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AUTOREGULATION OF HIND-LIMB BLOOD FLOW IN CONSCIOUS DOGS

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SUMMARY

1. We evaluated the efficiency of blood flow autoregulation of the hind-limb vascular bed of eleven conscious dogs during: (a) resting conditions; (b) graded levels of treadmill exercise; and (c) increases in oxygen consumption produced by the administration of 2,4-dinitrophenol (DNP).

2. Blood flow to the left hind limb was measured with an electromagnetic flow probe on the left external iliac artery. Hind-limb perfusion pressure was measured from a catheter in the deep femoral artery and was controlled via an externally inflatable occlusion cuff positioned just distal to the flow probe. Arterial pressure was measured in the abdominal aorta. Experiments were performed 5–16 days after instrumentation. Hind-limb pressure–flow (*P–F*) relationships were evaluated by decreasing hind-limb perfusion pressure in 4–5 small sequential 'square-wave' steps of 10–15 mmHg each while measuring flow. Each step decrease in perfusion pressure was maintained for 2 min.

3. The efficiency of autoregulation was quantified by calculating the closed-loop gain of flow regulation (G_c) at each decrement in perfusion pressure utilizing the equation: $G_c = 1-[(F_0-F_n/F_0)/(P_0-P_n/P_0)]$ where F_0 and P_0 are the starting (control) flows and pressures prevailing prior to decreasing perfusion pressure, and F_n and P_n are the new flows and pressures at each decrement in perfusion pressure. A G_c value < 0 indicates a predominantly passive *P–F* relationship, while a G_c of 1 is perfect autoregulation of flow.

4. When the dogs were at rest, decrements in hind-limb perfusion pressure were accompanied by almost equivalent decreases in flow, i.e. no autoregulation occurred, and G_c averaged $-0{\cdot}177 \pm 0{\cdot}044$ over the pressure range from 100–40 mmHg. During all levels of treadmill exercise (on gradients of 0, 7, or 21 %), however, positive G_c values were found that averaged from $0{\cdot}258 \pm 0{\cdot}046$ at a gradient of 0 % to $0{\cdot}392 \pm 0{\cdot}041$ at a gradient of 21 % and were significantly different from G_c values found during rest at perfusion pressure ranges from 90–40 mmHg.

5. The administration of DNP directly into the hind-limb circulation increased hind-limb blood flow from 199 to 481 ml/min. In the presence of DNP, G_c values were positive over perfusion pressure ranges from 100–40 mmHg and averaged $0{\cdot}473 \pm 0{\cdot}054$.

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6. These data demonstrate that hind-limb blood flow is not autoregulated in resting dogs, but that significant autoregulation is manifest during conditions that increase oxygen consumption. These findings support the general contention that hind-limb blood flow is not only regulated in accordance with tissue metabolic demands, but that the precision of blood flow control is also influenced by the metabolic state of the tissue.

INTRODUCTION

Autoregulation is the intrinsic ability of an organ to maintain a relatively constant blood flow over a range of perfusion pressures via local active changes in vascular resistance to flow; it has been observed to some degree in virtually all tissues and organs of the body (Johnson, 1964). Interest in skeletal muscle blood flow autoregulation and its mechanisms has grown continuously since the early observations of Roy & Brown (1879) and Bayliss (1902) because it is hypothesized that autoregulation is one of the mechanisms that operates to match nutrient delivery with tissue metabolic demands (Granger, Goodman & Cook, 1975). Autoregulation has also been implicated in the genesis of hypertension (Arendshorst, 1979; Coleman, Samar & Murphy, 1979).

The pressure–flow (*P–F*) relationship of the hind-limb vascular bed of anaesthetized animals has been described as passive, essentially rigid, or autoregulatory (Jones & Berne, 1964*b*). The reason different investigators have arrived at different conclusions is not entirely clear, but several studies have provided insight into predicting conditions during which autoregulatory behaviour predominates over passive behaviour. Data presented by Stainsby (1962), Jones & Berne (1964*a*), and Goodman, Einstein & Granger (1978) demonstrate an enhancement of hind-limb skeletal muscle blood flow autoregulation during conditions that increase oxygen consumption, such as muscular contraction. These observations indirectly support a metabolic mechanism for autoregulation.

Despite its theorized central role in cardiovascular homoeostasis, no previous work concerning autoregulation of skeletal muscle or hind-limb blood flow has been performed in conscious animals. Anaesthetic agents are known to alter many aspects of cellular metabolism and function (Bunker & Vandam, 1965), and to alter markedly cardiovascular regulatory systems (Altura, Altura, Carella, Turlapaty & Weinberg, 1980). Therefore, we deemed it important to determine conditions during which autoregulation of the hind-limb vascular bed of conscious dogs occurs in the absence of the modifying influences of anaesthetic agents. In the present study, we evaluated the ability of the hind-limb circulation of conscious dogs to autoregulate during: (1) resting conditions; (2) graded levels of treadmill exercise; and (3) increases in oxygen consumption produced by the administration of 2,4-dinitrophenol. Our data demonstrate that the hind-limb vascular bed *P–F* relationship is passive in dogs at rest, but that autoregulatory behaviour predominates when oxygen consumption is increased.

METHODS

Experiments were performed on eleven female mongrel dogs (body wt. 13–18 kg) selected according to their ability and willingness to run untethered on a treadmill. Dogs unable to run freely by the third day of coaching (five out of sixteen dogs) were rejected. The dogs were trained for 10 days prior to surgery to stand quietly on a treadmill for 1 h and to run on a treadmill at speeds of 3 and 5·5 km/h on gradients of 0, 7, and 21 %.

For surgery, the dogs were anaesthetized with sodium pentobarbitone (30 mg/kg, I.V.), intubated, and mechanically ventilated. Access to the blood vessels supplying the left hind limb was made through a mid-line abdominal incision. Sterile technique was observed and sharp dissection was used throughout the operation. A non-cannulating electromagnetic flow probe transducer (Carolina Medical Electronics, Inc., U.S.A.) was positioned around the left external iliac artery close to its origin from the aorta. An externally inflatable occlusion cuff, of the type described by Stephenson & Donald (1980), was implanted around the artery 1 cm distal to the flow probe. Arterial blood pressure was measured in the lower abdominal aorta through a catheter (0·078 in i.d. × 0·0125 in o.d.) inserted from the left internal iliac artery. Hind-limb perfusion pressure was measured from a catheter (0·040 in i.d. × 0·085 in o.d.) inserted into the deep femoral artery with its tip advanced to the junction of this artery with the external iliac artery. Hind-limb venous blood samples were obtained from a catheter (0·062 in i.d. × 0·095 in o.d.) inserted into the left deep circumflex iliac vein with its tip advanced 2 cm into the left external iliac vein. All catheters were made from Silastic tubing (Dow Corning, U.S.A.).

To eliminate as much collateral circulation to the left hind limb as possible, both circumflex iliac, the left umbilical, the left pudendoepigastric, and the median sacral arteries were ligated. Our intent was to isolate the inflow to the hind limb as completely as possible to minimize the effects of major collaterals.

All catheters, the occlusion cuff tubing, and the flow probe lead exited the abdominal cavity through the lower left quadrant of the abdominal wall and were routed subcutaneously to exit through the skin on the dorsum just caudal to the scapula and 3 cm to the left of mid line.

Each dog was administered 0·5 mg/kg of morphine sulphate every six hours for twenty-four hours after surgery to alleviate pain and one million units of Penicillin G daily for five days after surgery as a prophylactic measure. The catheters were flushed daily and filled with saline containing 1000 u./ml of heparin and 100000 u./ml of Penicillin G. The exteriorized ends of the catheters were closed with stainless-steel plugs. The skin wounds were washed daily with surgical scrub (Betadine) and treated with a topical antibacterial ointment (Neosporin). When the dogs were in their cages, the exteriorized flow probe connexion and catheters were protected by foam-rubber pads held in place with wide elastic bandages and each animal wore a canvas body jacket.

Zero blood flow base line was established at the beginning of each experiment and checked periodically by inflating the occlusion cuff with 12 lb/in^2 air pressure. Each flow probe was calibrated *in situ* at the completion of all experiments while the animals were anaesthetized. For calibration, the left external iliac artery was cannulated with a 3·8 mm i.d. Mayo iliac artery cannula (American V. Mueller, U.S.A.). This artery was then perfused using a calibrated controlled-flow roller pump (Sarns, U.S.A.) at flow rates up to 600 ml/min. The blood inflow to the pump was derived from a carotid artery. The relationship between the blood perfusion rate and the blood flow recording was linear at all flows tested.

Blood pressures were measured with Statham strain gauge transducers (P23dB) that were carried by the dogs in a canvas harness that was worn around the thorax. Zero pressure reference was taken at heart level. Heart rate was measured using a Beckman cardiotachograph that was triggered by an electrocardiographic signal. The signal from the flow probe on the external iliac artery was transduced with a Carolina flow meter (Carolina Medical Electronics, Inc., U.S.A.) and mean flow was recorded through a low-pass filter with a 1·2 s time constant. All variables were recorded on a Beckman R-611 polygraph.

Protocol: Experiments were performed on conscious dogs, 5–16 days after surgery. The following standard protocol was used to evaluate hind-limb *P–F* relationships during all conditions studied. Experiments were not started until all recorded variables were stable for at least 10 min. Zero flow base line was established during this stabilization period and hind-limb venous blood samples (1 ml) were withdrawn for blood gas and pH analysis. Perfusion pressure to the hind limb (measured via

the deep femoral artery) was then decreased from the base-line value in 4–5 small sequential 'square-wave' steps of 10–15 mmHg while measuring the flow. The starting pressures and flows were different for each animal studied and no attempt was made to decrease hind-limb perfusion pressure to any predetermined exact value. Hind-limb perfusion pressure was held constant at each new decrement for 2 min by manually adjusted, partial inflation of the occlusion cuff using a 10 ml plastic syringe. The flow value at each decrement in perfusion pressure was obtained from the most stable portion of the flow trace during the last 30 s of each pressure decrement. The lowest decrease in perfusion pressure was to approximately 35 mmHg. Negative pressure was then applied to the occlusion cuff to allow free perfusion of the hind limb. This standard protocol was used to evaluate hind-limb *P–F* relationships during rest and exercise on four separate mornings.

Hind-limb *P–F* relationships were first obtained while the dogs were standing quietly on the treadmill (resting conditions). After a 10 min recovery period, hind-limb *P–F* relationships were determined during three levels of treadmill exercise (gradient of 0 % at 3 km/h, gradient of 7 % at 5·5 km/h, and a gradient of 21 % at 5·5 km/h) that were always performed in the order of increasing severity. At each level of exercise, hind-limb venous blood samples were obtained after 5 min of exercise. Evaluation of *P–F* relationships was started after approximately 6 min of exercise when the hind-limb flow and perfusion pressure had obtained the new steady-state values. Approximately 10–15 min were required to record the *P–F* relationship at each level of exercise. Thus, each animal exercised for about 20 min at each exercise level. A 10 min rest period was allowed between the first two episodes of exercise. After exercise at the gradient of 21 %, each animal was given a 30 min rest. Venous blood samples were then withdrawn and the hind-limb *P–F* relationships determined by the standard protocol while the dogs stood quietly at rest on the treadmill (exercise recovery conditions).

Randomly, on either the second or third morning of study, the experiments were performed in six of the dogs after establishing blockade of autonomic nervous system ganglia using hexamethonium. Hexamethonium was administered intravenously at an initial dose of 10 mg/kg which was followed by a sustaining dose of 0·1 mg/kg . min. The adequacy of ganglionic blockade was shown by three observations: (1) the usual, immediate increase in heart rate accompanying the start of exercise was markedly attenuated; (2) the reflex bradycardia caused by intravenous injection of 0·5 mg of phenylephrine was abolished; and (3) the reflex tachycardia produced by intravenous injection of 0·5 mg of nitroglycerin was abolished.

On the last day (fourth) that the above rest–exercise–recovery protocol was performed, the dogs were returned to their cages for three hours and then studied in the late afternoon to evaluate the effects of an exercise independent increase in hind-limb oxygen consumption upon *P–F* relationships. After obtaining stable pressure and flow recordings, hind-limb venous blood samples were withdrawn for blood gas and pH analysis. While the dogs were standing at rest on the treadmill (control conditions), the standard protocol for evaluating hind-limb *P–F* relationships was performed twice with 10 min intervening between each episode. Then, to increase the rate of hind-limb oxygen consumption, 2,4-dinitrophenol (DNP) was administered as a constant infusion at a rate of 50 μmol/min via the catheter in the deep femoral artery. The infusion was maintained for 20 min or until body temperature had increased 2 °C, at which time a venous blood sample was obtained. With the dogs still standing at rest, the standard protocol for evaluating hind-limb *P–F* relationships was performed twice with a 10 min recovery between each episode.

Data Analyses. Data were analysed to determine if the efficiency of hind-limb blood flow autoregulation is changed by an increase in oxygen consumption produced by either treadmill exercise or the administration of DNP. To display graphically the *P–F* relationships, the prevailing hind-limb pressures and flows obtained during each condition of study (i.e. rest, exercise, exercise recovery, and DNP) were averaged for all trials from all dogs. In a similar manner, the pressures and flows obtained during each sequential decrement in hind-limb perfusion pressure, for each condition of study were averaged. These are shown in Figs. 2 and 4.

The efficiency of autoregulation was quantified by calculating the closed-loop gain of flow regulation at each decrement in perfusion pressure utilizing the equation:

$$G_c = 1 - \left[\frac{F_0 - F_n}{F_0} \Big/ \frac{P_0 - P_n}{P_0}\right]$$

where F_0 and P_0 are the starting (control) flows and pressures prevailing prior to the beginning of decreases in perfusion pressure, F_n and P_n are the new flows and pressures at each decrement in

perfusion pressure, and G_c is the closed-loop gain of flow regulation. If G_c is zero, flow varies proportionately with perfusion pressure and the net result of interacting factors results in behaviour typifying a rigid system. G_c values less than zero are indicative of predominately passive behaviour, while G_c values greater than zero indicate a predominately autoregulating vascular bed. If G_c is one, flow is independent of pressure indicating perfect efficiency of autoregulation; this method of calculating G_c is similar to that used previously by Morff & Granger (1982).

For comparison of the efficiency of autoregulation between the resting condition (designated as control) and the experimental situations (exercise and DNP), the data were collated and analysed within assigned ranges of perfusion pressure. The ranges of perfusion pressures chosen were $\geqslant 101$, 100–91, 90–81, 80–71, 70–61, 60–51 50–41, and $\leqslant 40$ mmHg. In this manner, *P–F* relationships between control and experimental situations could be evaluated at similar perfusion pressures in the absolute terms of mmHg without further data transformation.

G_c was calculated for each condition studied at each decrement in perfusion pressure and assigned to its appropriate perfusion pressure range. The gains obtained during rest (prior to exercise) within each range of perfusion pressures were compared statistically to the gains obtained during exercise (0, 7, and 21 %) and the recovery period within the corresponding range of perfusion pressure using the Dunnett test for multiple comparisons with a control (Dunnett, 1955; Zar, 1974). For example, if the starting perfusion pressure during exercise at a gradient of 7 % was 108 mmHg and the first controlled decrement in pressure was to 95 mmHg, the gain calculated would contribute to the values within the range of 91–100 mmHg.

The data obtained during resting conditions just prior to the administration of DNP, served as control data for comparison to data obtained in the presence of DNP (experimental group). Student's *t* test for non-paired values (Zar, 1974) was used to evaluate possible statistical significance between control and experimental groups for the calculated gains of flow regulation within each assigned pressure range. Data are presented as mean values $\pm$ S.E. of mean. For all statistical analyses, *P* values of $< 0{\cdot}05$ were considered significant for differences.

RESULTS

Each of the eleven dogs studied developed minor subcutaneous swelling on each side of the mid-line abdominal incision that subsided several days after surgery. The dogs were otherwise in good health throughout the study and each was willing to run untethered on the treadmill by the second post-operative day. The dogs ate well, maintained their weight, and exhibited normal vigour. The rectal temperatures were between 37·9 and 39·6 °C, and no infections were encountered. In two of the dogs, data collection was interrupted prematurely because of rupture of the occlusion cuff. Post mortem *in situ* evaluation demonstrated that all other cuffs gave complete occlusion against perfusion pressures up to at least 250 mmHg. Post mortem examination also revealed that all major arteries of the instrumented left hind limb were fully patent and free of thrombi.

In conscious dogs standing at rest, a 30 s occlusive inflation of the cuff on the external iliac artery caused down-stream pressure (measured via deep femoral artery) to decrease to a plateau value of $8{\cdot}9 \pm 0{\cdot}8$ mmHg. Occlusion pressures this low demonstrate that little collateral flow reached the left hind-limb circulation (Green, Cosby & Radzow, 1944).

Fig. 1 depicts a stable polygraph tracing of hind-limb perfusion pressure and hind-limb blood flow obtained during rest (upper panel) and during exercise at a gradient of 7 % (5·5 km/h). During resting conditions, each controlled decrement in perfusion pressure was accompanied by an almost equivalent decrement in blood flow. This general pattern of passive *P–F* behaviour was typical of that which occurred in most experiments during the rest and recovery periods. During exercise, however,

flow was better maintained during decreases in perfusion pressure. The lower trace (Fig. 1) represents one of the more powerful examples of autoregulatory behaviour that occurred during exercise. During exercise, the initial (first 30 s) flow response to changes in perfusion pressure followed no consistent pattern even within a given animal for a particular condition. In some responses, flow decreased during the initial decrement in perfusion pressure and then increased to remain at a stable plateau

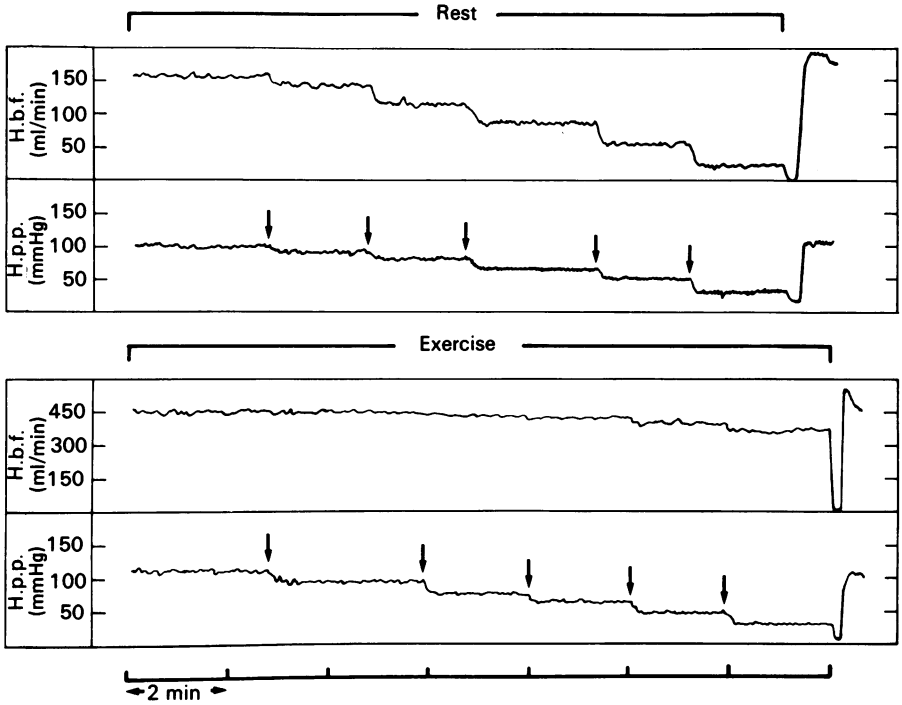

Fig. 1. Polygraph recording of changes in dog hind-limb blood flow (h.b.f.) during controlled decreases in hind-limb perfusion pressure (h.p.p.) while at rest (upper panel) and during treadmill exercise (lower panel) at a gradient of 7 %, 5·5 km/h. Each arrow indicates the initiation of a new decrease in perfusion pressure.

within 30–40 s. Other responses showed no initial decrease in flow, while in a few, flow actually increased subsequent to decreases in perfusion pressure. In general, no consistent initial pattern was revealed and the flows reported here were taken from the most stable portion of the flow trace recorded during the last 30 s of each 2 min pressure decrement.

Fig. 2 shows the hind-limb *P–F* relationships obtained while the dogs were standing on the treadmill at rest, during graded levels of treadmill exercise, and 30 min post exercise (recovery). During rest and recovery conditions, the *P–F* relationships were similar at all pressures tested and all points were below the line of isoresistance drawn from the starting pressures and flows to the origin of the graph. During exercise, however, the hind-limb *P–F* relationship was consistently above the line of isoresis-

tance. With increasing levels of exercise, the hind-limb *P–F* relationship became more concave to the pressure axis which suggests an increasing efficiency of autoregulation at the increasing levels of exertion.

The efficiency of autoregulation, i.e. G_c for each condition studied (rest, exercise at a gradient of 0 %, etc.) at each decrement in perfusion pressure is shown in Fig. 3. During resting conditions, G_c was negative (average $= -0{\cdot}177 \pm 0{\cdot}044$) within seven

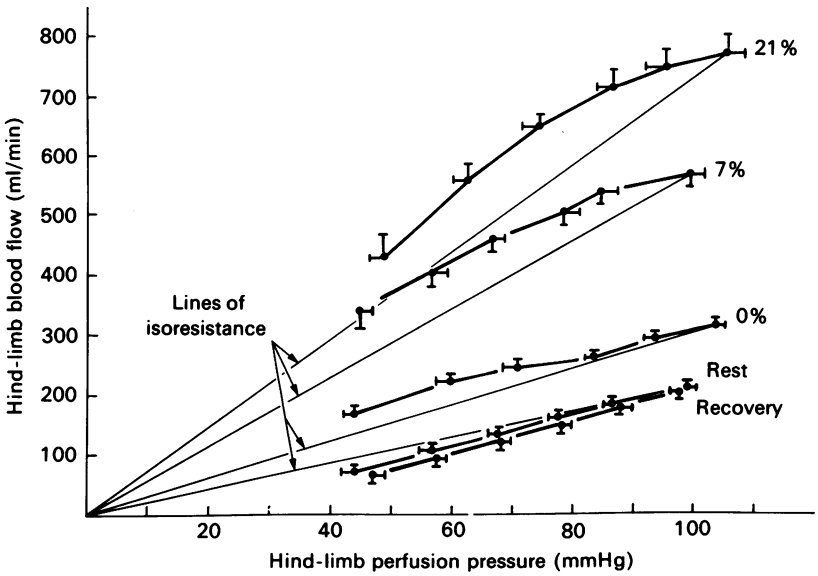

Fig. 2. Hind-limb pressure–flow (*P–F*) relationships obtained during rest, during treadmill exercise on gradients of 0, 7 and 21 % and after 30 min of rest following exercise (recovery). Each point represents the average *P* and *F* obtained at each sequential decrease in hind-limb perfusion pressure. The lines of isoresistance were drawn from the average *P–F* point that prevailed before starting the decreases in pressure.

of the eight pressure ranges. The G_c values obtained during the recovery period were consistently negative (average $= -0{\cdot}359 \pm 0{\cdot}038$) and were not different from the rest values within any pressure range (Dunnett test). During all levels of exercise, however, the G_c values obtained at perfusion pressure ranges from 41–50 mmHg to 91–100 mmHg were positive. The G_c values during exercise at a gradient of 0 % (average $G_c = 0{\cdot}258 \pm 0{\cdot}046$), 7 % (average $G_c = 0{\cdot}381 \pm 0{\cdot}032$), and 21 % (average $G_c = 0{\cdot}392 \pm 0{\cdot}041$) tended to decrease as the perfusion pressure decreased. During all levels of exercise, the G_c values obtained at perfusion pressure ranges from 41–50 mmHg to 81–90 mmHg were significantly greater than the G_c values obtained at rest (Dunnett test).

In the six dogs given hexamethonium, the pattern of autoregulatory efficiency was similar to that obtained in neurally intact animals. During rest conditions, the G_c value averaged from all pressure levels was $-0{\cdot}052 \pm 0{\cdot}112$ (number of observations, n, = 34) and became positive during all levels of exercise (0 % exercise

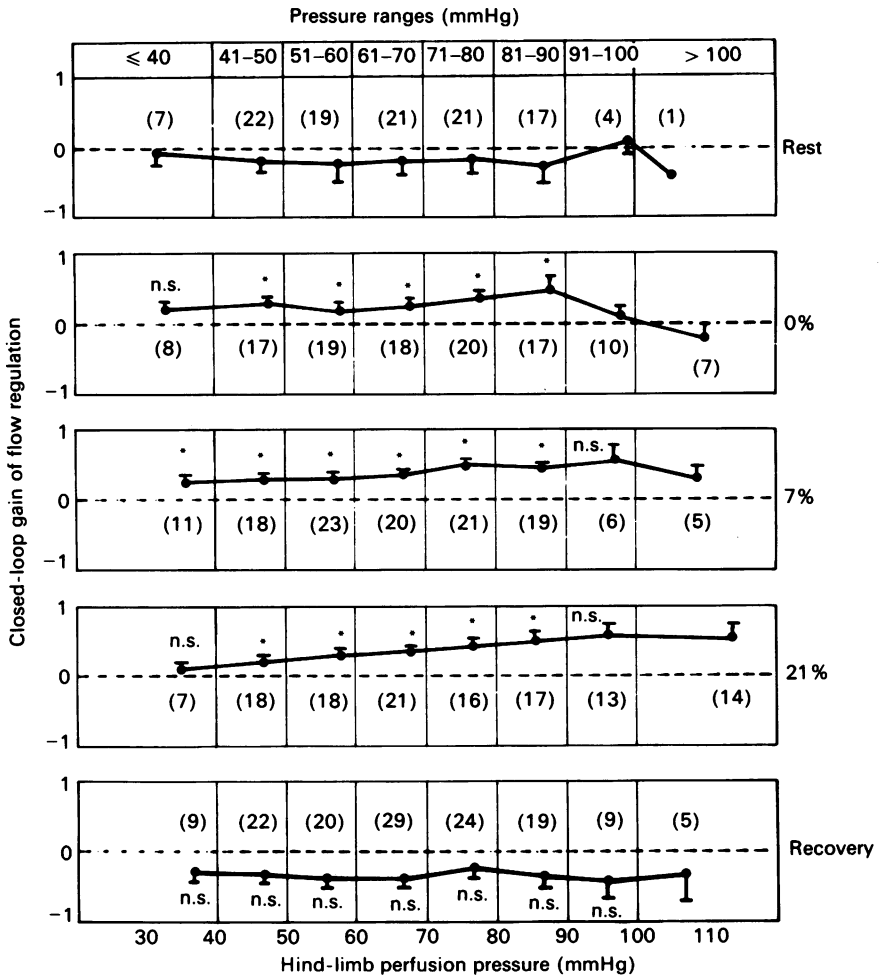

Fig. 3. Closed-loop gain of flow regulation during rest, during treadmill exercise on gradients of 0, 7 and 21 % and after 30 min of rest following exercise (recovery). Each point represents the average gain obtained within each pressure range shown at the top of the figure. Numbers in parentheses are the number of observations made within each pressure range for each condition studied. (*, $P < 0{\cdot}05$ from rest; n.s., not significant from rest.)

$G_c = 0{\cdot}504 \pm 0{\cdot}095$, $n = 45$; 7 % exercise $G_c = 0{\cdot}424 \pm 0{\cdot}104$, $n = 33$; 21 % exercise $G_c = 0.278 \pm 0{\cdot}100$, $n = 28$). The average G_c value during the recovery period in the presence of hexamethonium was $-0{\cdot}232 \pm 0{\cdot}091$, $n = 40$.

Fig. 4 shows the hind-limb *P–F* relationships before (control) and during the administration of DNP. DNP caused hind-limb blood flow to increase from 199 ± 14 ml/min to 481 ± 20 ml/min. DNP increased rectal temperature $2{\cdot}1 \pm 0{\cdot}1$ °C and caused obvious increases in ventilatory frequency. The control *P–F* relationship was below the line of isoresistance at all decrements of perfusion pressure. In the presence of DNP, the hind-limb *P–F* relationship was above the line of isoresistance at all decrements of perfusion pressure and became concave to the pressure axis.

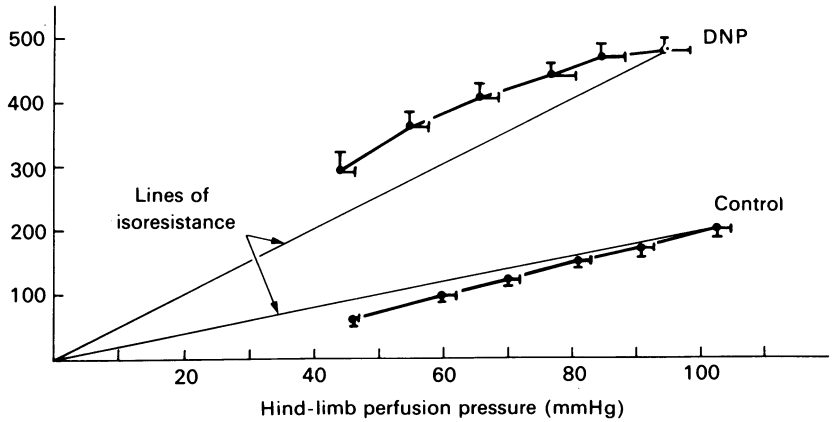

Fig. 4. Hind-limb *P–F* relationships obtained before (control) and during the administration of 2,4-dinitrophenol (DNP). Each point represents the average *P* and *F* obtained at each sequential decrease in hind-limb perfusion pressure. The lines of isoresistance were drawn from the average *P–F* point that prevailed before starting the decreases in pressure.

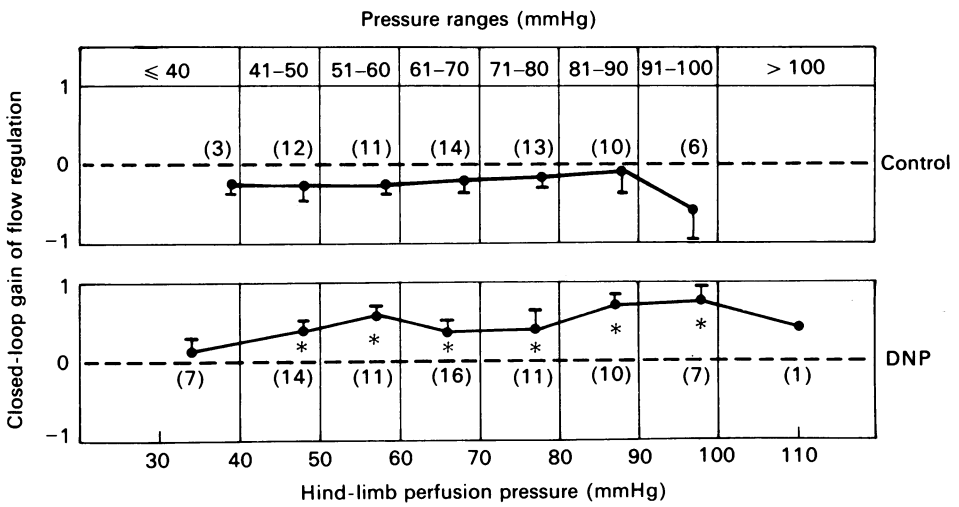

Fig. 5. Closed-loop gain of flow regulation before (control) and during the administration of 2,4-dinitrophenol (DNP). Each point represents the average gain obtained within each pressure range shown at the top of the Figure. Numbers in parentheses are the number of observations made within each pressure range for each condition represented. (*, $P < 0{\cdot}05$ for control *vs.* DNP.)

Fig. 5 shows the collated data from all nine animals that received DNP for hind-limb perfusion pressure *versus* G_c. The G_c values for the control data were negative (average $= -0{\cdot}201 \pm 0{\cdot}056$) at each pressure range. In the presence of DNP, the G_c values were positive within each pressure range (average $= 0{\cdot}473 \pm 0{\cdot}054$) and were significantly greater than the control G_c values over the six pressure ranges from 41–50 mmHg to 91–100 mmHg (Student's *t* test).

Fig. 6 shows the relationship between hind-limb venous blood P_{O_2} measured just prior to the first decrement in perfusion pressure and the G_c values obtained during the first step decrement in perfusion pressure for all conditions studied. This Figure demonstrates that the efficiency of autoregulation, as evaluated by G_c, increased as the hind-limb venous P_{O_2} values decreased. The G_c values of only the first step decrement in perfusion pressure were used in Fig. 6 because the venous blood P_{O_2} was

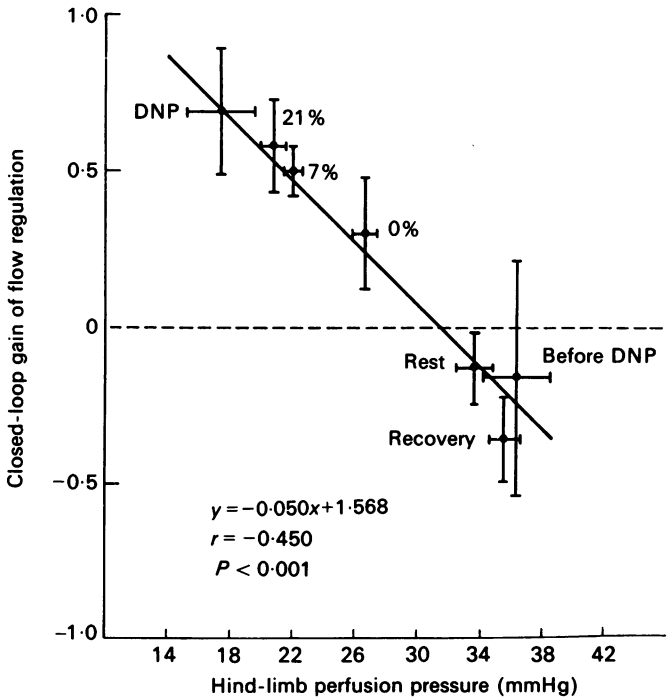

Fig. 6. Relationship between the hind-limb venous P_{O_2} measured just prior to the first decrement in perfusion pressure and the closed-loop gain of flow regulation obtained during the first step decrement in perfusion pressure. The linear regression equation was calculated by the method of least squares from 101 data points.

measured only during the steady state just prior to the first decrement in perfusion pressure. The relationship between hind-limb venous blood P_{O_2} and G_c was adequately described by a simple linear regression ($n = 101$, $r = -0{\cdot}450$, $P < 0{\cdot}001$). For each 1 mmHg decrement in venous blood P_{O_2}, G_c increased by 5% [$G_c = -0{\cdot}050$ (P_{O_2}) + 1·568].

DISCUSSION

From our results, we conclude that the hind-limb vascular bed of conscious dogs at rest displays a passive *P–F* relationship in response to short-term (2 min) changes in perfusion pressure. During conditions of increased oxygen consumption, however, the hind-limb *P–F* relationship is predominantly autoregulatory. These findings support the concept enunciated by Goodman *et al.* (1978), that skeletal

muscle blood flow is not only regulated in accordance with tissue metabolic requirements, but that the precision of blood flow control is also influenced by the metabolic state of the tissue. The finding that autoregulation is readily demonstrable during 'resting' conditions in tissues that normally have a relatively high metabolic rate, such as the heart (Drake-Holland, Laird, Noble, Spaan & Vergroesen, 1984) and brain (Johnson, 1978), also supports this concept.

The exact mechanism(s) mediating autoregulation has not been elucidated, but two major hypotheses have evolved (Johnson & Henrich, 1975). First, the myogenic (pressure related) hypothesis attributes the constancy of blood flow during changes in perfusion pressure to a direct effect of changes in transmural hydrostatic pressure on vessel wall tension that affects vascular smooth muscle reactivity (Baez, 1968; Johnson & Wayland, 1967; Koch, 1964). Secondly, the metabolic (flow related) hypothesis proposes that the microvascular segments regulating flow are controlled by the release of vasoactive agents whose concentrations are determined by metabolism (Granger *et al.* 1975). The myogenic and metabolic hypotheses are not mutually exclusive, and both mechanisms could operate in concert as determinants of autoregulation.

The results of the present study indirectly support the operation of a metabolic mechanism of autoregulation in the hind-limb vascular bed. Other studies also demonstrated that the efficiency of hind-limb autoregulation increased with increases in metabolism (Stainsby, 1962; Jones & Berne, 1964*a*; Goodman *et al.* 1978; Granger *et al.* 1976). Granger *et al.* (1975) have proposed a two component control system that explains how the efficiency of autoregulation and the rate of metabolism could be coupled. Their model proposes that the precapillary elements (first component) are more sensitive to changes in tissue P_{O_2} (or metabolites) than are the flow-regulating arterioles (second component). Thus, during conditions when hind-limb metabolism is relatively low (rest), oxygen delivery is maintained during decreases in perfusion by dilation of precapillary elements and the consequent increase in functional capillary density. When metabolic rate is increased, e.g. during exercise, the precapillary element oxygen delivery reserve will have already been utilized and oxygen delivery, and thus flow, will be maintained by dilation of the less sensitive arterioles (second component). In accord with this model is the observation that vascular elements exerting control over capillary exchange capacity are more sensitive to metabolic changes than are the resistance vessels (Granger *et al.* 1976). Thus, Granger's two-component model appears to account for the passive hind-limb *P–F* relationship observed in conscious dogs during rest and the autoregulatory behaviour found during exercise or the administration of DNP.

Previous work by Jones & Berne (1964*b*) demonstrated that acute or chronic denervation did not alter the extent of autoregulatory adjustments. Our findings that blockade of autonomic ganglia with hexamethonium did not alter hind-limb *P–F* relationships corroborates the hypothesis that our data represent local, intrinsic phenomena that were independent of neural reflexes. This independence of hind-limb vascular bed *P–F* relationships from neural reflexes was not surprising for two reasons. First, in muscles at rest or during low work loads, substantial reduction in hind-limb perfusion initiated no neural cardiovascular responses (Wyss, Ardell, Scher & Rowell, 1983). Secondly, numerous studies (Bulbring & Burn, 1939; Bowman, 1959;

Remensnyder, Mitchell & Sarnoff, 1962) have shown that vasoconstriction, resulting either from direct stimulation of sympathetic nerves or the administration of norepinephrine, is diminished during muscular activity.

The hind-limb *P–F* relationship obtained under any given circumstance is the net result of the interaction of passive elastic properties and active changes in vascular smooth muscle (Burton & Stinson, 1960). Because the vascular system is elastic, autoregulation during decreases in perfusion pressure represents not only active vasodilation necessary to oppose pressure-induced flow changes characteristic of a rigid system, but also the increased resistance caused by passive narrowing of vascular conduits as perfusion pressure decreases. The absence of autoregulation during resting conditions does not preclude the possibility that local vasodilation was present; it only demonstrates that the net effect of several interacting factors resulted in passive behaviour predominating.

The *P–F* relationship of the hind limb as a whole reflects an interaction between the individual components of the hind-limb vascular bed. Blood flow supplied to the hind limb via the external iliac artery supplies both skin and skeletal muscle in a parallel coupled circuit (Sparks, 1978). Although autoregulation may occur in skin (Henrikson, Nielson, Paaske & Sejrsen, 1973), this tissue has most often been depicted as a passive vascular bed (Green & Rapela, 1964). Because of the parallel vascular coupling between skin and skeletal muscle, the absence of autoregulation in the whole hind limb during resting conditions could be accounted for by entirely passive behaviour in the skin circulation with either passive or modestly autoregulatory behaviour in skeletal muscle. Our data do not differentiate the behaviour of the individual vasculatures.

A direct practical interest in autoregulation emanates from its possible role in hypertension (Coleman *et al.* 1979). The autoregulatory theory of hypertension postulates that hypertension is initiated by salt and water retention that causes an increased extracellular fluid volume that leads to an increase in cardiac output via the Frank–Starling mechanism. The increased cardiac output leads to perfusion of tissues in excess of metabolic demands and to an initial increase in blood pressure. This initial rise in blood pressure is thought to cause an increase in vascular resistance through mechanisms that are presumably similar to those involved in normal short-term autoregulation of peripheral blood flow. In the present study, we evaluated flow adjustments that occur during short-term (2 min) decreases in perfusion pressure. Our findings that the hind-limb *P–F* relationship is passive during resting conditions should not be extrapolated to predict events during long-term alterations in perfusion pressure. Flow responses during long-term changes in perfusion pressure could be quite active and demonstrate a high gain of autoregulation (Granger & Guyton, 1969). Meininger, Routh & Granger (1984) have recently demonstrated an autoregulation-mediated increase in vascular resistance of the splanchnic circulation of rats during the onset of two-kidney, one-clip renal hypertension.

Anaesthetic agents are known to have profound effects upon cellular metabolism and cardiovascular function (Altura *et al.* 1980). Because autoregulation of hind-limb and skeletal muscle blood flow is probably linked to metabolic events, data derived from anaesthetized animals concerning the mechanisms of autoregulation should be

interpreted with caution. Of particular relevance to the assessment of blood flow regulation are the observations that chloralose–urethane and barbiturate anaesthesia dilate arterioles and depress or abolish spontaneous arteriolar vasomotion (Faber, Harris & Wiegman, 1982; Colantuoni, Bertuglia & Intaglietta, 1984). The present inability to define the predominant mechanism(s) mediating autoregulation (Morff & Granger, 1982) may be related to anaesthetic-induced changes that can alter the hierarchy of control systems as a function of the anaesthetic agent used and/or the depth of anaesthesia present at a given point in time. Future work in conscious animals, free of the artifacts of anaesthesia and recent surgery, will hopefully lead to a clearer understanding of the mechanisms and pathophysiological roles of autoregulation.

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