Responses of Dog’s Pad Vessels to Epinephrine Following Sympathectomy or Denervation

By Theodore Cooper, M.D., Ph.D., Vallee L. Willman, M.D., and Alrick B. Hertzman, Ph.D.

Augmented reactivity of the skin vessels of the hind limbs of dogs to doses of l-epinephrine in the physiologic range could not be detected following conventional sympathectomy. Larger doses of epinephrine resulted in exaggerated reactions. Augmented reactivity of these vessels to physiologic doses of epinephrine was detectable if total cholinergic as well as adrenergic denervation of the limb was accomplished.

Therapeutic failure following lumbar sympathectomy often occurs in cases in which organic changes in the blood vessels of the lower extremities have been minimal. Several possible explanations have been advanced for these failures: Incomplete section of sympathetic trunks or restricted ganglionectomy, the presence of accessory sympathetic pathways outside the abdominal distribution of the sympathetic trunks, regeneration of sympathetic vasoconstrictor fibers, the syncytial nature of the vascular neuroeffector units which would permit a few fibers extensive neural control, and denervation supersensitivity to a circulating constrictor substance.

If the latter phenomenon accounts for the observed recovery of tone, then the vessels on an operated limb might be more responsive to epinephrine in a physiologic concentration. Duff has emphasized this point, but could not demonstrate a consistent pattern of change in manual vascular responses to epinephrine or norepinephrine given intraarterially after therapeutic sympathectomy, particularly when physiologic amounts were used.

A major difficulty in the study of the vascular results of sympathectomy by conventional hemodynamic methods is the failure to identify precisely the vessels which determine the level of flow through a particular region. Microscopic observations indicate that the noninnervated vascular smooth muscle in the precapillary sphincter and metarteriole is quite autonomous and that it is more sensitive to epinephrine than that in the innervated arterioles. Hence, epinephrine in physiologic concentration may decrease blood flow principally through constrictor action on the noninnervated muscle. If so, sympathectomy should have no effect on the apparent reactivity of the precapillary and metarteriolar muscle of the “denervated” vascular bed when tested by physiologic doses of epinephrine. A postulated increase in the reactivity of arterial or arteriolar muscle might make either muscle sensitive to epinephrine in the concentration to which the noninnervated muscle reacts. In the latter case, the effects would be additive and should show in an exaggerated increase in the resistance to flow.

The present study was concerned with comparing the reactivity of the vascular beds of the footpads of the two hind feet of dogs tested with a standard dose of epinephrine after conventional unilateral lumbar sympathectomy. The standard dose, given intravenously, gave an arterial blood concentration in the “physiologic” range.
SENSITIZATION TO EPINEPHRINE

METHODS

The vascular responses in the nonpigmented hind footpad were followed by recording the cutaneous volume pulses with the photoelectric plethysmograph. Volume pulse amplitude was expressed as per cent of the photoelectric current. Calibration experiments on the human digit have shown that the volume pulse amplitude correlates closely with arterial blood flow over a wide range. Therefore, in situations which do not involve changes in central hemodynamics, the operation of the technic is essentially equivalent to the application of the hydraulic analogy to the local cutaneous region.

This technic offered significant advantages: observation of a strictly cutaneous vascular bed, absence of trauma or of irritation to nerve fibers, lack of interference in any way with the circulation, and opportunity for repeated observation with no surgical preparation. Therefore, photoelectric photometry as a method for comparing responses in vascular beds offers the great advantage of eliminating the uncertainty respecting the topographic distribution of blood flowing in a main artery, as the femoral, and the trauma resulting from various manipulations designed to limit the measurements of flow to cutaneous or muscle vascular beds. The high level of vasomotor activity in the footpad and the great rates of flow in this vascular bed permitted an advantageous application of photoelectric photometry to the comparison of the vascular responses. However, the photometric records did not permit the precise identification of the vessels responding to a particular procedure, but they did represent the integrated result of changes in tone of the pad’s vascular smooth muscle. The exact location of the reacting muscle elements was not identified.

The injections of L-epinephrine were made into a foreleg vein and were comparable in all experiments. A dose of 1 × 10⁻⁸ Gm./Kg. body weight was selected for study since exploratory experiments demonstrated that this dose does not alter the central arterial pressure pulse and consistently elicits a small but measurable constriction in the footpad. The absence of a significant difference in the magnitude of the constriction between the two sides is interpreted as no change in the reactivity of the footpad vessels to the dose of epinephrine as a result of the lumbar sympathectomy. The rate of “wash-in” of the injection was constant in all experiments, but the volume varied with the weight of the dog hence the rate of inflow of epinephrine was the same in all experiments. Blood dilution may have been variable. However, deliberate manipulations of the rate of epinephrine inflow did not significantly change the magnitude of the vascular response in the footpad so long as the total dose was the same. Single injections were preferred to constant infusion in order to minimize effects of prolonged constriction on the rate of removal of the drug from the region.

The intravenous injection of 1 × 10⁻⁸ Gm./Kg. body weight should result in an arterial blood concentration of not more than 1 × 10⁻¹¹ Gm./ml. This value was arrived at from calculations utilizing the concept of a single mixing chamber as a closed circuit. It agrees favorably with data from studies on the fate of intravenously injected epinephrine in the dog. This level is well within the resting physiologic range of arterial epinephrine concentration reported for the dog (1 × 10⁻⁶ Gm./ml.).

All observations were made on dogs during various levels of anesthesia with sodium pentobarbital. Of the 22 dogs used, 18 were subjected to a unilateral lumbar sympathectomy which consisted of removal of the chain from above the renal vessels to below the sacral prominence. All tissue removed was studied histologically to verify the removal of the chain. Preoperative observations were made on the dogs scheduled for sympathectomy. The postoperative observations were made at various intervals after operation (from 10 days to 20 weeks), hence all of the postoperative data were obtained after adequate time had elapsed for the restoration of vascular tone and “sensitization.” Unilateral sciatic and femoral neurectomy was performed on three of the unoperated dogs. The femoral nerve was isolated for section at the inguinal ligament; the sciatic nerve was isolated by a posterior approach, care being taken to section both major divisions after their egress from the pelvis. A 1 cm. length of tissue was removed from each divided nerve trunk. The operated legs were protected from ulceration by pressure and friction by a soft cast prepared from orthopedic sheet wadding and adhesive tape. Observations were made on these animals 16 to 21 days after operation.

RESULTS

All experiments were done in a constant temperature room (20 ± 1 C.). Volume pulse amplitude before the injection was plotted against the minimal amplitude during the constriction (fig. 1). This procedure is essentially similar to that proposed by Green for describing changes in peripheral resistance. If all points fall along a line as in

*Epinephrine kindly supplied by Winthrop Laboratories, New York, N. Y.
576

COOPER, WILLMAN, HERTZMAN

Fig. 1. Top left. Individual responses of the pad vessels of unoperated dogs to 1-epinephrine 1 \times 10^{-8} \text{Gm./Kg.} body weight given intravenously. Slope (b) of regression line = 0.84. Top right. Individual responses of the pad vessels of the nonoperated sides of dogs with chronic unilateral lumbar sympathectomy to 1-epinephrine 1 \times 10^{-8} \text{Gm./Kg.} body weight given intravenously. Slope (b) of regression line = 0.87. Bottom left. Individual responses of the pad vessels of the chronically sympathectoimized hind limbs to 1-epinephrine 1 \times 10^{-8} \text{Gm./Kg.} body weight given intravenously. Slope (b) of regression line = 0.90. Bottom right. Comparison of the regression lines which express the responses of the pad vessels of unoperated dogs, the normal side of sympathectomized dogs and the sympathectomized sides, to 1-epinephrine 1 \times 10^{-8} \text{Gm./Kg.} body weight given intravenously.

these figures, the slope of this line expresses the reactivity of the vessels with respect to the particular concentration of the injected substance, and then "corrects" for the level of dilatation which may obtain at the moment of injection.

Comparison of these figures does not demonstrate any essential difference in the intensity of the constrictions in the footpad of the nonoperated dog (fig. 1, Top left), of the nonoperated side of the sympathectomized dog (fig. 1, Top right), or of the sympathectomized side (fig. 1, Bottom left); the slopes of the regression lines were 0.84, 0.87 and 0.90 respectively. These slight differences were not statistically significant (fig. 1, Bottom right).

The volume pulse amplitude at the time of injection varied between .04 and 2.38 in the footpad of the nonoperated side and between .05 and 2.38 on the operated side. The corresponding averages were 0.46 and 0.65 respectively. (Values in per cent of photoelectric current.) Differences on the two sides were
not significant statistically. These measurements do not demonstrate any systematic dilator effect of the operation or of the anesthesia at the time of the injection and indicate an apparent recovery of vascular tone on the operated side.

The possibility that vasomotor reflexes were elicited in the innervated foot by the injection of the small dose of epinephrine was excluded by the performance of three cross circulation experiments in which the blood supply of the recipient's foot was derived from the donor, but the recipient foot's innervation was still intact. In these preparations, neither constrictor nor dilator responses were elicited in the cross perfused recipient pad vessels by the intravenous injection of epinephrine (1 × 10⁻⁸ Gm./Kg.) into the recipient dog. Likewise, no responses were detected in the pad vessels of the normally perfused leg of the recipient dog when epinephrine was injected intravenously into the donor dog.

When much larger doses of epinephrine (1.3 × 10⁻⁸ Gm./Kg.) were used, the reduction in volume pulse amplitude was much greater on the sympathectomized side. Whether this implies greater reactivity would require consideration of any possible difference on the two sides in the intensity of vasomotor reflexes elicited by the injection of the drug.

In 29 observations on 3 dogs in which complete denervation of the foot was done by neurectomy (femoral and sciatic nerves), the percentage decrease in volume pulse amplitude on injecting epinephrine (1 × 10⁻⁸ Gm./Kg.) was about 60 per cent greater than in the nonoperated or sympathectomized dogs. The volume pulse amplitudes at the time of injection were consistently less on the operated side than on the control side, indicating a recovery and augmentation of vascular tone on the operated side.

DISCUSSION

In our opinion, these studies indicate that conventional lumbar sympathectomy does not result in an augmented reactivity of the pad vessels of the dog to physiologic doses of epinephrine.

Several possibilities should be considered in explanation of failure to demonstrate increased responses of the pad's vascular bed to intravenous injection of epinephrine in doses of 1 × 10⁻⁸ Gm./Kg. following a conventional lumbar sympathectomy: First, the dose employed may not excite those resistance vessels which receive an innervation. The inability of Ederstrom and his colleagues to demonstrate an altered pattern of saphenous vein outflow in response to small doses of intravenous epinephrine or norepinephrine in the chronically sympathectomized limb may have a similar explanation. As these investigators demonstrated, the absence of regional flow differences does not preclude the possibility of altered reactivity of the smooth muscle of arteries. Second, completeness of sympathetic denervation may be essential to a sufficient increase in reactivity to become detectable by the procedure used in this study. Such denervation may not be complete after conventional sympathectomy because of the presence of variable accessory pathways. Hence the increased reactivity following neurectomy might be due to extension of the sympathetic denervation. Neurectomy, however, also removes influences other than sympathetic constrictor fibers. Cholinergic vasodilators fibers if present would also be eliminated. The presence of specific cholinesterase in arteriovenous anastomoses of human digits would suggest their presence, although their existence in the hind footpad of the dog has not been established. Sensitization of the vessels of the rabbit's ear to epinephrine has been demonstrated to follow the surgical removal of cholinergic fibers to the ear. Neurectomy would also result in the removal of cholinergic dorsal root fibers. It is apparent that although sympathectomy may remove some cholinergic fibers, neurectomy would effect complete cholinergic as well as adrenergic denervation. Caution must be exercised, therefore, in the interpretation of total neurectomy as merely an ex-
tension of conventional sympathectomy. Third, the hemodynamic approach may not permit the detection of consistent subtle changes which may be present as a result of sympathectomy.

Factors contributing to the augmented responses of the sympathectomized footpad vessels to large doses of epinephrine were not defined by this study.

These data suggest that physiologic concentrations of endogenous epinephrine or epinephrine-like substances in the blood stream do not contribute to therapeutic failure following clinical lumbar sympathectomy.

**SUMMARY**

Following unilateral lumbar sympathectomy, the reactivity of the vascular beds of the footpads of the two hind feet of dogs to a standard physiologic dose of intravenously administered 1-epinephrine was evaluated by photoelectric plethysmography. No significant differences in the responses of the vessels on the sympathectomized and the intact sides of 1 X 10^-8 Gm./Kg. body weight of drug was demonstrated. In doses of 1.3 X 10^-6 Gm./Kg. given intravenously, 1-epinephrine produced a stronger reaction on the operated side. The pad vessels of limbs which had been totally denervated by femoral and sciatic neurectomy showed augmented reactivity to epinephrine at the smallest dosage range. These data emphasize the importance of the possible role of noninnervated terminal vessels, the influence of the extent of the anatomic denervation, the possible influence of the removal of cholinergic influences following denervation procedures and the influence of alterations in central hemodynamics as well as the quantity of circulating humoral substances in evaluating the phenomena of denervation sensitization and the augmentation or return of vascular tone following sympathectomy.

**SUMMARIO IN INTERLINGUA**

Post sympathectomia lumbar unilateral, le reactivitate del vasculatura plantar in ambe pedes posterior de canes tractate con un dose physiologic standard de 1-epinephrina per via intravenose esseva evolutate per plethysmographia photoelectric. Nulle significative differentias esseva demonstrate inter le responsas del vasculatura al latere sympathectomisate e del vasculatura al latere intacte quando le droga esseva administrate in un dosage de 1 X 10^-8 g per kg de peso corporee. In doses de 1.3 X 10^-6 g per kg de peso corporee, administrate per via intravenose, l-epinephrine produciva un plus forte reaction al latere operate. Le vasculaturas plantar de gambas completate disnervate per neurectomia femoral e sciatic exhibiva augmentos del reactivitate a epinephrina in le plus miere doses empleate. Iste constatationes sublinea le importantia del rolo possibile de non-innervate vasos terminal, del influentia exercite per le extension del disnervation anatomic, del influentia possibile del elimination de factores cholinergic post interventiones disnervatori, e del influentia de alterationes in le hemodynamica central tanto ben como del quantitate del circulante substantias humoral pro le phenomenos de sensibilisation post disnervation e le augmento o retorno del "tono" vascular post sympathectomia.

**REFERENCES**


6. Deff, R. S.: Effect of adrenaline and nor-


Responses of Dog's Pad Vessels to Epinephrine Following Sympathectomy or Denervation
THEODORE COOPER, VALLEE L. WILLMAN and ALRICK B. HERTZMAN

Circ Res. 1959;7:574-579
doi: 10.1161/01.RES.7.4.574

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1959 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/7/4/574

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/