Effect of temperature on reactivity of isolated cutaneous veins of the dog

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Vanhouette, Paul M., and John T. Shepherd. Effect of temperature on reactivity of isolated cutaneous veins of the dog. Am. J. Physiol. 218(1): 187-190. 1970—Excised segments of dogs' saphenous veins were perfused, with autologous blood or Krebs-Ringer solution, at constant flow. Changes in driving pressure were used to measure venomotor responses. The reactivity of the veins to electric stimulation and various vasoactive agents was tested at 37 C, and the effects of changes in temperature (25-43 C) were investigated. When the veins were constricted by electric stimulation, norepinephrine, 5-hydroxytryptamine, acetylcholine, or adenosine triphosphate, cooling the perfusate from 37 C augmented the constriction and warming depressed it. Cooling the vein in the absence of external stimulation did not cause the vein to constrict. When constricted with potassium chloride or barium chloride, the veins relaxed with cooling and constricted further with warming, indicating that the contractile process of the venous smooth muscle is depressed by cooling and facilitated by warming. Thus, the thermal effect on the venoconstriction induced by electric stimulation or drugs is not due to the direct effect of temperature on the contractile machinery itself.

In the intact dog, the cutaneous veins are sensitive to local temperature changes: cooling augments and warming depresses the venomotor reaction to adrenergic stimulation (14-16). Webb-Peploe (13) showed that the sensitizing effect of cold on the smooth muscle of the veins cannot be explained by inhibition of norepinephrine reuptake by the nerve terminals. Since the venoconstrictions elicited by 5-hydroxytryptamine and potassium chloride were also potentiated by cooling, he suggested that the temperature changes may be affecting either excitation-contraction coupling or the process of contraction itself.

Since further analysis of the mechanism underlying the temperature effects is difficult in the intact dog, we conducted a series of experiments on cutaneous veins perfused at constant flow in an organ bath. The reactivity of the veins to electric stimulation and to various vasoactive agents was tested and the effects of altering the perfusate temperature were examined. Cooling the perfusate augmented and warming depressed the venoconstrictions caused by electric stimulation, norepinephrine, 5-hydroxytryptamine, acetylcholine, and adenosine triphosphate. In the absence of any external stimulation, cooling the perfusate did not cause venoconstriction. By adjusting the dose of potassium or barium chloride in the perfusate so that a vasoconstriction was obtained, the veins could be relaxed with cooling and constricted with warming.

METHODS

The experiments were performed on isolated saphenous veins from mongrel dogs (weight, 17-25 kg) anesthetized with intravenously administered pentobarbital sodium (20 mg/kg). A venous segment (length, 8 cm) was dissected in situ and the collaterals tied off near to their origin. Each end of the cut segment was secured to the tip of a thin-walled glass cannula with an outside diameter similar to the inside diameter of the vein. The preparation was placed horizontal in a chamber filled with Krebs-Ringer bicarbonate solution (NaCl, 118.3 mM; KCl, 4.7 mM; MgSO4, 1.2 mM; KH2PO4, 1.2 mM; CaCl2, 2.5 mM; NaHCO3, 25.0 mM; and glucose, 11.1 mM) maintained at 37 C and aerated with a 95 % O2-5 % CO2 gas mixture. The distance between the two cannulas was adjusted so that the length of the segment was similar to its length in vivo.

The vein was perfused, in the direction of the flow in the intact animal, at constant flow (100 ml/min) with either autologous blood taken from the median sacral artery of the donor animal or with the same Krebs-Ringer solution as used in the chamber. The perfusion circuit consisted of a roller pump and a depulsator and heat exchanger placed in the pump outflow line. The inflow temperature, measured by a thermistor probe (Yellow Springs Instrument Co.) inserted between the heat exchanger and the inflow cannula, and the inflow and outflow pressures, measured by pressure transducers (Statham P23De) connected to sidearms of the glass cannulas, were continuously recorded. Since flow was constant, an increase in driving pressure (inflow pressure minus outflow pressure) was caused by constriction of the venous smooth muscle and a decrease, by relaxation.

Electric stimulation. The technique for electric stimulation of isolated saphenous veins has been described previously (11, 12). Two rectangular platinum electrodes (90 x 10 mm, 0.5 mm thick) were placed parallel to the vein; this provided a homogeneous electric field with simultaneous activation of the whole venous segment. Electric impulses consisted of square waves (8 v, 2 msec) provided by a direct current power supply and switching transistor (RCA 2N-3055) triggered by a Grass stimulator (model S4). This provided supraliminal stimulation, and hence the magnitude of the evoked venoconstriction was dependent only on the frequency of stimulation.
Pharmacologic stimulation. The agents used were: L-norepinephrine bitartrate (Levophed, Winthrop Laboratories), acetylcholine hydrochloride (Roche Laboratories), 5-hydroxytryptamine (Aldrich Chemical Co., Inc.), adenosine triphosphate magnesium salt (Mann Research Laboratories, Inc.), potassium chloride, and barium chloride. To ensure adequate mixing, the agents were added to the perfusate, upstream from the roller pump, by means of a Harvard infusion pump. For each drug, the number of tested preparations reported in the results section refers to saphenous segments taken from different dogs.

Temperature changes. Altering the temperature of the water flowing through the heat exchanger produced rapid and reproducible changes in the temperature of the perfusate. Usually the perfusate was suddenly warmed from 37 to 43 C and then gradually cooled. At each temperature (approximately 43, 37, 30, and 25 C), the driving pressure was allowed to stabilize and then the venomotor reaction was studied.

RESULTS

In preliminary experiments, six saphenous veins were first perfused with blood and then with Krebs-Ringer solution. Electric stimulation and norepinephrine were used to constrict the veins. A similar augmentation of the constriction occurred with cooling and relaxation with warming each perfusate. Therefore, in the main series of experiments, the veins were perfused only with Krebs-Ringer solution.

Temperature and basal venomotor tone. In 29 saphenous vein segments from 20 dogs, the perfusate temperature was changed in the absence of venoconstrictor stimulation. In 16 segments, cooling or warming the perfusate had no effect on the driving pressure (Fig. 1). In eight segments, cooling resulted in minimal increases (maximum, 2 mm Hg from an initial pressure of 10 mm Hg) in driving pressure; in five veins, warming the perfusate caused a slight increase (maximum, 5 mm Hg from an initial pressure of 12 mm Hg) in driving pressure.

Temperature and electrically induced venoconstriction. In the same 29 segments, a sustained venoconstriction was induced by low-frequency electric stimulation. The frequency of stimulation was adjusted to obtain comparable increases in driving pressure, at a perfusate temperature of 37 C, in the different preparations. When the venoconstriction reached its plateau, the temperature of the perfusate was altered (Fig. 1). Cooling the perfusate gradually resulted in progressively increased venoconstriction, whereas warming caused relaxation. This effect of local temperature could be reproduced repeatedly on the same preparation. When the perfusion pressure was increased by increasing the rate of flow through the segment (Fig. 1), to the level achieved by the continuous electric stimulation, no effect of temperature variations was evident. Figure 2 compares, in 10 veins taken from different dogs, the effects of similar changes in temperature imposed during a sustained electric stimulation and in the absence of venomotor activation. In four of these dogs, both cephalic and saphenous veins were studied and gave similar responses.

Temperature and norepinephrine. In 11 saphenous vein segments the venoconstriction induced by addition of nor epinephrine to the perfusate also showed dependency on local temperature. However, the potentiation with cooling was less pronounced than that observed for similar temperature changes induced during continuous electric stimulation of the same veins (Fig. 3). The data presented in Fig. 3 were selected from those eight segments in which the increase in driving pressure at 37 C was similar with norepinephrine and electric stimulation.
When venoconstriction was obtained in these preparations by adding the norepinephrine either to the outer bath or to the perfusate, the same dependency on temperature was observed.

Temperature and 5-hydroxytryptamine. In five vein segments the sustained venoconstriction evoked by 5-hydroxytryptamine was depressed by warming and augmented by cooling the perfusate (Fig. 4). In the same preparation, the magnitude of the change was similar to that obtained during continuous low-frequency electric stimulation.

Temperature and acetylcholine. In seven vein segments the venoconstriction caused by acetylcholine was potentiated by cooling and depressed by warming the perfusate (Fig. 4). In two segments the potentiation by cooling could not be maintained, and in one other segment the depressing effect of warming was followed by a progressive return toward the driving pressure obtained at 37°C.

Temperature and adenosine triphosphate. In seven vein segments, infusion of large doses of the magnesium salt of adenosine triphosphate resulted in slight, transient increases in driving pressure. These venoconstrictions, although not comparable to the reactions observed with the other vasoconstrictor agents, were also potentiated by cooling and depressed by warming the perfusate (Fig. 4).

Temperature and potassium chloride. In six vein segments, potassium chloride was infused at 0.25 mg/min. This caused, at 37°C, a marked increase in driving pressure; cooling the perfusate decreased the pressure. This decrease was preceded in four preparations by a small, transient increase. Warming the perfusate increased the constriction caused by potassium chloride (Fig. 5). With infusion of potassium chloride at 0.25 mg/min, the vein no longer reacted to electric stimulation. Five minutes after the infusion of potassium chloride was ended the vein segment showed its normal sensitivity to electric stimulation and temperature.

Temperature and barium chloride. The reactions to infusion of barium chloride were investigated in six vein segments. A small dose of barium chloride did not alter the perfusion pressure but potentiated the reaction to electric stimulation imposed at constant intervals (Fig. 6). When the potentiation reached its plateau the same stimulation frequency as used in control conditions was tested; notwithstanding the obvious potentiation of the reaction, cooling the perfusate still increased the reaction and warming depressed it.

In larger doses, barium chloride produced a significant venoconstriction which was reduced by cooling and potentiated by warming the perfusate (Fig. 7).
DISCUSSION

The venoconstriction caused in vitro by electric stimulation and various classic vasoactive drugs was augmented by cooling and reduced by warming the perfusate, confirming observations in the intact animal (14, 15). Hence, as indicated by previous in vivo experiments (16), the response is a local one and no reflex arc is involved. Since the temperature sensitivity of the venomotor reaction was still present when Krebs-Ringer solution was used instead of autologous blood to perfuse the veins, the response is not due to temperature-dependent variations in the physical or chemical properties of the blood.

Changes in viscosity of the Krebs-Ringer solution with changes in temperature are unlikely to account for more than a small fraction of the observed changes in perfusate pressure because, in the absence of active constriction at 37 C, there was no change in perfusate pressure with alterations in perfusate temperature.

Changes in the pH of Krebs-Ringer solution do not exceed 0.06 pH unit for a temperature change from 15 to 20 C. Since changes of the order of 0.2 pH unit are required to cause small but significant alteration in the reactivity of the saphenous veins of the dog (8, 10), the small changes in pH in the present experiments cannot explain the marked potentiation of venomotor reactions during cooling.

The constriction of isolated saphenous vein segments of the dog caused by electric stimulation is abolished by bretylium tosylate, reserpine pretreatment, or alpha-adrenergic blockade (11). Similar findings are reported for other isolated venous tissue (5, 6). Thus, electric stimulation causes venoconstriction by release of norepinephrine from nerve terminals in the vein wall. The observation that the potentiation by cooling of the venoconstriction induced by infusion of norepinephrine was less than that induced by electric stimulation is puzzling, particularly since in vivo experiments show the opposite relationship (16). We have no explanation for this. One obvious difference between the in vivo and in vitro studies is the recirculation of the infused norepinephrine in the intact dog.

The thermal sensitivity is not specific for adrenergic substances. It was also seen with other agents which constrict cutaneous veins—5-hydroxytryptamine (3), acetylcholine (3), and adenosine triphosphate. These different agents may be assumed to initiate contraction by their action on the cell membrane (4).

Webb-Peploe (13), in three in vivo studies on two dogs, noted that cooling potentiated potassium chloride-induced venoconstriction, but the resultant cardiac arrhythmias prevented detailed studies. The present experiments with potassium chloride show that it has complex actions but, when large doses were used to depolarize the cell membrane (1), cooling decreased and warming increased the venoconstriction in all the saphenous segments. Barium is believed to release intracellularly bound calcium in smooth muscle cells (4, 7). The higher intracellular calcium concentration can account (9) for the increased reactivity to electric stimulation observed in our experiments. With larger doses, barium probably causes the contractile protein to shorten directly (4, 7), which explains the venoconstriction we observed. This venoconstriction, like that caused by potassium, was reduced by cooling and increased by warming.

When these observations with potassium and barium are combined with those of Bohr and associates (2), who showed that the rate of tension development of glycerol-extracted vascular smooth muscle increased directly with temperature, it can be concluded that the contractile process of venous smooth muscle is depressed by cooling and facilitated by warming. Thus, the potentiation by cooling of the venoconstriction induced by electric stimulation and drugs is unlikely to be due to the direct effect of temperature on the contractile machinery of the smooth muscle cell. The observation that cold potentiated the action of various drugs but not that of potassium chloride suggests that temperature may affect the excitation mechanisms of the venous smooth muscle cell.

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