Sympathetic Vasoconstrictive Responses during Exercise- or Drug-Induced Vasodilatation

A TIME-DEPENDENT RESPONSE
By Derek J. Rowlands and David E. Donald

ABSTRACT
The effect of exercise on the vasoconstrictive response to sympathetic stimulation was studied by observing the changes in perfusion pressure in dogs' hind-limbs perfused at constant flow. During steady-state simulated exercise, a time-dependent increase occurred between 10 and 30 minutes in the magnitude of response to lumbar trunk stimulation or carotid sinus hypotension. This was present (although to a reduced degree) in drug-induced vasodilatation and in exercising limbs perfused at constant pressure. The time-dependent change was not due to (1) repetition of stimulus, (2) artifact from muscle electrodes, (3) circulating vasoactive substances, (4) skin circulation, or (5) pH, Pco₂, or temperature of the perfusing blood. When norepinephrine was used as the vasoconstrictive stimulus, no time-dependent change was noted. After stabilization, the frequency-response curve of exercising muscle exceeded that at rest at all blood concentrations of norepinephrine and at all save the lowest frequencies of stimulation. It was concluded that vascular smooth muscle can develop greater tension from greater initial length. However, during exercise some factor which operates between the sympathetic nerve ending and the receptor site on vascular smooth muscle initially tends to depress this enhanced ability to develop tension.

ADDITIONAL KEY WORDS vascular responses vascular smooth muscle limb perfusion constant-pressure perfusion peripheral resistance simulated exercise dogs

Muscular exercise is accompanied by a redistribution of blood flow, which is mediated chiefly through alterations in the caliber of the resistance vessels. In the active muscles there is an immediate dilatation of the resistance vessels that is related to the intensity of the work load and is attributed to the action of vasodilative metabolites. There is also substantial evidence that exercise is accompanied by an increase in reflex sympathetic vasoconstrictive drive (1). This can be demonstrated in nonexercising areas and results in a reduction in blood flow to these regions (2, 3). The mechanisms whereby this locally induced vasodilatation is coordinated with the centrally controlled vasoconstriction to produce the blood flow appropriate to the metabolic needs of the active muscle is a matter of some interest. On this point, several studies (4, 5) have shown that in active muscle there is almost complete abolition of the vasoconstrictive response of the resistance vessels to sympathetic drive, and this permits full expression of the locally induced vasodilatation.

These findings would imply one of the following: (1) the sympathetic nerves to the resistance vessels in the active muscles do...
not share the general increase in sympathetic activity; (2) the dilated smooth muscle of the resistance vessel is less responsive to a given vasoconstrictive impulse; or (3) there is some interference with the sequence of events between liberation of the excitant substance at the nerve ending and its arrival at the receptor site in the smooth muscle of the resistance vessel. This study was undertaken to describe further the response of the resistance vessels to sympathetic drive during exercise. The results indicate the dependence of the findings in such investigations on (1) the perfusion technique used (constant pressure or constant flow), (2) the magnitude (frequency) of the sympathetic drive used, (3) the degree of dilatation induced, and (4) the duration of the exercise.

Methods

The basic preparation consisted of dogs' hindlimbs perfused with autologous blood at constant flow. Frequency-response curves (F-R curves) were used to compare the changes in vascular resistance effected by sympathetic nerve stimulation with the limbs at rest and during simulated exercise. The F-R curves were constructed from observations of the changes in perfusion pressure during electrical stimulation of the lumbar sympathetic trunk at graded frequencies.

Mongrel dogs weighing 12 to 19 kg were anesthetized with sodium pentobarbital (Nembutal), 30 mg/kg, given intravenously; additional doses were given as required during the procedure. In a few animals (specified later), morphine-chloralose was used (morphine, 2.5 mg/kg; chloralose, 80 mg/kg). Auffed endotracheal tube was inserted and respiration with 100% oxygen was maintained with a respirator set at 12 cycles/min, with the stroke volume adjusted to give a peak inspiratory pressure of 14 cm H2O. Arterial blood P02 ranged between 300 and 400 mm Hg and Pco2 between 35 and 44 mm Hg. Heparin, 2.5 mg/kg, and atropine, 0.2 mg/kg, were given initially; additional doses of 20 mg of heparin and 2 mg of atropine were given hourly. All dogs were supported by the slow infusion of heparinized donor blood.

Most of the experiments were performed under conditions of constant flow, using a 360° roller pump whose output could be varied between 20 and 500 ml/min. The inflow to the pump was taken from the abdominal aorta below the level of the renal arteries. To reduce collateral circulation, the third to seventh lumbar arteries and the deep circumflex iliac and cava1 mesenteric vessels were ligated and severed bilaterally. The aorta and umbilical, internal iliac, and median sacral arteries were ligated below the origin of the external iliac arteries. The contralateral external iliac artery was ligated. The circulation to the paw was occluded by means of a tight ligature applied above the ankle joint. The pump outflow was fed through a depulsating chamber and the heat exchanger into the external iliac artery of the leg being studied. The perfusion pressure was recorded from the external iliac artery, distal to its cannulation, by a Statham P23Db strain-gauge transducer and a Honeywell Visocorder. Flow was measured from the volumetrically calibrated roller pump and also by an electromagnetic flowmeter-cannulating transducer (Carolina Medical Electronics, Model EMP300, 8 inch) inserted into the perfusion line between the depulsator and heat exchanger. The flowmeter could be bypassed through a parallel circuit for repeated zero assessment. The flowmeter was calibrated by known flows before and after the experiment.

Simulated exercise was induced by means of electrical impulses delivered from a second S4 Grass stimulator into the muscles of the hindlimb through four needles (two positive and two negative). One needle of each polarity was inserted into the anterior thigh muscles on each side of the femoral neurovascular bundle, the third needle was inserted into the muscles just above the knee joint, and the fourth needle was inserted into the posterior thigh muscles close to the sciatic nerve. The stimulated leg was secured at the ankle by a cord. Morphine, 8-msec square-wave impulses were delivered at 5/sec. The voltage was set between 5 and 30 v according to the flow required. Because of the relatively high internal resistance of the stimulator, the voltages actually delivered to the muscles were appreciably less than the voltages indicated by the settings.

The basic experiment consisted of comparing the F-R curves obtained at rest to those obtained during simulated exercise. Thirty-three ex-
periments of this type were carried out in 22 dogs. The initial pump flow to the limb in the resting state was adjusted to bring the mean perfusion pressure to between 80 and 100 mm Hg. In general, this was close to the central arterial pressure. This same flow was used for all subsequent measurements in the resting state for that limb, except that occasionally after a period of exercise, a minor adjustment in flow was necessary to ensure the same perfusion pressure. Sup- plemental stimuli of increasing frequency were then applied to the lumbar sympathetic trunk, and the changes in perfusion pressure were recorded. Sufficient time was allowed between periods of stimulation for the perfusion pressure to return to the control value. At the onset of muscular exercise the pump flow was increased to maintain the perfusion pressure equal to that at rest. After stability had been reached during the first few minutes of exercise, the flow was main- tained constant throughout the exercise period. Generally, a single frequency was selected and repeated at intervals of 5 minutes until a stable response was obtained. Only then was the complete F-R curve obtained. In some instances a 3- to 4-point F-R curve was obtained within the first 10 minutes of exercise for comparison with that obtained after the response had stabilized. After a recovery period of 10 to 20 minutes, a sec- ond resting F-R curve was obtained. Exercise flows ranged from 100 to 470 ml/min. In some dogs it was possible to obtain F-R curves at two or more different exercise flows.

In a number of experiments, vasodilatation was induced by adenosine triphosphate (ATP) or acetylcholine instead of by muscle stimulation. In three dogs, norepinephrine was infused as the vasoconstrictive stimulus at rest and during sim- ulated exercise. Blood concentrations were cal- culated from the amount of norepinephrine de- livered by the infusion syringe into the known arterial pump flow perfusing the hindlimb. At each concentration the infusion was continued until its pressure had stabilized. Drugs were in- fused into the inflow line to the roller pump to promote mixing.

In some experiments a second limb was per- fused separately as a control. Sometimes a fore- limb was perfused through a cannula placed in the brachial artery, and sometimes the contra- lateral hindlimb was perfused, with the inflow to the separate pumps being taken from the can- nulated proximal end of the abdominal aorta by means of a Y connection. The pump outflow line on each side was fed into the appropriate external iliac artery. With the bilateral hindlimb perfu- sion, it was necessary to dissect free the muscular insertions from the symphysis of the pelvis and from the pubic bone on one side. If this precau- tion was not taken, the application of stimuli to the muscle electrodes of the test limb caused mus- cular contractions in the control hindlimb also. When the hindlimb was used as control, stimuli of identical voltage, duration, and frequency were applied to the sympathetic trunk on the control side simultaneously with those on the test side, both sympathetic trunks having been dissected in the same way.

In five dogs, vasoconstriction was induced reflexly by carotid sinus stimulation (Moissejeff technique) (5). Morphine-chloroform anesthesia was used, the lumbar trunk was not dissected out, and a perfused forelimb was used as a non- exercising control.

In 11 experiments in which a constant-pressure perfusion technique was used, F-R curves were obtained at rest and during exercise. In these, the pump inflow was fed from the aorta in the usual manner. The pump outflow was led into a blood reservoir, which was suspended at a variable height above the dog. An outflow line of wide- bore tubing led from this reservoir to the distal end of the cannulated external iliac artery. A switching relay was used to control the pump flow so that the height of the perfusing column of blood remained constant within ± 1 cm. The actual perfusion pressure was measured in the external iliac artery, as in the other experiments. In view of the flow-dependent pressure drop across the tubing connecting the reservoir and the artery, the height of the reservoir was increased during the higher flows of exercise and drug-in- duced vasodilatation, to maintain the limb per- fusion pressure at the same level. In some experi- ments, the apparatus was so arranged that a changeover from constant pressure to constant flow, or vice versa, could be achieved within seconds. A number of experiments were performed to study the effects of only pump perfusion of the hindlimb and electric stimulation of nerve and muscle. These are presented in Results.

Calculations

In the studies done at constant flow, changes in pressure reflect changes in the vascular resistance of the perfused limb. To permit comparison of sympathetically in- duced changes in resistance at rest with those during exercise, the results have been ex- pressed as percent change in resistance (which, under conditions of constant flow, has the same value as percent change in pres- sure). The same percent reduction in cir- cumference (and therefore in radius) of the resistance vessels when the limb was resting.
and when it was exercising would result in
the same percent change in resistance (or
pressure) despite the widely different flows.
Conversely, widely different values for abso-
lute increase in peripheral resistance would
be obtained for the resting and exercising
states, owing to the widely different initial
resistance values, even if the percent change
in vessel circumference (and therefore pre-
sumably in the length of the contractile ele-
ment) was the same in the two situations. At
constant flow, the percent increase in resis-
tance (ΔR%) is given by:

$$ΔR% = \frac{(P_{max} - P_o) \times 100}{P_o}$$

in which ΔP% = percent increase in pressure;
P₀ = initial perfusing pressure before sympa-
thetic stimulus; and P max = maximal perfusing
pressure during sympathetic stimulus.

The results of the experiments performed
at constant pressure have not been expressed
as ΔR% for two reasons. (1) It was occasional-
ly possible to stop flow completely during
sympathetic stimulation, giving an infinite re-
sistance which could not be handled with the
other data. (2) To calculate ΔR% would have
required the assumption that the pressure-
flow curve for the vascular bed was strictly
linear. In this situation, therefore, the changes
have been expressed as percent change in
flow (ΔF%)

$$ΔF% = \frac{(F_0 - F_{min}) \times 100}{F_0}$$

in which F₀ = initial flow before sympathetic
stimulus; and F min = minimal flow during
sympathetic stimulus.

Results

Stability of Preparation.—Since most of the
experiments took 2 to 5 hours to complete, it
was necessary to discover if the response of
the limb resistance vessels to sympathetic
stimulation changed during this period. In
two dogs, F-R curves were plotted repeated-
ly, under resting conditions, over 4 to 5 hours.
The change in the F-R curve with time in
these two dogs was minimal and consisted of

a slight reduction in the response at the
higher frequencies.

There were thus no gross changes in sen-
sitivity over several hours of perfusion. How-
ever, in three animals there were spontaneous
changes in the perfusion pressure (and there-
fore in the resistance) of the perfused limb.
If the prestimulation perfusion pressure of
the limb did not remain within ±10 mm Hg
from the chosen starting pressure, it became
impossible to analyze the results because re-
sponses to different frequencies of stimulation
would be measured at different initial resis-
tances and pressures. It was not considered
adequate to adjust flow to produce identical
starting pressures because this would not
compensate for differences in the resistance.
If stability of the starting pressure within
the above limits could not be achieved, the data
were discarded.

Effect of Anesthesia.—The effect of the
additional doses of anesthetic (80 to 120
mg of sodium pentobarbital) on the response
to sympathetic stimulation was studied eight
times in four dogs. The additional doses re-
sulted in a mean decrease in response of 16%
(rang 0 to 29%), and this lasted for a mean
period of 15 minutes (range, 0 to 22 min-
utes). Therefore, supplementary doses of

![Graph showing vasoconstrictive response to graded stimulation of lumbar trunk at rest (o) and during simulated exercise (x) in a dog's hindlimb perfused at constant flow. Resting and recovery flow, 60 ml/min; exercise flow, 215 ml/min; control mean perfusion pressure, 80 mm Hg in each situation. Recovery responses (x) were obtained between 7 and 30 minutes from end of exercise.](http://circres.ahajournals.org/)}
sodium pentobarbital were kept to a minimum, and they were well diluted and given slowly. Test sympathetic stimulations were made before and after any such additional doses of anesthetic.

Frequency-Response Curves in Resting and in Exercising Limbs.—Figure 1 shows typical F-R curves in a limb at rest and during exercise. The general features of the curve for the resting state were similar to those described many times and for various sympathetically innervated tissues. A small but definite response could generally be obtained at 0.2 cps. With increase in frequency, there was a rapid increase in response, generally reaching 80% of maximum at about 4 cps. The increase in the response was considerably smaller as frequency was increased above this rate. The F-R curve in the exercising limb differed, generally showing a smaller response to the lowest frequencies of stimulation and a larger response to all other frequencies. These differences between the F-R curves of exercise and those of rest were seen in all 33 experiments.

The changes in the form of the F-R curve concurrent with induced muscular activity in the limb were reversible, as shown by the curve obtained at rest after a period of exercise (Fig. 1). However, the time course of the recovery varied considerably from one animal to another and, at times, in the same animal. Usually, measurements taken 20 minutes after a period of exercise gave F-R curves very similar to the initial resting curves. Occasionally, the general features of the F-R curve during exercise persisted for as long as measurements were made (up to 70 minutes) after the end of an exercise period, despite restoration of the flow and pressure to values very close to those before exercise.

Time-Dependent Change in Frequency-Response Curves during Exercise.—The exercise curve shown in Figure 1 includes no points resulting from measurements made during the first 25 minutes of exercise because, despite an apparently steady level of exercise (as evidenced by the unchanged muscle stimulus, flow rate, and perfusion pressure), the response to a given sympathetic stimulus did not become stable for 25 to 30 minutes. In nearly all experiments, the response increased appreciably between 10 and 30 minutes of exercise. This increase in response was not uniform at all frequencies; thus, F-R curves obtained during the first 10 minutes of exercise differed markedly in shape from those made later. Examples of early and late F-R curves are shown in Figure 2. At the higher frequencies (to the right of line B in Fig. 2) the response early in exercise was greater than that at rest and there was only a small further increase with continued exercise. At medium frequencies (between lines A and B) the initial exercise response was less than at rest, but a substantial time-dependent increase occurred, and the final response was greater than in the resting state. At the lowest frequencies (to the left of line A) the response early in exercise was much less than in the resting state, and a small time-dependent increase occurred, but the response during exercise at no time reached the level of that achieved at rest.
In practice, to determine when the response had stabilized, an intermediate frequency was chosen (usually between 2 and 8 cps), and the response to this frequency was observed at intervals of 4 to 5 minutes from the onset of exercise. When the response to this frequency was stable with respect to time, the response to other frequencies was determined. Figure 3 is a reproduction of actual tracings obtained during an experiment and illustrates such a time-dependent change in response to the same stimulus during continued steady exercise. In general, no appreciable change from the initial exercise response occurred in the first 10 minutes. Rapid changes over the next 10 to 30 minutes consisted of a small or large increase in the magnitude of the response. Reference to Figure 2 will indicate that, in each experiment, the magnitude of the change in response depended mainly on the test frequency selected. Usually, after 30 minutes no further systematic changes in the magnitude of the response occurred during continued exercise up to 90 minutes. Once or twice, however, at the higher flows used, increases in the response to a given stimulus continued for up to 50 minutes.

In the remainder of this report, all results described as being obtained during exercise refer to results after stability of response had been demonstrated, unless otherwise stated.

Relationship of Frequency-Response Curves during Exercise to Degree of Vasodilatation Induced.—The extent of the difference between the F-R curve for the exercising limb and that for the resting limb was related to the degree of vasodilatation induced. Figure 4 shows mean curves for four ranges of exercise. The higher the flow achieved, the more nearly linear was the F-R curve during

![Graph of STIMULUS FREQUENCY 6/SECOND](chart_url)

Reproduction of tracings obtained during experiment using dog's hindlimb perfused at constant flow. Pressure and electromagnetic flowmeter tracings have been erased for the sake of clarity. Vasoconstrictive responses to lumbar trunk stimulation at 6 cps were recorded at rest, at intervals during 65 minutes of exercise, and 10 minutes after termination of exercise. Perfusion flows at rest, during exercise, and recovery were 84, 285, and 78 ml/min, respectively.
exercise. The higher flows were thus associated with greater responses at the higher frequencies but lesser responses at the lower frequencies.

Repeated Sympathetic Stimulation. Since it was necessary (because of the time-dependent change) to apply a sympathetic stimulus every 4 to 5 minutes during exercise, it was conceivable that the increased responses later in exercise (and therefore the form of the "stable" F-R curve in the exercising state) were dependent on the previous repeated stimulation. To test this, in two dogs both hindlimbs were perfused; muscle electrodes were placed in both hindlimbs and stimulating electrodes on both lumbar sympathetic trunks. F-R curves were first obtained with both limbs at rest. Exercise was commenced in both limbs simultaneously, and the muscle stimuli and the induced flows were similar in the two limbs. On one side, a repeated lumbar sympathetic stimulus was applied in the usual way to determine when the response became stable. During this initial period, no sympathetic stimuli were applied to the contralateral sympathetic trunk. When a stable response had been demonstrated on one side, F-R curves for the exercising state were determined simultaneously for both sides. The results were identical for the two sides.

Effect of Muscle Electrodes and Stimulation. It was conceivable that the electrical stimulation used to activate the skeletal muscle was augmenting the effect of the sympathetic drive to the vascular smooth muscle by stimulating the latter directly. Furthermore, in all the experiments at least one "muscle" electrode was positioned close to the sciatic nerve, which carries 90% of the sympathetic outflow to the vascular bed of the hindlimb (7). It was therefore possible that the stimuli used to effect muscular contraction were also inducing activity in the sympathetic component of the sciatic nerve. In two experiments, the same stimulus was applied to the muscle electrodes after administration of a neuromuscular blocking agent (gallamine triethiodide [Flaxedil], 5 mg/kg) in a limb perfused at constant flow. There was no significant change in perfusion pressure, and muscular activity was reduced to small fibrillar movements confined to the immediate neighborhood of the muscle electrodes. In addition, the form of the F-R curve during stimulation of the muscle after neuromuscular blockade was indistinguishable from that in the resting state.

These experiments demonstrated that (1) practically all of the muscular activity had been induced via the motor nerves rather than directly via the muscle; (2) there was no significant contribution to the vascular response as a result of direct electric stimulation of the vascular smooth muscle; and (3) no appreciable stimulation of sympathetic vasoconstrictor fibers in the sciatic or femoral nerve was being produced from the muscle electrodes.

Role of Skin Circulation. Since there are appreciable differences in behavior and control between skeletal muscle vessels and skin vessels, it was necessary to determine whether the change in F-R curves during exercise was due to changes in the cutaneous vascular bed.
In two animals, the hindlimb was completely skinned; F-R curves from these animals showed differences between the exercising and resting states which were indistinguishable from those shown in the animals with an intact skin circulation.

Role of Circulating Vasoactive Substances during Simulated Exercise.—To investigate the possibility that the simulated exercise of these experiments resulted in the release of vasoactive substances into the arterial circulation, in two dogs both hindlimbs were perfused, and muscular exercise was simulated in one hindlimb. If induced exercise reflexly resulted in the release into the arterial circulation of vasoactive substances (such as catecholamines), then these substances should reach both the exercising and the resting hindlimb in the same blood concentration. Any potentiation of the vasoconstrictor response to lumbar sympathetic stimulation ought therefore to be equally apparent on each side. The results from the two dogs are shown in Figure 5. With both hindlimbs perfused in the resting state, graded stimuli were simultaneously applied to each lumbar trunk and the vasoconstrictive responses were recorded. In dog A, F-R curves for the two limbs at rest differed in magnitude and shape, but in dog B they were similar. Exercise was then simulated in the left limb while the right remained at rest. Again, graded stimuli...
Sympathetic vasoconstrictive responses to graded stimulation of lumbar sympathetic trunk during constant-flow perfusion of dogs' hindlimbs. Composition of arterial blood perfusing the limb was altered by ventilating the animal with different gas mixtures: Left, 95% O₂-5% CO₂; Right, 85% O₂-15% CO₂. Note similarity of vasoconstrictive responses in the control (o) and test (x) states despite wide differences in venous pH and Pco₂.

Also, shown in right panel for comparison is F-R curve obtained during exercise (•) of limb after recovery from previous test. Vasoconstrictive responses during exercise are greater, although pH and Pco₂ are similar to those induced by breathing 85% O₂-15% CO₂.

Frequency-Response Curves during Drug-Induced Vasodilatation.—In seven experiments in seven dogs, ATP was infused into the resting hindlimbs to produce vasodilatation comparable to that during muscular

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**SYMPATHETIC VASOCONSTRICTION IN EXERCISE**

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**FIGURE 6**

Sympathetic vasoconstrictive responses to graded stimulation of lumbar sympathetic trunk during constant-flow perfusion of dogs' hindlimbs. Composition of arterial blood perfusing the limb was altered by ventilating the animal with different gas mixtures: Left, 95% O₂-5% CO₂; Right, 85% O₂-15% CO₂. Note similarity of vasoconstrictive responses in the control (o) and test (x) states despite wide differences in venous pH and Pco₂. Also, shown in right panel for comparison is F-R curve obtained during exercise (•) of limb after recovery from previous test. Vasoconstrictive responses during exercise are greater, although pH and Pco₂ are similar to those induced by breathing 85% O₂-15% CO₂.
Figure 7
Vasokonstrictive responses to stimulation of lumbar sympathetic trunk at rest (○) and during vasodilatation (●,●) induced by infusion of ATP (triangles) and by exercise (circles) in dogs' hindlimbs perfused at constant flow. Left, time-dependent change in vasokonstrictive response to stimulation of lumbar trunk at 4 cps; ATP and exercise flow, each 240 ml/min. Note stable response after 30 minutes of vascular dilatation in each situation. Right, F-R curves at rest and during exercise (flow, 230 ml/min) and ATP infusion (flow, 235 ml/min). Curves were recorded after response to test frequency had become stable with respect to time.

Figure 8
Sympathetic vasokonstrictive responses to graded stimulation of lumbar sympathetic trunk in control state and during induced vascular dilatation in dogs' hindlimbs perfused at constant flow. Left, mean (±SE) responses in five dogs in which ATP was used. Average flow during vasodilatation was 250 ml/min (range, 230 to 250 ml/min). Right, mean responses from similar series of experiments in five dogs in which acetylcholine was used. Average flow during vascular dilatation was 325 ml/min (range, 230 to 400 ml/min).

After exercise. Once the desired increase in flow had been attained, the infusion rate was adjusted during the procedure to maintain the perfusion pressure constant prior to stimulation of the lumbar sympathetic trunk. F-R curves for sympathetic stimulation were plotted for the resting, nondilated state and for the resting, dilated state. Considerable difficulty was experienced in maintaining a stable baseline (the same degree of vasodilatation over 30 to 60 minutes) during ATP infusion. This contrasted with the relative ease with which this could be achieved by simulated exercise. In two dogs it was completely impossible to keep the perfusion pressure within reasonable limits (∆ 10 mm Hg).
during the ATP infusion, and the results from these dogs have been discarded. In Figure 7 are shown the results from experiments in two of the remaining five dogs in which, in the same limb, vasodilatation of equal magnitude was induced by the infusion of ATP and by exercise. The vasconstrictive responses in the two states are similar both in the change with time in the response to a given stimulus (dog A) and in the relationship of the F-R curve in the resting state and the vasodilated state (dog B). The mean results (± SE) for the five dogs are plotted in Figure 8. The F-R curve in the dilated state resembles that obtained during exercise, but the increases from the F-R curve for the resting, nondilated limb are not as gross as those induced by exercise.

In five experiments on five dogs, acetylcholine was used as the vasodilator and atropine was withheld. There was some difficulty in maintaining a stable dilatation, but this was appreciably less than with ATP. The resulting F-R curves (Fig. 8) show the same general features as those obtained with ATP and during exercise. However, the increases in response from control values at the higher intensities of stimulation were not so pronounced as in the studies with ATP and during exercise. Time-dependent changes similar to but less marked than those of exercise occurred in three of five experiments with ATP-induced vasodilatation and in one of five experiments with acetylcholine-induced vasodilatation.

Response to Carotid Sinus Stimulation in Resting and Exercising Limbs.—Since the technique was not adequate to provide reproducible, graded stimuli, a single degree of stimulation was used on each occasion. The carotid sinus pressure was held at 200 mm Hg, independent of the central aortic pressure. This produced maximal inhibition of the central vasconstrictive drive to the two perfused limbs. The carotid sinus pressure was then decreased abruptly to 30 mm Hg and held there for 1.5 minutes. Several stimuli were applied in the resting state. Exercise was then commenced in the hind-limb in the usual manner and the responses in the resting and the exercising limb to the same change in carotid sinus pressure were observed at intervals during the exercise. Because the sensitivity to a given electrical stimulus applied to the lumbar sympathetic trunk did not change in a resting limb during exercise of another limb, it could be assumed that, as long as the response to carotid sinus stimulation in the resting limb remained unaltered, the drive from this stimulus to the exercising limb would presumably also be unaltered.

In two dogs, carotid sinus hypotension resulted in increases in hindlimb perfusion pressure of 50 and 60 mm Hg in the resting control state and of 100 and 160 mm Hg during exercise. The simultaneous increases...
study, constant-flow perfusion has been used to compare the response of vascular smooth muscle in the resting and exercising states, and constant-pressure perfusion has been used to show the net result of these and other changes.

Throughout the investigation, no frequencies of sympathetic stimulation higher than 16 cps were used because it is well established that maximal responses are achieved at or below this frequency and because in many cases mean arterial pressures of 300 mm Hg or higher were achieved at or below this frequency, especially in the exercising state, and it was thought unwise to expose the vessels to higher pressures.

The evidence of the present study indicates that the vascular smooth muscle of the resistance vessels in the canine limb is capable of developing a greater tension during exercise of the limb than during rest. This appears to be true whether the cause is electrical stimulation of the lumbar sympathetic trunk, central vasoconstrictive drive produced by carotid sinus hypotension, or intra-arterial infusion of norepinephrine. Furthermore, there is good evidence both from individual animals and from the grouped results from 22 animals (Fig. 4) that this is more marked at the higher levels of exercise. Working with isolated strips of arteries, Speden and later Sparks and Bohr showed that the isometric contraction from a standard stimulus (norepinephrine or electricity) increased with stretch of the arterial strip.

It could be argued that, if the augmented response during exercise is due solely to the increased length of the vascular smooth muscle elements, then augmentation ought to be even more apparent during drug-induced vasodilatation in which the perfusion-metabolism ratio is unusually high and the influence of products of metabolism should be minimal. However, it should be stressed that difficulty was experienced in maintaining a steady state of dilatation (especially with ATP), and neither ATP nor acetylcholine precisely mimicked the peripheral vascular changes of exercise.

If vascular smooth muscle can develop greater tension in response to certain frequencies of lumbar trunk stimulation during the dilatation caused by exercise than it can at rest, the following questions arise: (1) Why is the response to low frequencies of stimulation less during exercise than at rest? (2) What is the nature of the time-dependent change in sensitivity observed early in exercise? It is important to note that even in the absence of a time-dependent change (F-R curves during the first 10 minutes of exercise) the vasoconstrictive response to higher frequencies of stimulation was greater during exercise. It is therefore suggested that the ability of the vascular smooth muscle to develop tension is greater throughout the exercise period but some other factor tending to depress the response is initially present and then becomes less important after 10 to 20 minutes of continued exercise. Some idea of the possible nature of this factor can be gained by comparing the exercise response to lumbar trunk stimulation with that to infusion of norepinephrine. The main differences are that when norepinephrine is the stimulus, the vascular response is greater at all levels of stimulation and no time-dependent change is seen. Since there seems to be no reason to believe that the amount of norepinephrine liberated per nerve stimulus at any given frequency is different during the simulated exercise than it is at rest (and this is further supported by the observations in resting limbs during exercise in other limbs when changes in carotid sinus pressure are the stimulus), it is suggested that the locus of the time-dependent change may lie between the sympathetic nerve ending and its receptor site.

De la Lande and colleagues concluded from a study of the responses of rabbit ear vessels to intraluminal and extraluminal norepinephrine that the smaller response to extraluminal norepinephrine was due to a loss of the drug by uptake into storage sites in the sympathetic nerve endings as the norepinephrine diffused from adventitia to media. Thus the difference be-
in pressure in the nonexercising forelimbs remained close to the control values of 43 and 55 mm Hg. In two dogs, the reflex increase in perfusion pressure in each limb in the resting state averaged 80 mm Hg. During exercise, in each dog there was an initial decrease in the magnitude of the reflex pressure increase, to 46 and 44 mm Hg. As exercise continued, the response in the exercising limbs increased and stabilized at 87 and 67 mm Hg. The simultaneous reflex responses in the nonexercising limbs remained around 80 mm Hg. In one dog, the average reflex increase in pressure in the resting forelimb and hindlimb was 80 and 45 mm Hg, respectively. The initial response in the exercising hindlimb was 30 mm Hg; it increased to 60 mm Hg after 10 minutes of exercise and remained at about this value. In the nonexercising forelimb the simultaneous reflex increase in perfusion pressure was also less than in the control state and averaged 57 mm Hg during the period of hindlimb exercise. Figure 9 shows the result of one experiment in which the exercising limb showed a major increase in response to vasoconstrictive drive.

Response to Infusion of 1-Norepinephrine.—Four experiments were performed in three animals with intra-arterial infusion of 1-norepinephrine as the vasoconstrictive stimulus. During exercise the infusion rate was increased so that the same calculated blood concentrations of norepinephrine reached the perfused limb in the exercising and in the nonexercising forelimb.
resting states. Figure 10 shows the results expressed as the mean (± SE) of the four experiments. At all concentrations of noradrenaline used, the response was greater in the exercising than in the resting limb. No time-dependent change in the response was seen.

Changes in F-R Curves during Exercise and with Constant-Pressure Perfusion.—The mean (± SE) of the F-R curves obtained at rest and during exercise in 11 dogs studied under conditions of constant-pressure perfusion is shown in Figure 11. The results differed from those obtained during constant-flow perfusion; even at the highest frequencies of stimulation, the vasoconstrictive response during exercise rarely exceeded that observed in the resting state. Although the curves for the resting and the exercising state are similar in shape to the corresponding curves at constant flow, the maximal response to sympathetic stimulation in the exercising state approaches that in the resting state only at the highest frequencies used—the "crossover" point of F-R curves during exercise and those during rest is at a higher frequency under conditions of constant pressure than it is under conditions of constant flow. Time-dependent increases in the response to a given frequency were seen in 6 of 11 experiments, but the magnitude of these increases was less than in the constant-flow studies.

Discussion

In assessing the magnitude of vasodilative or vasoconstrictive responses to stimuli applied to a vascular bed, the function to be measured is resistance to flow. This can be measured at constant pressure, at constant flow, or in the "intact" preparation in which neither pressure nor flow remains constant. The third is the normal "physiologic" state and undoubtedly expresses the net result of the stimuli applied under the given conditions. Difficulty arises when an attempt is made to compare such responses in two different physiologic states (for example, rest and exercise). The use of a resistance estimate such as P/F—in which P is the driving pressure (arterial pressure minus venous pressure) and F is the flow—at widely different values for F assumes a linear pressure-flow relationship which is not found in practice except over small ranges of flow. The true resistance estimate required for a comparison of the efficacy of vasoconstrictive stimuli during rest and during exercise would be given by the slope of the tangent to the pressure-flow curve at the given values for P and F. This would present a complex and time-consuming technical problem, and most studies have simplified matters by making comparisons at constant P or at constant F. Each has its advantages and drawbacks, but it is important to realize that the two techniques do not always give the same results. When a constant-flow technique is used, vascular distensibility limits the response to vasoconstrictive drive. Because in these experiments the range of pressures covered was the same at rest and during exercise, the results in the two conditions would be directly comparable if vascular distensibility were the same. Folkow and Lofving (8) have shown that distensibility is actually increased during vasodilatation; despite this, pressure responses were greater during exercise than at rest in this study. When a constant-pressure technique is used, the response to the vasoconstrictive stimulus is a reduction in flow. The consequent decrease in the perfusion-metabolism ratio leads to the accumulation of vasodilative metabolites, which itself should tend to limit the vasoconstrictive response. In addition, a given percent reduction in flow is likely to result in a greater accumulation of metabolites during exercise than during rest, and thus in a greater limitation of response. The data from this study suggest that, at comparable levels of exercise, metabolic factors limit vasoconstrictive responses to a greater extent than does vascular distensibility. Burton and Stinson (9) have shown that when perfusion at constant flow is used, the increase in perfusion pressure in response to a vasoconstrictive drive is a very good measure of the increase in active tension of the vascular smooth muscle. In the present
between F-R curves during exercise and rest could lie in a change in the balance between release and inactivation of the neurogenically released norepinephrine. An increase in the rate of uptake of norepinephrine by the nerve, of inactivation by catechol-O-methyl transferase, or of discharge into the bloodstream due to the greater number of patent blood vessels and the reduced intercapillary distance would reduce the quantity of norepinephrine arriving at the receptor site. It is likely that the reduction would be relatively greater at the lower than at the higher frequencies of stimulation. However, this reduction would be offset by the increase in response due to an increase in the length of smooth muscle fiber during vasodilatation, and the final F-R curve would be the result of these two antagonistic processes.

Another possibility might be a disturbance of the spatial relationships between sympathetic nerve endings and vascular smooth muscle. Electron microscopic studies (14, 15) have revealed areas of relatively close proximity of adrenergic nerve axons and smooth muscle, with interdigitating of their processes. While these cannot definitely be identified as receptor sites, no areas of closer apposition of nerve and muscle have been seen. Even at these sites, the minimal distance separating nerve and muscle appears to be of the order of 900 A, roughly three times greater than equivalent distances for junctions of motor nerve and striated muscle. If this is so, then the diffusion distance and the volume of fluid between adrenergic nerve and smooth muscle could be important in determining response to a given nerve stimulation. Alternatively, since adrenergic fibers are not seen to pass deep into the smooth muscle coat when it has three or four layers, conduction of the "stimulus" from superficial to deep muscle fibers could be similarly affected. It is conceivable that with the onset of exercise the intimate relationship of nerve and muscle is disturbed initially but later returns toward normal. This could cause an initial depression in response to nerve stimuli at all but the most potent levels (frequencies) of stimulation, whereas the response to intra-arterial norepinephrine (when the tissues are allowed to equilibrate to a given concentration of norepinephrine) would be constant with respect to time.

Whatever the mechanism of the time-dependent change, the fact that it occurs and that its effect is not uniform at all frequencies means that, in any comparison of the effects of sympathetic vasoconstrictive stimuli in the exercising and in the resting limb under the conditions of the present experiments, the frequency of stimulation and the duration of exercise both must be considered.

Time-dependent changes were seen under constant-pressure conditions in 6 of 11 experiments but were smaller and less constantly seen than under constant-flow conditions. Thus, during constant-pressure perfusion, the F-R curve made early in the exercise period was almost linear but had a slight convexity to the abscissa (frequency). The stable F-R curve, although similarly almost linear, had a slight concavity to the abscissa. The mean result of 11 experiments, as shown in Figure 11, leaves no doubt that under conditions of constant-pressure perfusion the overall effect of exercise on the vasoconstrictive response to sympathetic drive was one of depression. This is in keeping with the previous observations of Remensnyder and associates (4) and Kjellmer (5). However, the present studies demonstrate that sympathetic vasoconstrictive responses of considerable magnitude can be obtained during exercise, particularly at higher intensities of stimulation. The difference in F-R curves during exercise at constant pressure (generally depressed compared to the resting curve) and at constant flow (generally augmented compared to the resting curve) must be accounted for by the strong negative feedback produced by the rapid decrease in the perfusion-metabolism ratio during the sympathetic stimulation in the former situation, because conditions before the stimulus are similar, whichever perfusion technique is used. This was confirmed in those animals in which the experimental technique permitted rapid interchange.
between constant-flow and constant-pressure perfusion. It is of interest to observe that the shapes of the F-R curves in the resting and exercising states are similar in both perfusion systems, although the points at which exercise and rest curves cross are quite different.

Therefore, even under constant-pressure conditions the roughly linear shape of the curve in the exercising state as opposed to the roughly hyperbolic shape in the resting state may have implications in the integrated control of the circulation during exercise. Thus, not only could the same central sympathetic drive result in maximal flow reduction in resting muscle beds and much less in exercising areas, but also, if a situation arises in which the central demand of the whole organism temporarily becomes more important than the local needs of the exercising tissue, an increase in the frequency of sympathetic stimulation would produce a graded (almost linear) response in the exercising muscle bed while maintaining almost total response in the resting muscle bed.

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DEREK J. ROWLANDS and DAVID E. DONALD

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