Segmental Responses of Dog Paw Vasculature

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ABSTRACT

The production of constrictive responses limited to either arterial or venous segments of the vasculature of the dog paw was tested by stimulating various peripheral nerves of the hind leg. Resistance changes of the total vasculature of the paw, and of three of its component series-coupled segments (arterial, small vessel, and venous) were studied sequentially before and during stimulation of the sciatic nerve and of three of its branches (tibial, deep, and superficial fibular nerves). Stimulation characteristics (15 to 90 volts, 1-3 msec duration, 25/sec, and 30-second stimulation periods) were chosen to produce maximum vasoconstrictive responses. Tibial and deep fibular nerve stimulations produced constriction of the arterial segment but not of the venous segment. Superficial fibular nerve stimulation produced constriction of the venous segment but not of the arterial segment. All nerve stimulations produced constriction of small blood vessel segments. Thus, responses could be limited to either arterial (plus small vessel) or venous (plus small vessel) segments. Inability to localize response within small-vessel segments made it impossible to determine whether any peripheral nerve produced constrictive responses limited strictly to either pre- or postcapillary segments. No relationship was evident between control resistance values and the subsequent changes in resistance occurring in response to the standard stimulations.

ADDITIONAL KEY WORDS

small-vessel innervation arteries peripheral nerve stimulation vasoconstrictor response small-vessel response small-vessel pressures sympathetic stimulation series-coupled circuits

Many recent studies on small blood vessels have been directed toward a quantitative analysis of pre- and postcapillary resistance changes. Exact apportionment of responses to either pre- or postcapillary segments as well as more precise localization of responses within a given segment has proved difficult. A possible contribution to interpretation of these studies might be offered by experiments in which vasomotor responses could be restricted to selected segments of a vasculature. Zimmerman (1) in showing that constrictive responses could be confined predominantly to precapillary segments of the vasculature of the dog paw by stimulating the tibial nerve has demonstrated that partial segmentalization of responses of the vasculature is possible. The purpose of the present study was to determine whether a constrictor response confined to postcapillary segments of the dog paw vasculature could be produced by stimulating other peripheral nerves.
A schematic illustration of the dog hindpaw showing the four peripheral nerves and the individual series-coupled vascular segments. Blood flowed from A to B in the arterial segment; from C to D in the small-vessel segment; and from E through the venous drainage of the paw in the venous segment. The leg and the paw are not severed. See text for additional information.

Methods

All dogs were given morphine sulfate (3 mg/kg sc), and one-half hour later anesthetized with alpha-chloralose (100 mg/kg iv). With the animal supine on the operating table, a midline incision was made in the neck to allow tracheal cannulation (for positive pressure respiration), cannulation of the left common carotid artery (to pass a sound down to the arch of the aorta to record central arterial pressure), and cannulation of the left external jugular vein (to establish an intravenous drip for administration of alpha-chloralose, succinylcholine chloride, and heparin). Sustaining doses of alpha-chloralose (one-fourth the initial dose) were repeated hourly. Succinylcholine chloride was given at an initial dose of 1 to 2 mg/kg, and repeated hourly to permit electrical stimulation of peripheral nerve trunks without eliciting skeletal muscle contraction. Alveolar ventilation was maintained with a Harvard Apparatus positive-pressure respirator equipped with an intermittent hyperinflation valve attachment. Gas analyses of arterial blood or expired air or of arterial blood pH were not made. Respiratory minute volumes were calculated from the data of Kleinman and Radford (2). Heparin, given at an initial dose of 2 mg/kg, was repeated at one-half hour intervals at one-half the initial dose.

Responses of the total vasculature described in detail below and of three series-coupled segments constituting the vascular bed of the hindpaw were studied sequentially during electrical stimulation of the sciatic nerve or of one of its three major branches. The vascular segments and the peripheral nerves are illustrated in Figure 1. Each vascular segment of the right experimental paw was subjected sequentially to a constant inflow by pumping blood from a vessel of the opposite extremity with a Harvard Apparatus peristaltic pump. Only one pump was required because only one vascular segment, whether it was the total segment or one of the separate series-coupled segments, was studied at a time. Pump output was monitored with either a Medicon K-2000 microchannel electromagnetic flowmeter or a liquid-filled drop counter. Arterial blood was pumped into total, arterial, and small-vessel segments. Venous blood was pumped into the venous segment. During all experiments the ipsilateral femoral artery was occluded a few minutes prior to the recording period. The femoral artery occlusion was released during recovery periods between successive stimulation periods. Ten minutes, or more if required, were allowed for recovery and establishment of control states between successive stimulation periods. Collateral inflow was further reduced by ligating the planter branch of the saphenous artery. In some experiments, ties were placed around all tissues except major blood vessels and nerves at a level a few centimeters proximal to the talocrural joint, and the tibial bone cavity was packed with bone wax. These latter procedures did not alter the responses from those seen when saphenous and femoral arteries only were occluded, and thus, in most experiments, this technically more complicated and potentially more damaging surgical procedure was omitted. Inflow and outflow press...
SEGMENTAL VASCULAR RESPONSES

Pressures across each segment were measured, and the resistance of each segment was calculated by dividing the pressure drop across the segment by the flow through that particular segment. Pressure and flow data used to calculate resistances occurring during peripheral nerve stimulation were measured near the end of the 30-second stimulation periods. These resistances were expressed as percent changes from prestimulation values. A more detailed description of each of the segments follows.

The total vascular segment included all three series-coupled segments, and represented the entire paw vasculature. It extended from the arterial inflow at the dorsal pedal artery to the venous drainage at the lateral saphenous vein. Blood from the opposite femoral artery was pumped at a constant rate into the distal stump of the dorsal digital artery of the experimental paw. Inflow was monitored by the electromagnetic flowmeter. Dorsal pedal and digital artery and dorsal metatarsal and lateral saphenous vein pressures were recorded simultaneously. Dorsal pedal arterial pressure was recorded from the inflow tubing as close as possible to the point of cannulation. Digital artery and metatarsal vein pressures were recorded from the sides of plastic catheter loops inserted into each vessel.

The arterial segment extended from the dorsal pedal artery to the digital artery. Blood from the opposite femoral artery was pumped at a constant rate through this segment, and then through a variable resistance into a beaker for collection of polyethylene tubing. Outflow from the digital artery was measured with a liquid-filled drop counter. Inflow pressure was recorded from the inflow tubing as close as possible to the point of cannulation. Digital artery and metatarsal vein pressures were recorded from the sides of plastic catheter loops inserted into each vessel.

The small-vessel segment extended from the digital artery to the digital vein. This segment was perfused at a constant rate by pumping blood from the contralateral dorsal pedal or digital artery into the distal stump of a medial digital artery. Inflow was monitored with a liquid-filled drop counter. Inflow pressure was recorded from the sidearm of the drop counter apparatus. Outflow from the digital vein was collected in a beaker for periodic return to the animal. Outflow pressure was measured from the sidearm of the opened plastic catheter loop in the digital vein. During recording and stimulation periods both metatarsal and lateral saphenous vein were opened to the atmosphere.

The venous segment extended from the metatarsal vein centrally to the saphenous vein, or included the larger vein constituting the venous drainage of the paw. Blood was pumped from the opposite lateral saphenous vein through a liquid-filled drop counter into the proximal stump of the divided dorsal metatarsal vein and flowed centrally through the venous drainage of the paw. An occluding cuff on the contralateral leg proximal to the level of venous cannulation provided a small reservoir of venous blood to be pumped into the metatarsal vein. The pump was adjusted to give a resting pressure of 30 to 40 mm Hg. In experiments in which separate branches of the sciatic nerve were stimulated, the occluding cuffs were applied near the ankle level. Thus, the venous segment undergoing constriction could have extended centrally only as far as the ankle. In experiments in which the sciatic nerve was stimulated, the venous segment undergoing constriction could have extended further centrally. The effect this had on subsequently recorded responses will be discussed.

The sciatic nerve was exposed and crushed over a length of a few centimeters as near as possible to its emergence from the pelvis. Silver wire electrodes were then looped approximately 1 cm apart around the nerve trunk at the mid-thigh level. Both the sciatic nerve and the electrodes then were enveloped loosely in plastic sheeting to insulate the stimulating electrodes from the surrounding muscles. The sciatic nerve was exposed by a medial incision on the lower thigh a few centimeters proximal to the talocrural joint and was isolated from connective tissue and accompanying blood vessels. Both deep and superficial branches of the sciatic nerve were exposed by a dorso-lateral incision on the lower thigh a few centimeters proximal to the talocrural joint. To avoid temperature changes and dehydration, all three branches of the sciatic nerve were left in their normal position until time for stimulation. They were then placed on Harvard Apparatus stimulating electrodes positioned to avoid undue stretch of nerve trunks and speed of stimulating current to surrounding tissues.

All nerves were stimulated with a Grass-S4 square-wave stimulator through an isolation unit. All stimulations were made at a pulse duration of 1 to 3 msec, a frequency of 25 Hz/sec, and a total stimulation period of 30 seconds. Stimulation voltages were 90 v for the sciatic trunk and 15 to 70 v for separate branches of the sciatic nerve. All blood pressures were recorded with Statham P23C pressure transducers on an Electronics for Medicine Oscillographic Recorder. Significant differences between vascular responses occurred during peripheral nerve stimulations.
The changes in resistance of each vascular segment produced by stimulation of each of the four peripheral nerves. Resistances (percent changes from the control value) are plotted along the ordinate. Each vertical bar represents one animal. Horizontal rows of histograms show responses of the arterial, small-vessel, venous, and total vascular segments. Vertical columns of histograms show responses to stimulation of the sciatic, tibial, deep fibular, and superficial fibular nerve stimulations. The dashed line (with accompanying numerals) across each histogram represents the mean of that histogram, and it joins the usual symbol for ± SE. Histograms in horizontal rows are for the same animal groups, and the animals within each group are arranged in identical order. Histograms arranged in vertical columns are for animals from different groups, except for three dogs, each identified by a different number of crosses.

to different nerve stimulations were tested for by the Student t-test (3).

Results

Responses of the paw vasculature to sympathetic stimulation were studied in 16 dogs. Because of the length of time necessary to complete the experimental preparation and because of technical difficulties in some experiments—such as damage to a particular small blood vessel or nerve trunk—in most cases it was impossible to record responses from all segments of the vasculature to stimulation of each of the four peripheral nerves. Responses of any given segment were not accepted for comparison unless responses to stimulation of all four peripheral nerves had been recorded and were believed to represent responses from near normally responding vascular segments. Comparisons were made of responses of the total vascular segment from 13 dogs, of the arterial segment from 8 dogs, of the small-vessel segment from 7 dogs; and of the venous segment from 10 dogs.

The histograms in Figure 2 illustrate resistance changes in total vascular segments, and in each of the three series-coupled segments during stimulation of each of the four peripheral nerves.

Although individual responses varied widely, some vascular segments showed no response to stimulation of certain peripheral nerves and apparently were not innervated by nerves contained in these nerve trunks. When each segment was considered separately, the following facts appeared to be true. The total vascular segment showed the greatest increases in resistance in response to stimulation of the sciatic nerve. This was closely followed by the response to deep fibular nerve stimulation, and by the progressively smaller responses of the tibial and superficial fibular nerves. Responses to sciatic and deep fibular nerve were not statistically different at the 0.05 level of probability. All others were significant at the 0.01 level. The arterial segment showed a striking degree of variation in individual response. Although the mean values indicated a greater response to stimulation of the tibial and deep fibular nerves than to stimulation of the sciatic nerve, this was not statistically different at the 0.05 level of probability. Resistance changes in response to superficial fibular nerve stimulation were negligible. The small-vessel segment showed the most uniform, as well as the smallest, response to stimulation of each of the four nerves. Responses to each of the nerves were not statistically different at the 0.05 level of probability. The venous segments showed responses to stimulation of sciatic and superficial fibular nerves only.
The responses were statistically different at the 0.01 level of probability.

When the responses of individual vascular segments to stimulation of each nerve were compared, the following facts were apparent:
(1) Each of the peripheral nerves produced responses in total and small-vessel segments.
(2) Superficial fibular nerve stimulation produced responses in all segments except the arterial.
(3) Both tibial and deep fibular nerves produced responses in all segments except the venous.
(4) The percent changes in resistance of the individual segments in response to sciatic nerve stimulation, when arranged in decreasing order of responses, were the arterial, venous, and small-vessel segments.
(5) Although results from individual animals were arranged in histograms to give a progressively increasing response to sciatic nerve stimulation, this same order of response was not present when separate branches of the sciatic nerves were stimulated.

As mentioned in the description of methods, venous segments were anatomically comparable when each branch of the sciatic nerve was stimulated. Each branch was stimulated near the talocrural joint, and thus only venous segments distal to this level would have been stimulated. Sciatic nerve stimulation could produce constriction of venous segments central to the talocrural joint. In all cases metatarsal and lateral saphenous vein pressures were measured. Figure 2 shows that superficial fibular nerve stimulation produced greater venous constrictive responses than did sciatic nerve stimulation. A reasonable explanation of this difference is that the venous segment between the metatarsal and lateral saphenous veins was passively distended by increased resistance in the veins central to the lateral saphenous vein when the sciatic nerve was stimulated. This would not have occurred in response to superficial fibular nerve stimulation.

Comparisons of histograms in their vertical alignment, although statistically less valid, substantiates the interpretations mentioned above. Only one of the three animals in which responses had been recorded from each of the four vascular segments to stimulation of each of the four peripheral nerves (and thus was common to all histograms in Fig. 2) showed a pattern of response exactly similar to that followed by the means of each histogram in response to sciatic nerve stimulation. Responses of the remaining two dogs differed only by greater changes in resistance of the total segment than of the arterial segment during sciatic nerve stimulation. The agreement was better in response to stimulation of each of the individual branches of the sciatic nerve. Here all three animals followed the general pattern of response of the means.

The wide scatter of data included in Figure 2 not only renders interpretation of results more difficult, but also raises the question whether the magnitude of the response was determined primarily by the existing state of the vasculature. Figure 3 shows control resistance values immediately prior to the stimulation period for each of the segments shown.
in Figure 2. The data are arranged in the same order as in Figure 2. Once again, there was marked scatter in the data. There was no difference at the 0.05 level of probability in the resting resistance of any of the segments. Although there were individual exceptions, the shape of each histogram making up the horizontal columns was similar. This indicates that the control vascular resistance of any given segment, whether high or low, tended to remain at that level throughout the duration of the experiment. It is also apparent that there was little relationship between the shapes of the corresponding histograms in Figures 2 and 3. This indicates that the initial resistance had little effect on the response to the test stimulus. To test the relationship between resting resistance and percent change in resistance more closely, these two parameters are plotted in Figure 4. In this figure percent change in resistance is plotted along the ordinate against initial resistance along the abscissa for each segment. No relationship between the two parameters was evident in any of the plots.

Discussion

The results obtained in this study indicate that the tibial nerve contains a major innervation of both arteries and small vessels. While Zimmerman (1) reported that a smaller fraction of the innervation of these vessels is contained in the deep fibular nerve, the present study indicates that similar fractions of the innervation must be contained in tibial and deep fibular nerves. Whereas Zimmerman stated that innervation of venous segments is included in nerves coursing along veins and scattered in connective tissue, this study showed that a major fraction of the venous innervation is contained in the superficial fibular nerve. A possible explanation for the discrepant interpretations of results may be that Zimmerman stimulated only the lumbar sympathetic trunk and tibial nerves. Abboud and Eckstein (4) reported similar results from the vasculature of the dog forepaw. They reported that only one peripheral nerve, the radial, produced significant venous constriction.

The present study indicates that super-
facial fibular nerve stimulation produces appreciable constriction of venous segments without demonstrable arterial participation. It also shows that tibial and deep fibular nerve stimulation produces marked constriction of arterial segments without demonstrable venous participation. A decision whether stimulation of any given nerve produces a constriction limited strictly to either pre- or postcapillary segments is not possible because the small-vessel segment responded quite similarly to stimulation of all peripheral nerves. Although attempts are under way to extend the present recording technique to smaller segments of the venous drainage, extension to smaller arteries does not seem feasible.

One of the most striking aspects of the data obtained in this study is the wide variability of response of arterial and venous segments to our standard stimulus. The wide range of values is not unique to this study because similar scatter has been noted in previous studies (5, 8). The present study permitted evaluation of one possible contributory factor, i.e., the influence of the existing state of the vasculature (as judged by the control resistance) on the responses to a standard stimulus. The stimulation characteristics were chosen to produce maximal vascular responses (7, 8), and it might be argued that the constrictor responses obtained were so complete and overwhelming that a maximal state of constriction was obtained regardless of the starting level. If this were true, an inverse relationship between initial and peak resistance changes should have occurred, with absolute resistance values reached during stimulation nearly constant for any given segment. Although differences in resistance of individual vascular segments (due to differences in conductance, geometry, etc., of the beds) would not have been identical, a strong indication of an inverse relationship should have been present. The data in Figure 4 indicate that there was no apparent relationship between control resistance and change in resistance in response to peripheral nerve stimulation. This is difficult to explain in view of the wide range of control resistances, and the published data which show that the initial tone of the vessels influences subsequent responses (9-12).

The above discussion serves to focus attention on resting resistance values of each vascular segment. The grand averages of resting values in Figure 3 for the total, arterial, small-vessel, and venous segments were respectively, 1.3, 12.6, 12.5, and 2.3 resistance units (mm Hg X ml/min). The resistance value of the total bed agrees well with previously published values (13). Resistance values of individual segments fail to agree well with the data of Haddy, because they pertain to segments containing few parallel-coupled channels. The resistance of each of the series-coupled segments was larger than that of the total vascular bed. Probably this was because the resistance of each series-coupled segment with the exception of the small-vessel segment, represented the resistance of only a few parallel-coupled segments. As would be expected, the venous segment exhibited the smallest resistance of the separate series-coupled segments. Arterial and small-vessel resistances were similar, but appreciably larger than small-vein resistances. Their similarity was probably due to the fact that the small-vessel segment included approximately the entire vasculature of the digit, i.e., was composed of many parallel-coupled segments while only a few parallel-coupled channels were included in the arterial segments.

Histograms in Figure 2, showing the percent change in resistance of each vascular segment in response to sciatic stimulation, indicates that arterial segments had the largest percent changes in resistance. In a progressively decreasing order were the total, venous, and small-vessel resistances. Mean percent changes for arterial, total, venous, and small-vessel segments were, respectively, 1400, 722, 130, and 16. Absolute resistance changes occurring in each vascular segment were calculated by multiplying mean control resistances by mean percent changes in resistance. The calculated values for changes in
resistance in response to sciatic nerve stimulation for arterial, total, venous, and small-vessel segments were, respectively, 1763, 9.4, 3, and 2 resistance units (mm Hg x min/ml). These resistance changes expressed as absolute units were in the same sequence when arranged in order of decreasing values as those expressing the percent change in resistance, but the magnitudes of absolute resistance changes were quite different. This was because absolute changes in resistance of arterial segments differed from other segments by a factor of 100, or more, while the remaining vascular segment values were all similar. This agrees with previous findings (14) that during the response to high-frequency sympathetic stimulation, the largest fraction of the constrictor response occurred in arteries proximal to, and larger than, arterioles. The fact that resistance changes in arterial segments were so large compared with those of all other beds, again demonstrates the high resistance obtainable when recording from segments containing few parallel-coupled channels. Previous studies from this laboratory have indicated that the digital artery segment represents a vascular circuit capable of producing much higher resistances than other parallel-coupled circuits within the paw. Both of these factors must have contributed to the high resistances seen in the arterial segment. The fact that arterial segments, extending from the dorsal pedal artery to the digital artery, could generate such high resistances is not surprising when one considers the comparatively thick muscular walls of these medium-sized arteries. It is, however, surprising that such small resistances were developed by small-vessel segments. The term small-vessel segment is perhaps an inappropriate term when the diameter of some vessels included in this segment are considered. The small-vessel segment, extending from digital artery to digital vein, contained approximately the distal one-half of the digital artery, which in dogs in this study had outside diameters varying from 3 to 1 mm. The digital arteries thus were appreciably larger than arterioles, and in view of their relatively thick walls should have been capable of generating resistance changes of the order of magnitude of that developed by arterial segments. Others (1, 15) have noted relatively minor small-vessel constrictive responses to sympathetic nerve stimulation. Although adrenergic terminals have been described in various vessels (16), there is insufficient information to allow an explanation of the variability of vascular response on the basis of difference in innervation density. The findings of Abbboud and Deutsch (17) indicate that density of innervation, rather than amount of smooth muscle present in different vascular segments, may be involved. They reported that small arteries and veins, in contrast to the small-vessel segment, responded to injections of vasoactive agents such as norepinephrine but not to sympathetic nerve stimulation.

Results obtained in this study provide some indication of gross distribution of peripheral branches of the sciatic nerve within the paw. The arterial segments receive innervation from both tibial and deep fibular nerves. The small-vessel segment apparently receives innervation from all three peripheral branches of the sciatic nerve. The venous segment included in these studies apparently receives innervation from the superficial fibular nerve only.

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References

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