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Control of A–V Shunt and Capillary Circuits in the Dog Hindpaw (40092)

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The distribution of blood flow in peripheral vascular areas is known to be influenced by neural (1–4), myogenic (5) and hormonal (6, 7) mechanisms. The dog hindpaw is unique for studying these controls because there are two major vascular circuits present connected in parallel. These are the nutritional or capillary circuits and the nonnutritional or arterio-venous anastomosis circuits. The dog hindpaw vasculature receives its innervation from three separate nerves that affect the arteries, small vessels and veins in distinctly different combinations (8, 9). The deep fibular nerve and the tibial nerve are reported to constrict arterial and small vessel segments when stimulated. Superficial fibular nerve stimulation causes small vessel segment and venous segment constriction.

The present study is to determine the effects of stimulation of each of these nerves at several frequencies on blood flow and volume distribution under conditions of controlled perfusion pressure.

Methods. Seventeen mongrel dogs averaging 18 kg in weight were pretreated with 10 mg/kg morphine sulfate and anesthetised with 15–20 mg/kg pentobarbital. The right hind paw was neurally and vascularly isolated at the ankle joint. The cranial tibial artery, saphenous vein, the tibial nerve, superficial fibular nerve and deep fibular nerve were isolated. The nerves were doubly ligated and sectioned. The skin flap produced by the isolation procedures was used as a seal for an air-filled volume recorder (plethysmograph).

The animals were given 10 mg/kg heparin. The saphenous vein was cannulated so that the blood flow passed through a scintillation detector. Venous outflow pressure was altered by a Starling Resistor for CFC measurements. The cranial tibial artery was perfused under controlled pressure conditions from a femoral artery by means of tubing having sidearms for measurement of perfusion pressure and injection of indicators. The perfused blood was kept at 37° by means of a heat exchanger. During the cannulation procedure, blood flow to the hindpaw was interrupted for less than one minute.

Stimulations of the superficial fibular nerve (60 volts, 0.3 msec duration), the deep fibular nerve (40 V, 0.3 msec duration), and the tibial nerve (60 V, 0.3 msec duration) were made at 0.1, 1, 5, and 11 Hz. Stimulation frequencies higher than 11 Hz reduced blood flow to levels where the indicator data were not reproducible. Elapsed time of stimulation of each nerve averaged nine minutes. Only experiments in which the blood flow change was maintained for the entire stimulation period are included.

The duration of the experiments was variable. Sufficient postsurgical time was allowed for the animals to stabilize and the hindpaws to become isovolumetric. It was unnecessary to immobilize the animals with a curarelike drug since stimulation of the nerves did not consistently elicit movement of the paw and in those instances where some movement did occur, the data was not different from paws that did not show contractions.

All measurements were made during control periods and during each period of nerve stimulation and recorded on an Electronics for Medicine DR-8 recorder. Systemic arterial pressure, paw perfusion pressure and venous outflow pressure were monitored using Statham P23AA transducers. Total paw volume changes were measured with the air-filled plethysmograph connected to a Statham P23-2D-300 pressure transducer. The sensitivity of the system was 2 cm of recorder deflection per milliliter change in paw volume. Arterial inflow was monitored with a Carolina Medical electromagnetic flowmeter. Venous outflow was measured by a timed collection of the effluent in a graduated cylinder (a separate collection was made for each injected isotope). Blood containing isotope was not returned to the dog but rather

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a continuous infusion of homologous, heparinized donor blood was made at approximately the same rate as venous outflow. The amount of donor blood infused was sufficient to maintain the dog's mean arterial pressure constant and averaged 1500 ml per experiment. Capillary filtration coefficient (CFC) was measured by rapidly increasing the venous outflow pressure 10 mmHg with the Starling resistor. This produces an initial rapid increase in paw volume due to venous distension followed by a slower increase due to outward filtration of fluid (5, 10). The following equation using this filtration slope from the plethysmograph record and the venous pressure change was used. The assumption was made that 80% of the venous pressure increment is transmitted to the capillaries:

$$CFC = \frac{100 \text{ (filtration rate)}}{0.8 \text{ (venous pressure change)(paw weight)}}$$

It is conceivable that the 0.8 factor would not be accurate under all conditions. Since venous compliance and radius may vary with experimental conditions the fraction transmitted would be expected to be greater with venous dilation and less with venous constriction.

Active vascular volumes were measured using red cells-^{51}Cr and albumin-^{131}I as indicators. The red cells were labeled as previously reported. A 0.25 ml aliquot of these cells (3–5 mCi ^{51}Cr) was injected as a bolus into a sidearm of the arterial cannula as was 0.25 ml of albumin-^{131}I (0.3–0.5 mCi ^{131}I). The indicators were injected separately and not simultaneously. The time-concentration curves of the venous effluent were monitored by a previously described hole-through scintillation detector and rate meter (10). Each injection was made in less than 1 sec and although this transiently changed pressure and flow the recording showed no persistent change in vascular resistance. Completion of the dilution curve was ascertained by return of the recording to background levels. Indicator recovery was subsequently measured by determining the radioactivity of a mixed sample of the venous effluent collected during the period of injection. Blood flow and vascular volume were calculated from the time-concentration curves by the method of Hamilton *et al.* (11). There was no distortion of the curves due to recirculation since all the indicators were collected in graduated cylinders and not returned to the animals. The volume of the system from the point of injection to the point of detection by the probe was calculated by multiplying the mean transit time (MTT) in seconds by the measured flow in milliliters per second. The vascular volume was then calculated by subtracting the volume of the inflow and outflow tubing from the total volume. The changes in vascular volume were compared to each other as well as to those noted from the volume recorder.

Capillary diffusion capacity (PS) was calculated from the extraction of 0.25 ml bolus injection containing 3–5 mCi of ^{86}RbCl using the following equation: $PS = -Q \ln (1-E)$ in which Q is the blood flow in milliliters per minute per 100 g tissue and E is the extraction of ^{86}RbCl. The extraction was measured by collecting the venous outflow in a graduated cylinder and subtracting the amount of indicator collected from the amount injected. Our method differs from that of Renkin (12) in that a bolus injection was used rather than a constant arterial infusion. Theoretically, E should be the same for either method if uptake by the cells can keep pace with transcapillary diffusion. If the tissue cell membranes limit interstitial clearance of the indicator, back diffusion would introduce an error when flow is changed from control. Comparison of the time-concentration curves of ^{86}Rb and albumin-^{131}I by the method of Martin and Yudilevich (13) indicate that back diffusion was minimal in these types of experiments.

Even though it has been reported by Renkin that PS may not adequately reflect changes in capillary surface area when flow is changing, the calculation was made under constant pressure perfusion. This was done to determine how PS changes compare with CFC changes which are not considered to be as blood flow dependent.

All data are reported with standard error of the means (SEM). Appropriately related points were evaluated with a paired-*t* test.

Results. Hemodynamic changes (Fig. 1). The hindpaws perfused under conditions of controlled pressure (93 ± 4 mmHg) exhibited progressive decreases in blood flow and in-

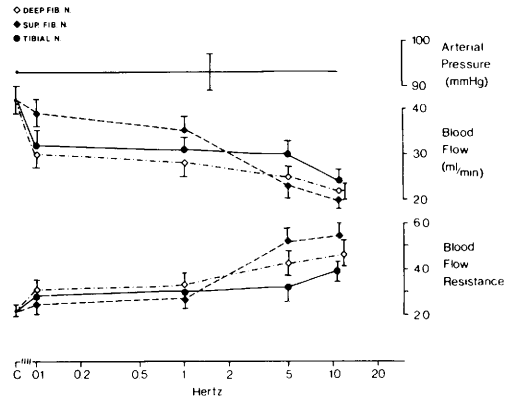

FIG. 1. Hemodynamic responses to several stimulation rates of the superficial fibular nerve, deep fibular nerve and tibial nerve during controlled pressure perfusion of 17 experiments. Brackets indicate ±SEM.

creases in resistance with stimulation frequency. The responses of all three nerves were similar.

Vascular and tissue volume changes (Fig. 2). Stimulation of the superficial fibular nerve at 0.1 Hz caused a small but significant ($P < 0.05$) decrease in total tissue volume but no change in vascular volume as measured by red cells-^{51}Cr and albumin-^{131}I. Total tissue volume increased at 1 Hz and 5 Hz ($P < 0.01$) and remained at that level at 11 Hz. Edema was easily observed at the higher frequencies. Vascular volume decreased minimally but insignificantly with increasing rates of stimulation.

Stimulation of the deep fibular nerve and the tibial nerve each caused progressive decreases in total tissue volume and vascular volume which were significantly less than control from 0.1 Hz to 11 Hz ($P < 0.05$).

Indicator Recovery (Fig. 3). The recovery of red cells-^{51}Cr and albumin-^{131}I was essentially complete at all stimulation frequencies of all three nerves, and, therefore, the data are reported as one group.

The recovery of ^{86}Rb decreased progressively for all three nerves as the stimulation frequency increased from 0.1 to 5 Hz. At 11 Hz the recovery continued to decrease with tibial nerve stimulation but increased with deep fibular nerve and superficial fibular nerve stimulation.

PS and CFC Changes (Fig. 4). The capillary surface area available for exchange was assessed by determining the capillary filtration coefficient (CFC) and by calculating the PS product. Tibial nerve stimulation increased PS and CFC progressively with stimulation rates up to 5 Hz and did not increase further at 11 Hz. Deep fibular nerve stimulation caused increases in PS and CFC values at 0.1 Hz and 1.0 Hz. While CFC increased at 5 Hz PS declined slightly but remained significantly above control ($P < 0.05$). At 11 Hz PS decreased to below control values and CFC decreased to the control level. When the superficial fibular nerve was stimulated the PS increased at 0.1 Hz and 1.0 Hz but then decreased at 5 Hz and 11 Hz to approximately the control levels. Stimulation of this nerve did not change the CFC from the control at any stimulation rate.

Discussion. The evidence for separate innervation of the series coupled arteries, small vessels and veins in the dog hindpaw (1, 8) would lead to the expectation that the nerves involved would individually influence blood

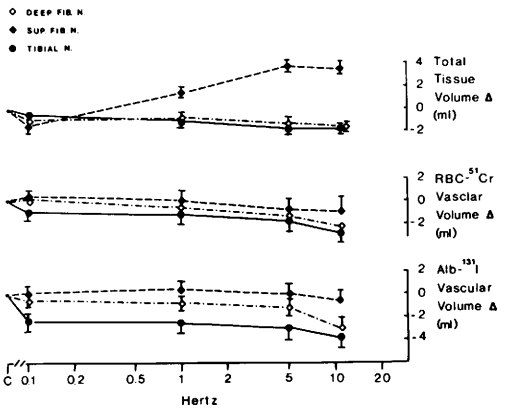

FIG. 2. Vascular and tissue volume changes with stimulation rates. Brackets indicate ±SEM.

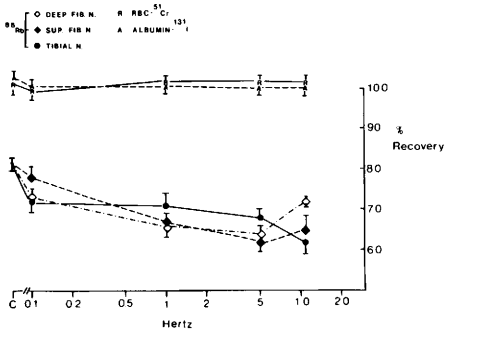

FIG. 3. Indicator recoveries with stimulation rates. Brackets indicate ±SEM.

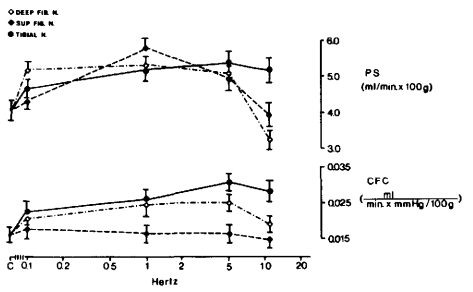

FIG. 4. PS and CFC with stimulation rates. Brackets indicate ±SEM.

flow and volume distribution as well. The data in this report indicate that there are differences between the effects of the nerves which also depend upon the frequency of stimulation of each nerve.

Superficial fibular nerve stimulation. Stimulation of the superficial fibular nerve under controlled pressure conditions caused decreased blood flow consequent to increased resistance. It has been demonstrated by Hammond *et al.* (9) who measured segmental resistance changes that stimulation of this nerve increased small vessel resistance somewhat and venous resistance to a much greater degree with no effects on arterial resistance. The data in this report would support these findings. The progressive increases in total tissue volume with no effect on vascular volume as stimulation frequency was increased would support strong venous constriction, with continued arterial inflow due to the relatively weak small vessel segment constriction. This would result in blood pooling in the small veins and capillaries. This pooling would elevate the venous pressure resulting in progressively greater outward capillary filtration of fluid as the stimulation rate was increased. The reduced downstream venous volume and the consequent pooling in the small veins apparently resulted in no net vascular volume change. Therefore, the change in total tissue volume represented extravascular fluid accumulation. It is difficult to assess the lack of agreement between the PS and CFC values. Since blood flow redistribution should be reflected by changes in these values one would be forced to conclude that on the basis of the CFC values little redistribution occurred at any stimulation frequency although there was a tendency to lower CFC at 11 Hz, indicating either increased precapillary sphincter constriction or less uniform perfusion of the capillary bed. Also, the CFC may not be accurate under these conditions of large vein constriction as a smaller percentage of the venous pressure change would be transmitted to the capillaries, and therefore the CFC may be underestimated. The PS values, however, increased at 0.1 and 1 Hz and then decreased at 5 Hz, and to below control levels at 11 Hz. The increased PS values might reflect a larger extraction of Rb^{86} due to the higher outward filtration of fluid than would occur when there is little net filtration. One could expect the extraction to be proportionally greater at 5 Hz and 11 Hz if extraction is filtration dependent since outward filtration was greatest at these frequencies. Blood flow decreased to very low levels at these rates of stimulation but the extraction did not increase proportionally. It is, however, difficult to accept redistribution of blood flow away from the capillary bed when large amounts of fluid are being filtered so that edema is frankly obvious. It has also been suggested by Renkin (12) that the PS is not valid when blood flow is changing. This would be particularly true at the low flows that exist with the higher stimulation frequencies.

Deep fibular nerve stimulation. Stimulation of the deep fibular nerve increased blood flow resistance and therefore reduced blood flow as the rate increased. Vascular and tissue volume changes were small but consistent decreases in each of these parameters occurred with increasing rate of stimulation. These would presumably be due to arteriolar constriction to a small degree and to passive reduction in venous capacity consequent to the reductions in blood flow. The PS and CFC values did change reasonably uniformly. When the nerve was stimulated at 0.1, 1, and 5 Hz there were significant increases in these values indicating that flow was redistributed due to constriction of the arteriovenous anastomoses to the capillaries. However, at 11 Hz the PS and CFC values both declined markedly. This would seem to be best explained as uneven distribution of flow through capillaries as there would be little reason to expect less constriction of the A-V anastomoses at this higher frequency. Rather, one would expect more constriction of these vessels.

Tibial nerve stimulation. Stimulation of the

tibial nerve at increasing frequencies resulted in progressive increases in resistance and decreases in blood flow. The progressive declines in tissue volume and vascular volume were similar to those caused by deep fibular nerve stimulation and could be best explained on the basis of resistance vessel constriction and passive reductions in venous capacity. PS and CFC values increased progressively with stimulation rate, indicating that the A-V anastomoses were constricted strongly by these stimulations redistributing blood flow to the capillary circuits. This conclusion is supported by the report of Davis and Hammond (1) that tibial nerve stimulation resulted in redistribution of blood flow away from the digit which is rich in A-V anastomoses.

Summary. The effects of stimulation of the three nerves containing the sympathetic innervation of the vasculature of the dog hindpaw were studied during controlled pressure perfusion. Stimulation at increasing frequency of the superficial fibular nerve resulted in progressively greater edema formation, apparently due to progressively increasing venous resistance with little evidence of blood flow redistribution. Increasing frequency of stimulation of the deep fibular nerve caused constriction of the arteriovenous anastomoses resulting in blood flow redistribution to the capillary bed. Tibial nerve stimulation at all frequencies resulted in blood flow redistribution to the capillary circuit due to constriction of arteriovenous anastomoses.

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1. Davis, D. L., and Hammond, M. C., Amer. J. Physiol. **215,** 496 (1968).
2. Dresel, P., Folkow, B., and Wallentin, I., Acta Physiol. Scand. **67,** 173 (1966).
3. Menninger, R. P., and Baker, C. H., Amer. J. Physiol. **230,** 797 (1976).
4. Menninger, R. P., and Baker, C. H., Amer. J. Physiol. **230,** 1561 (1976).
5. Baker, C. H., Amer. J. Physiol. **218,** 674 (1970).
6. Baker, C. H., Amer. J. Physiol. **216,** 368 (1969).
7. Daugherty, R. M., Scott, J. B., Emerson, T. E., Haddy, F. J., Amer. J. Physiol. **214,** 611 (1968).
8. Zimmerman, B. G., Circ. Res. **18,** 429 (1966).
9. Hammond, M. C., Davis, D. L., and Dow, P., Circ. Res. **23,** 1 (1968).
10. Baker, C. H., and O'Brien, L. J., Amer. J. Physiol. **206,** 1291 (1964).
11. Hamilton, W. F., Moore, J. W., Kinsman, J. M., and Spurling, R. G., Amer. J. Physiol. **84,** 338 (1928).
12. Renkin, E. M., Amer. J. Physiol. **197,** 1205 (1959).
13. Martin, P., and Yudilevich, D. L., Amer. J. Physiol. **207,** 162 (1964).

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