Cutaneous Venoconstrictor Response to Local Cooling in the Dog
UNEXPLAINED BY INHIBITION OF NEURONAL RE-UPTAKE OF NOREPINEPHRINE

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ABSTRACT

Studies were performed in dogs to determine if inhibition of neuronal re-uptake of norepinephrine was the cause of the enhanced response to sympathetic nerve impulses seen in the cutaneous veins when the temperature of the blood perfusing them was decreased. Changes in driving pressure of the saphenous vein perfused with blood at constant flow were used to measure venomotor responses. The increases in vеноconstrictor response to norepinephrine infusion caused by cooling the perfusate were similar in both hindlegs, one subjected to acute and the other to chronic sympathectomy. Blocking the reentry of norepinephrine into the sympathetic nerve endings by treating the vein with cocaine did not affect the augmentation by local cooling of the constrictor response to norepinephrine or electric stimulation of the lumbar sympathetic chain. The vеноconstrictor responses to norepinephrine, epinephrine, 5-hydroxytryptamine, and molar potassium chloride were all augmented by local cooling. The results suggest that the sensitizing effect of cold on the smooth muscle of the veins cannot be explained by inhibition of norepinephrine re-uptake by the nerve terminals.

ADDITIONAL KEY WORDS

sympathetic nerves and veins
sympathectomy and veins
smooth muscle and cold
5-hydroxytryptamine and veins
cocaine
saphenous vein

Previous studies (1, 2) have shown that the cutaneous veins of the dog constrict when the temperature of the blood perfusing them is decreased. This constriction is due to an enhanced response of the venous smooth muscle to sympathetic nerve impulses. The vеноconstrictor caused by norepinephrine infusion is similarly increased by a decrease in perfusate temperature (3).

Organs with an extensive sympathetic innervation take up circulating norepinephrine (or epinephrine) and store most of it in a chemically unchanged form in the granulated vesicles of sympathetic nerve endings (4). This uptake can proceed against a concentration gradient and involves both diffusion and an active transport mechanism (5, 6). The uptake and storage might be impaired by a reduction in local temperature, allowing a higher concentration of norepinephrine (whether infused or released from nerve terminals by stimulation) to reach the alpha-receptor sites, thus accounting for the increased sensitivity of the venous smooth muscle to sympathetic nerve impulses and circulating norepinephrine.

Three series of experiments were conducted to test this hypothesis:

(1) A comparison was first made of the effects of changes in perfusate temperature on the vеноconstrictor responses to various vasoactive substances, some of which are not taken up by the nerve endings. The substances used were norepinephrine, epinephrine, 5-
hydroxytryptamine, and molar potassium chloride.

(2) The effects of local changes in temperature on the venoconstrictor response to norepinephrine were assessed in chronically and acutely denervated limbs in the same animal, because chronic denervation has been shown to cause the catecholamine storage sites to deteriorate (7).

(3) The effect of changes in venous perfusate temperature on the constrictor responses to sympathetic nerve stimulation and norepinephrine infusion was examined in the same vein before and after treatment with cocaine because this drug prevents uptake of norepinephrine into the storage sites (7).

The data suggest that the sensitizing effect of cold on the smooth muscle of the superficial veins cannot be explained by inhibition of norepinephrine uptake by the nerve terminals.

Methods

Mongrel dogs weighing 15 to 25 kg were anesthetized with intravenously administered thiopental, 15 mg/kg, and chloralose, 80 mg/kg body weight, and were artificially ventilated with oxygen. The lateral saphenous vein was cannulated at the ankle and perfused at constant flow (flow rate, 100 ml/min) by a roller pump with blood taken from the median sacral artery. Perfusion and femoral vein pressures were measured, and during experiments (which lasted as long as 15 minutes), the common iliac artery was occluded. Previous analysis of the method (8) has shown that any alteration in driving pressure, that is, the difference between perfusion (inflow) and femoral vein (outflow) pressures, is due to a change in the venomotor activity of the lateral saphenous vein. A heat exchanger was placed in the pump outflow line, and the temperature of the blood entering the vein was monitored by means of a thermocouple probe. Esophageal temperature also was measured. Changing the temperature of the water flowing through the heat exchanger resulted in rapid and reproducible changes in venous perfusate temperature (1). In those experiments in which the lateral saphenous veins of both hindlegs were perfused, two pumps and two heat exchangers were used, the two heat exchangers being connected to separate sources of water.

The drugs used in the study were l-norepinephrine bitartrate (Levophed, Winthrop Laboratories), epinephrine (Adrenalin chloride, Parke, Davis and Company), 5-hydroxytrypt-

amine (serotonin creatinine sulphate, Abbott Laboratories), tyramine hydrochloride (Abbott Laboratories), cocaine hydrochloride (10 µg/ml in normal saline), and potassium chloride (75 g/liter in normal saline). Fresh dilutions in normal saline were prepared for each experiment. The doses of norepinephrine are given in terms of the base. The doses of all other agents are expressed in terms of their salts. All test drugs were injected or infused by means of a Harvard infusion-withdrawal pump upstream to the roller pump to ensure adequate mixing.

Treatment of the Vein with Cocaine

The roller pump was arrested, and the vein was slowly perfused with 200 ml of normal saline containing 10 µg cocaine hydrochloride per ml during a 5-minute period. After this treatment, the vein was again perfused with blood by the roller pump.

Lumbar Sympathectomy

Chronic.—At a separate operation, 3 to 3½ weeks before the main experiment, the left lumbar sympathetic chain was dissected free and removed from immediately below the diaphragm to the level of the sacrum.

Acute.—The sympathetic chain was divided at the level of the second or the third lumbar vertebral bodies and dissected free down to the level of the sixth lumbar vertebral body.

The sympathetic fibers to the veins of the hindleg leave the spinal cord in the first to fourth lumbar nerves, and run in the main sympathetic trunk from the third to sixth lumbar ganglia, leaving the sympathetic trunk to join the sciatic nerve by the rami to the sixth and seventh lumbar nerves and the first and second sacral nerves (9, 10). The procedures previously outlined, therefore, should have interrupted the venomotor fibers that traverse the sympathetic trunk. The completeness of both types of sympathectomy was confirmed by the absence of significant changes in venomotor tone on altering the temperature of the blood perfusing the vein, because the powerful venoconstriction that is normally seen on cooling the perfusate is dependent on an intact autonomic nerve supply to the vein (1, 2). The deterioration of the catecholamine storage sites resulting from chronic denervation was assessed by comparing the effects of the same dose of tyramine hydrochloride (0.75 mg) on the chronic and acutely denervated veins.

Lumbar Sympathetic Nerve Stimulation

The centrally divided lumbar sympathetic trunk was stimulated at the level of the fourth or the fifth lumbar vertebral body via a platinum bipolar electrode using a Grass stimulator (Model...
S4). Monophasic impulses of 8 v (supramaximal) and 1-msec duration were used at frequencies of 2 or 4 cps.

Results

Effects of Change in Perfusate Temperature on the Venoconstrictor Responses to Different Vasoactive Substances.—Figure 1 shows tracings from a representative series of experiments in one animal weighing 17 kg. After acute left lumbar sympathectomy, changes in perfusate temperature from 42° to 17°C caused an increase in left saphenous driving pressure of only 8 mm Hg (control panel). In the subsequent experiments, identical changes in perfusate temperature greatly augmented the venoconstrictor response to norepinephrine, molar potassium chloride, and 5-hydroxytryptamine.

In five dogs, the responses of the left lateral saphenous vein to infusions of norepinephrine and 5-hydroxytryptamine were compared at venous perfusate temperatures of 37°, 42°, and 27°C. The increase in driving pressure varied from dog to dog but could be compared if the responses at perfusate temperatures of 42° and 27°C were expressed as percentages of the response obtained at 37°C with each agent in each dog (Fig. 2).

In three dogs, a similar comparison was made between norepinephrine and epinephrine. With norepinephrine, the responses at 42° and 27°C averaged 38% and 309%, respectively, of those at 37°C, whereas with epinephrine the corresponding figures were 35% and 341%.

The experiments involving infusion of molar potassium chloride into the vein at a sufficient rate to cause venoconstriction were difficult to complete without causing serious cardiac arrhythmias or cardiac arrest. Three experiments, however, were completed in two dogs. A tracing from one of these experiments (Fig.
1) illustrates that changes in local perfusate temperature also affected the venoconstrictor response to potassium chloride.

**Effect of Changes in Perfusate Temperature on the Venoconstrictor Responses to Norepinephrine after Acute and Chronic Sympathectomy.**—In three dogs, all of which had had a left lumbar sympathectomy 3 to 3½ weeks previously, a right lumbar sympathectomy was carried out, and the effect of changes in perfusate temperature on the venoconstrictor response to norepinephrine was compared in the acutely denervated right leg and chronically denervated left leg in the same animal. Because of the changes in sensitivity to norepinephrine after chronic sympathectomy, the dose was adjusted so that the changes in driving pressure in each leg were not grossly different. A representative experiment in one of the three dogs is shown in Figure 3. In both legs, a change in perfusate temperature from 42° to 22°C augmented the response to norepinephrine. When the increase in driving pressure obtained by warming and cooling the venous perfusate was expressed as a percentage of the response at 37°C, the changes were similar in both legs in all three animals (Fig. 4).

Injection of 0.75 mg of tyramine hydrochloride into each vein at the termination of the experiments caused increases in driving pressure of 0, 39, and 8 mm Hg in the chronically denervated veins, as compared to 75, 132, and 98 mm Hg in the acutely denervated vessels (Fig. 3).
Effect of changes in perfusate temperature on saphenous venoconstrictor responses to norepinephrine in acutely denervated right leg and chronically denervated left leg. In three dogs, a total of ten experiments was performed in each leg, and the graphs show average results and ranges obtained in each dog.

Effects of Local Changes in Temperature on the Venoconstrictor Responses to Norepinephrine and to Sympathetic Nerve Stimulation before and after Treatment of the Vein with Cocaine.—In three dogs, the effect of changes in venous perfusate temperature on the
Effect of changes in perfusate temperature on saphenous venomotor responses to norepinephrine before and after blocking neuronal re-uptake of norepinephrine by treating the vein with cocaine. Five experiments before cocaine and five after cocaine administration in three dogs.

Effect of changes in perfusate temperature on saphenous venomotor responses to sympathetic nerve stimulation at 4 cps (dog B, left) and 2 cps (dog C, right), before and after blocking neuronal re-uptake of norepinephrine by treating the vein with cocaine.

venoconstrictor response to infusion of norepinephrine was assessed before and after treatment of the vein with cocaine. Figure 5, upper panels, shows tracings from a typical experiment in one dog weighing 18 kg, and Figure 6 gives the results obtained in all three animals. Treatment of the vein with cocaine invariably rendered it more sensitive to norepinephrine infusion, but did not alter either the enhancing effect of local cooling or the inhibiting effect of local warming on the response.

In two of these animals, it was also possible to assess the relationship between the local temperature and the response to sympathetic nerve stimulation before and after cocaine.
treatment, and similar results were obtained (Fig. 5, lower panels, and Fig. 7).

Discussion

There is valid evidence that circulating norepinephrine (or epinephrine) is taken up and stored in the granulated vesicles within adrenergic nerves and chromaffin cells (11), and its localization within these vesicles has been shown by several techniques (4, 12-14). The catecholamine storage granules from chromaffin cells are also capable of concentrating 5-hydroxytryptamine as well as norepinephrine and epinephrine from their medium (15). Granules prepared from sympathetic nerve endings at one time were believed to take up only catecholamines and certain beta-hydroxylated monophenols such as octopamine (16), but there is recent evidence that sympathetic nerves are also capable of taking up 5-hydroxytryptamine (17). Changes in the rate of uptake of norepinephrine, epinephrine, and 5-hydroxytryptamine by the nerve endings might thus explain the similarity of the effects of changes in local temperature on the venoconstrictor responses to these three agents. However, the finding that local cooling also enhances the venoconstrictor response to molar potassium chloride suggests that neuronal uptake contributes little or nothing to the temperature effects.

After chronic sympathetic denervation, the denervated organ loses its ability to take up norepinephrine-3H from the blood (18), and the catecholamine storage sites deteriorate (7). That changes in local temperature had similar effects on the response of acutely and chronically denervated saphenous veins is further evidence that uptake of catecholamine by nerve terminals does not have a major role in the effect of temperature on the venoconstrictor responses to catecholamine infusion. The greatly reduced response to tyramine in the chronically denervated vein, as compared to the acutely denervated contralateral vessel, was evidence that chronic denervation had been effective in depleting catecholamine stores in all three dogs.

Cocaine, which blocks the reentry of released norepinephrine into the sympathetic nerve ending (7, 19), enhanced the responses of the saphenous vein to both sympathetic nerve stimulation and infusion of norepinephrine. The venoconstriction induced by both methods, however, was still modified profoundly by changes in local temperature after treatment with cocaine (Figs. 5, 6, and 7), providing further evidence that the effect of such temperature change was not due to altered rate of uptake of norepinephrine by the nerve terminals.

The observation that changes in local temperature modified the venoconstrictor response to norepinephrine, epinephrine, and 5-hydroxytryptamine in the same way suggested either that the kinetics of drug-receptor interaction at both alpha and 5-hydroxytryptamine receptor sites are affected to the same extent by temperature changes, or that the temperature sensitivity of some later common step in the chain of reactions linking receptor activation to smooth-muscle contraction is responsible for the observed temperature effects. The venoconstriction induced by potassium chloride presumably occurred with the smooth muscle in a depolarized state, and the finding that local cooling also enhances the constriction induced by potassium favors the view that changes in temperature do not modify reactions occurring at the cell membrane, but rather act either on excitation-contraction coupling or on the process of contraction itself.

Keatinge (20), measuring the electric and mechanical responses of spiral strips of carotid artery from sheep to constrictor hormones and potassium-rich solutions at normal and low temperatures, found that cooling to 5°C prevented norepinephrine from causing either its electric or its mechanical effects, apparently blocking an early stage in its action on the cell. At 15°C, there was partial inhibition of both the electric and the mechanical response, and at 25°C, the initial change in membrane potential was as great as at 35°C, but the
subsequent plateau was usually lower; the mechanical response was smaller and slower at 25° than at 35°C. This is in contrast to our observation that the lateral saphenous vein constricted more powerfully at 27° than at 37°C in response to norepinephrine.

In Keatinge's experiments, cooling to 5°C did not prevent potassium-rich solutions from depolarizing the carotid artery cells, but the contractions at 5°C were approximately 5%, and at 15° to 20°C, approximately 25% of the size of those at 35°C. These results were interpreted as indicating that cooling seriously impaired excitation-contraction coupling, because the contractile machinery was still capable of functioning well at 5°C, as judged by the large contractions in response to strong electric stimulation at that temperature. In our three satisfactory potassium-infusion experiments in two dogs, the saphenous venoconstrictor responses at perfusate temperatures of 27° and 17°C were greater than at 37°C (Fig. 1).

The differences between the results of the in-vitro studies of Keatinge on carotid artery strips from sheep and the results of our in-vivo examination of the responses of the lateral saphenous vein of the dog might be due to the species difference or to differences in technique. However, they might represent genuine differences in the response to temperature change of the smooth muscle of a vessel that is not normally exposed to wide fluctuations in temperature (the carotid artery), as compared to the smooth muscle of a vessel that is exposed to a wide variation in both ambient and blood temperatures (the superficial limb vein). This possibility is supported by the recent work of Glover and associates (21), who removed ear and femoral arteries from anesthetized rabbits and perfused them simultaneously with Krebs' bicarbonate solution. They found that whereas the response of the femoral artery to injections of norepinephrine decreased steadily with cooling from 37°C and disappeared at about 13°C, the response of the ear artery (which contributes to temperature regulation in the rabbit) increased to a maximum at about 24°C and then decreased but did not disappear until 7°C.

Kaufmann and Fleckenstein (22) found that if the isolated atria or papillary muscles of the guinea pig were cooled from 37° to 17°C in Tyrode's solution containing 1.8 mM calcium, a threefold to sixfold increase in isometric peak tension occurred. But if the calcium concentration of the Tyrode's solution was increased to 14.4 mM, maximal activation of the contractile machinery already took place at 37°C so that only an inhibitory action of cooling could be seen. They concluded that at 37°C and in Tyrode's solution containing only 1.8 mM calcium, the availability of calcium ions is a rate-limiting factor in the activation of the contractile system, since, under these conditions, the duration of the action potential is not sufficiently great to allow a calcium entry of such a magnitude that the absolute maximum tension will be reached. However, a full calcium saturation of the contractile elements can be achieved either, at 37°C, by a considerable augmentation of the extracellular calcium concentration or, in normal Tyrode’s solution, by a prolongation of the action potential as happens in the cold. Such a mechanism, however, cannot be invoked to explain the increased venoconstrictor responses obtained with local cooling, since the enhanced response was also seen with potassium chloride which would have depolarized the venous smooth muscles.

Kaufmann and Fleckenstein's data suggested that in the temperature range 37° to 14°C, the calcium-binding capacity of the sarcoplasmic reticulum, and thus the rate of calcium inactivation within the myocardial cells, was not an important factor in the inotropic response to cold. Venous smooth muscle may well differ from cardiac muscle in this respect, and it seems likely that partial inhibition of calcium binding within the smooth muscle cell by cold may have a major part in the enhanced venoconstrictor responses obtained on local cooling of the vein.
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References


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