fails to

produce comparable decreases in resistance. These data and those of the present study suggest that, in the gracilis muscle, osmolality does not contribute importantly to the initial or late hyperemia but may have a role for a period of time shortly after its initiation. This may not apply to muscles of different composition and species (13).

The present study also shows that the increased arteriovenous difference for hydrogen also waned but was still present at the 120th min of exercise. Hydrogen ion sensitive electrodes implanted in rat gastrocnemius muscle indicate that contractions in excess of 1/sec first transiently decrease and then increase hydrogen ion activity (14), as if preexisting hydrogen ions were washed out by increased flow and then added to by increased metabolism. These findings suggest that the hydrogen ion does not participate in the initiation of dilation but might contribute to its maintenance with high frequency exercise.

The bioassay studies again show that venous blood from skeletal muscle increases its vasodilator activity during exercise (10, 15). They show in addition that submaximal, graded levels of active dilation in the donor muscle are not followed by comparable dilation in the bioassay muscle but that with severe exercise the dilation is more comparable and has a similar time course. These findings suggest that stable substances such as oxygen, hydrogen, potassium, and osmolality cannot entirely account for exercise hyperemia. The difference could equally well be explained by the Bayliss response or labile vasoactive substances since neither would affect resistance in the bioassay organ. Since the time course of the responses were similar (Fig. 4, Table II), the study fails to provide evidence for the participation of vasoactive substances to which the capillary wall acts as a significant diffusion barrier (16).

The mechanism of the increased vasodilator activity of the venous effluent from the gracilis muscle during steady state heavy exercise has recently received attention (17). The increased vasodilator activity can not be completely abolished by correcting the change in oxygen tension or pH alone but it completely disappears when they are both corrected. It is not necessary to correct any changes in potassium and osmolality.

Thus, these studies and those already in the literature suggest the hyperemia seen in the dog gracilis during moderate to heavy exercise in initiated by potassium efflux from the skeletal muscle cells and the Bayliss response. Shortly thereafter increased osmolality and hydrogen ion concentration contribute to the hyperemia. The influence of potassium and osmolality then gradually wane. Whether hypoxia, acting directly or indirectly, contributes to the initiation of the hyperemia is not clear but it apparently does contribute to its maintenance. Possible indirect actions of oxygen are via hydrogen, the adenine nucleotides, and adenosine. The Bayliss response may continue to contribute for the duration of the exercise.

Summary. A prolonged, 2-hr period of exercise hyperemia in the canine gracilis muscle was associated with initial increases in the arteriovenous differences for potassium, hydrogen and osmolality. However, that for hydrogen decreased and those for potassium and osmolality became negligible by the 120th min while blood flow remained elevated. Thus, potassium and osmolality do not appear to participate importantly in the maintenance of exercise hyperemia in canine gracilis muscle.

A bioassay muscle did not respond with comparable dilation when submaximal, graded levels of exercise hyperemia were induced in an upstream, donor muscle but did respond more comparably in terms of magnitude and time course when the exercise was more severe. Thus, stable vasoactive substances may not entirely account for exercise hyperemia and the study fails to provide evidence that the capillary acts as a significant barrier to the vasoactive substances.

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1. Haddy, F. J., and Scott, J. B., Physiol. Rev. **48**, 688 (1968).
2. Haddy, F. J., and Scott, J. B., Microcirculation. In press.
3. Mellander, S., and Johansson, B., Pharmacol. Rev. **20**, 117 (1968).
4. Scott, J. B., Rudko, M., Radawski, D., and Haddy, F. J., Amer. J. Physiol. **218**, 338 (1970).
5. Chen, W. T., Brace, R. A., Scott, J. B., Anderson,

D. K., and Haddy, F. J., Proc. Soc. Exp. Biol. Med. **140,** 820 (1972).
6. Mohrman, D. E., and Abbrecht, P. H., Fed. Proc. **32,** 374 (1973).
7. Mohrman, D. E., Cant, J. R., and Sparks, H. V., Circ. Res. **33,** 323 (1973).
8. Mohrman, D. E., and Sparks, H. V., Circ. Res. **33,** 337 (1973).
9. Sréter, F. A., and Friedman, S. M., J. Pharmacol. Exp. Ther. **131,** 158 (1961).
10. Scott, J. B., Daugherty, R. M., Jr., Dabney, J. M., and Haddy, F. J., Amer. J. Physiol. **208,** 813 (1965).
11. Brace, R. A., Scott, J. B., and Anderson, D. K., Physiologist **16,** 272 (1973).
12. Anderson, D. K., Roth, S. A., Brace, S. A., Radawski, D., Haddy, F. J., and Scott, J. B., Circ. Res. **31,** 165 (1972).
13. Mellander, S., Lundvall, J., Circ. Res. (Suppl. I) **28–29,** 39 (1971).
14. Gebert, G., and Friedman, S. M., J. Appl. Physiol. **34,** 122 (1973).
15. Haddy, F. J., and Scott, J. B., Circ. Res. (Suppl. I) **28–29,** 86 (1971).
16. Hilton, S. M., Circ. Res. (Suppl. I) **28–29,** 70 (1971).
17. Stowe, D. F., Owen, T. L., Chen, W. T., Anderson, D. K., and Scott, J. B., Fed. Proc. **32,** 436 (1973).

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