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The Effect of Cold on Adrenergic Neurotransmission in Canine Saphenous Arteries and Veins (42696)

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Abstract. The effect of severe cold (5 to 10°C) on adrenergic neurotransmission was compared in the isolated cutaneous (saphenous) artery and vein of the dog. The vein contracted to sympathetic nerve stimulation at temperatures as low as 10°C; higher temperatures were needed for the artery to contract. Both blood vessels contracted to exogenous norepinephrine at temperatures as low as 5°C. However, the contractile response to exogenous norepinephrine was less in the saphenous artery, and contractions to high K^+ solution were depressed by cooling more in the artery than in the vein. During electrical stimulation of the sympathetic nerves in saphenous arteries and veins previously incubated with labeled norepinephrine, progressive cooling from 37 to 5°C caused a sharp decline in overflow of [^{3}H]norepinephrine and its metabolites. However, overflow of labeled norepinephrine in both blood vessels continued at very cold temperatures. Thus the inability of the saphenous artery to contract to sympathetic nerve stimulation at 10°C can be explained by a greater sensitivity of the arterial smooth muscle to the direct depressant effect of cold, rather than to a differential release or metabolism or norepinephrine in the arterial wall or a loss of responsiveness to norepinephrine at very cold temperatures.

During exposure of the fingers to severe cold, Lewis (1) noted an initial constriction, followed by alternating periods of dilation and constriction which he called the "hunting reaction." Later Greenfield and Shepherd (2) demonstrated from plethysmographic observations that the finger veins remained constricted in cold water (0–6°C) at the same time that finger blood flow increased.

In an attempt to understand some of the complex local events which cause blood flow changes during severe cold, we previously investigated how cold effects adrenergic neurotransmission and smooth muscle reactivity in the isolated cutaneous (saphenous) veins of the dog (3). Despite the depressant effect of cold on the contractility of smooth muscle (4–6) and on the release of norepinephrine by the adrenergic nerves (7), saphenous veins contracted by sympathetic nerve stimulation at 37°C developed further tension when cooled to as low as 10°C (3). This potentiation during cold caused most likely by an enhanced affinity of postjunctional α-adrenoceptors to neuronally released norepinephrine, since contractions to exogenous norepinephrine were also increased by cooling (8). When extrapolated to the intact organism, these findings may explain why cutaneous veins remain constricted during severe cold. It is not known, however, why arterial vessels relax at the same temperature at which the veins constrict (2). Thus, the purpose of the present study was to compare the effect of cold on canine cutaneous arteries and veins.

Methods. Isolated saphenous arteries and veins were obtained from mongrel dogs anesthetized with sodium pentobarbital (30 mg/kg iv). The preparations were studied in a physiological salt solution (composition, m*M*: NaCl, 118.3; KCl, 4.7; $CaCl_2$, 2.5; $NaHCO_3$, 25; KH_2PO_4, 1.2; $MgSO_4$ 1.2; Ca EDTA, 0.026; and glucose, 11.1) aerated with a 95% O_2–5% CO_2 gas mixture.

1. Organ bath experiments: Isometric tension recording. Rings (4–5 mm long) were placed in organ baths (25 ml) filled with physiological salt solution and attached to force transducers (Statham UC2) for continuous isometric tension recording.

Electrical stimulation. Two platinum elec-

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trodes were placed parallel to the rings to stimulate the adrenergic nerve endings. Impulses (rectangular waves, 9 V, 2 msec) were provided by a Grass stimulator and a d.c. amplifier. At the beginning of each experiment, rings were placed at the optimal point of their length–tension relationship using standard electrical stimulation (16 Hz, 10 msec), and allowed to equilibrate for 30 min.

Cooling. The temperature of the system was altered by changing the temperature of the water circulating through water jackets surrounding the muscle baths. A thermistor probe (Yellow Springs Instrument Company) continuously monitored the temperature of the bath solution. The solution was maintained at 37°C, except when experiments were in progress.

Drugs. l-Norepinephrine bitartrate (Sigma) and phentolamine mesylate (Ciba) were added to the bath solution in 0.1-ml aliquots. The K^+ concentration of the physiological salt solution was altered by equimolar replacement of Na^+.

Experimental protocols. In some experiments, rings were contracted with electrical stimulation or norepinephrine at 37°C until a stable contraction was recorded. The bath solution surrounding the vessels was then cooled to 5°C for 10 min, increased to 10°C until tension had equilibrated, and then cooled again at 5°C. Throughout this period, the electrical stimulation or norepinephrine exposure was continued. The same protocol was followed for temperature changes between 10 and 15°C, and 15 and 20°C. In other experiments on the arteries, as previously performed in the veins (3), rings were contracted to norepinephrine or K^+ (in the presence of 10^{-5} to 3×10^{-5} M phentolamine), and then equilibrated for 20 min at 5, 10, 15, or 20°C (selected at random). The effect of cold on the response to ED_{50} concentrations (concentration of agonist causing half-maximal contraction) of norepinephrine and K^+ and the half-maximal frequency of electrical stimulation were studied for each blood vessel to allow for comparative responses.

2. Superfusion experiments. Longitudinal strips of saphenous arteries and veins (4–6 cm long, 2–3 mm wide) were incubated for 120 min in [^{3}H]norepinephrine (10^{-6} M). After incubation, the tissues were rinsed in fresh physiological salt solution and mounted for superfusion. The strips were suspended at 4 g of tension, allowed to equilibrate, and then were superfused by means of a roller pump at 3 ml/min with oxygenated physiological salt solution at 37°C. For electrical stimulation (4–6 V, 2 msec), two platinum wires (0.5 mm in diameter, 10 cm long) were placed parallel to and in contact with the strips.

After an initial washout period of 120 min, the superfusate was collected every 2-min over a 1-hr period (by means of a fraction collector) for estimation of the efflux of total radioactivity. The superfusate was then cooled (by means of a heat exchanger) to 5°C for 30 minutes, at which time electrical stimulation (5 Hz) was started and continued throughout the experiment. After 20 min, the temperature was increased to 10°C for 20 min, and further stepwise increases (to 15, 20, 25, 30, and 37°C; 20 min each) were imposed in a similar fashion during continuous stimulation.

At the end of the experiments with [^{3}H]norepinephrine, the strips were blotted dry, weighed, and the tritiated compounds were extracted with 1 N acetic acid containing 0.03 mM disodium EDTA and 5 mM ascorbic acid. Fractional release of tritiated compounds was calculated as the ratio of disintegrations per minute (dpm) released per unit time to the total dpm extracted plus dpm released.

To determine the amounts of intact [^{3}H]norepinephrine in the superfusate, samples for column chromatographic analysis were obtained by pooling 3 of the 2-min samples taken immediately before switching to 10, 15, 20, 25, and 30°C, respectively. In these samples, tritiated norepinephrine was separated from its major metabolites and radioactivity was measured as described previously (9).

Samples (1 ml) of the incubation solutions, superfusate, the extraction medium, and the fractions obtained during the column chromatographic procedure were added to 10 ml of Safety-Solve (Research Products International) and the radioactivity was measured in a liquid scintillation spectrometer (Beckman LS 8800); corrections for quenching were made by the external standard methods. All samples were counted for 10 min or until 10,000 cpm was reached.

3. Statistical analysis. Student's *t* test for paired or unpaired observations was used. Each experiment was performed on arteries or veins from six different dogs ($n = 6$), and results are expressed as grams of developed tension for tension recording experiments. P values smaller than 0.05 were considered to be statistically significant.

Results. *Isometric tension recording.* To stimulate low level sympathetic nerve stimulation, saphenous arteries were stimulated continuously at 3.5 Hz (half-maximal frequency) at 37°C. Temperature was then lowered to 5°C until tension stabilized, and then changed to 10°C. In similar experiments performed earlier in rings of saphenous veins, changing temperature from 5 to 10°C during continuous stimulation at 2.0 Hz (half-maximal frequency) caused a large and reversible contraction (3) (Fig. 1). However, in the saphenous artery stimulated at 3.5 Hz, raising the temperature of the bath solution from 5 to 10°C did not induce an active change in tension (Fig. 1A). The absence of contraction in the saphenous arteries was not secondary to a lack to viable innervation, as alternating temperature from 10 to 15°C and from 15 to 20°C during continuous electrical stimulation caused large and reversible increases in tension (Fig. 2A) and 13.9 ± 2.6 g of tension resulted from 16 Hz stimulation at 37°C.

However, the ability of the vein but not the artery to contract to electrical stimulation could reflect: (1) a more profound depressant effect of cold on the vascular muscle excitation–contraction process in arteries than veins; and/or (2) a differential release of norepinephrine from adrenergic nerve endings in arterial and venous muscle; a greater depression by cold of transmitter release in the arterial wall could result in an inability to contract to electrical stimulation at temperatures between 5 and 10°C. To investigate the first possibility, saphenous arteries were continuously exposed to norepinephrine ($10^{-6}M$) at 37°C and cooled to 5°C. At 5°C, active tension was still present and was enhanced by raising temperature to 10°C (Fig. 1A, Fig. 2B). These data provided evidence that the arterial muscle maintains its respon-

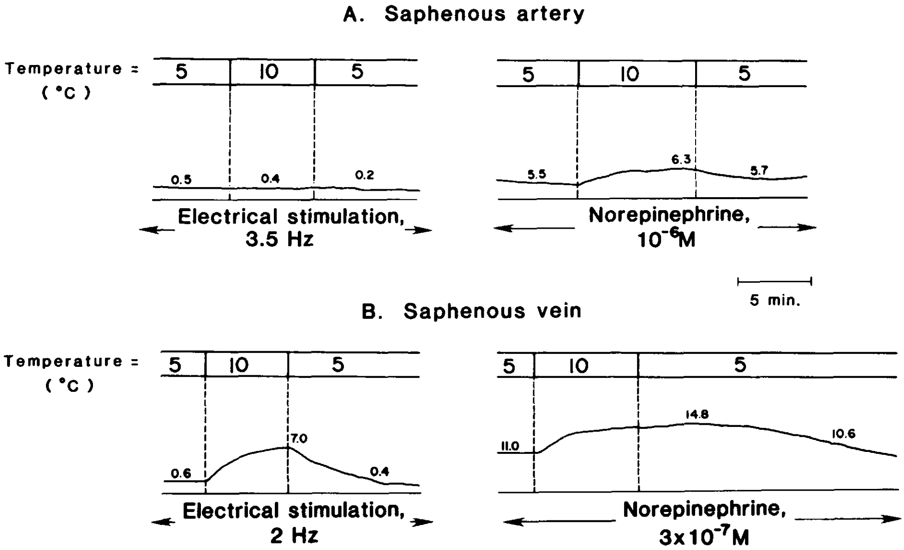

FIG. 1. Isometric tension recordings in a ring of saphenous artery and vein during continuous electrical stimulation (ED_{30} = 3.5 and 2.0 Hz, respectively) and continuous exposure to exogenous norepinephrine ($ED_{50} = 10^{-6}$ and 3×10^{7} *M*, respectively). Numbers above the tracings refer to grams of developed tension. Note that the saphenous artery did not contract to electrical stimulation when warmed from 5 to 10°C, but did contract to norepinephrine. In contrast, when warmed from 5 to 10°C, the saphenous vein contracted to both stimuli.

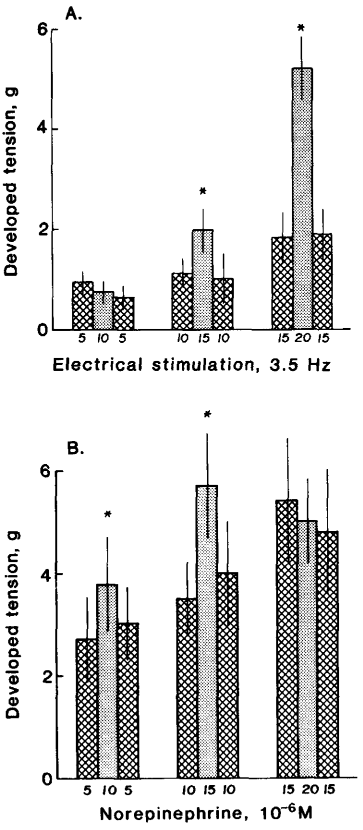

FIG. 2. Effect of changes in temperature on the response of six canine saphenous arterial rings to electrical stimulation (3.5 Hz) and to exogenous norepinephrine (10^{-6} *M*). Note the absence of an increase in tension during electrical stimulation when the artery was warmed from 5 to 10°C, but a significant and reversible increase in tension when warmed from 10 to 15°C and from 15 to 20°C. With exogenous norepinephrine (10^{-6} *M*), active tension was maintained at 5°C and a significant increase in tension occurred with warming from 5 to 10°C and at higher temperatures (* denotes significant increase in tension).

siveness to norepinephrine at very cold temperatures, as had been documented earlier in its venous counterpart (3) (Fig. 1B). Figure 3 provides further documentation of the ability of both saphenous arteries and veins to respond to norepinephrine. Vessels contracted to exogenous norepinephrine at 37°C contracted further when temperature was lowered to 20 and 15°C, and had maintained (artery) or enhanced (vein) tension development at 10°C. At 5°C, the artery maintained 17 ± 4.1% of its initial tension while the vein had 112 ± 8% of their original tension developed at 37°C. Although vascular tone of the saphenous artery was more profoundly affected by cold, both vessels contracted well to norepinephrine at 10°C. Thus, the inability of the saphenous artery to respond to the neuronally released norepinephrine induced by electrical stimulation at 10°C is not secondary to a loss of responsiveness to the catecholamine at very cold temperatures.

To determine if the direct depressant effect of cold on vascular muscle differed between saphenous arteries and veins, vessels were continuously exposed to ED_{50} concentrations of K^+ (20 m*M* in artery; 40 m*M* in vein) at 37°C, and then cooled to 5, 10, 15, or 20°C. Cold depressed the contraction to K^+ in a temperature-dependent manner (Fig. 4). At all lower temperatures studied, however, cold inhibited the K^+-induced contraction significantly more in the artery than in the vein, indicating a greater direct depressant effect of cold on arterial muscle.

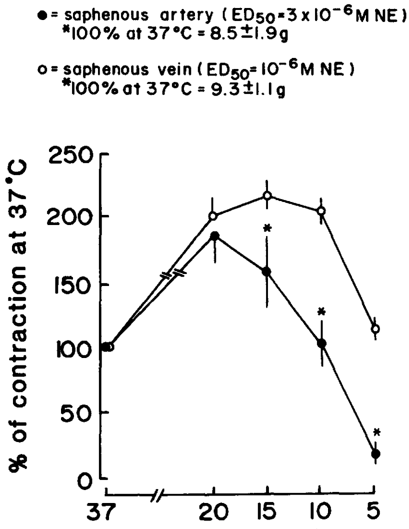

FIG. 3. The effect of cold on norepinephrine-induced contractions. ED_{50} concentrations of 3×10^{-6} and 10^{-6} were used to contract the saphenous artery and vein at 37°C, respectively. Cooling from 37°C to as low as 15°C augmented contractions in the artery and vein. At 10°C, tension remained augmented in the vein and was maintained in the artery. Both vessels sustained active tension at 5°C, although the response of the artery was depressed. Data shown as means ± SEM (* denotes significant difference between values for artery and vein).

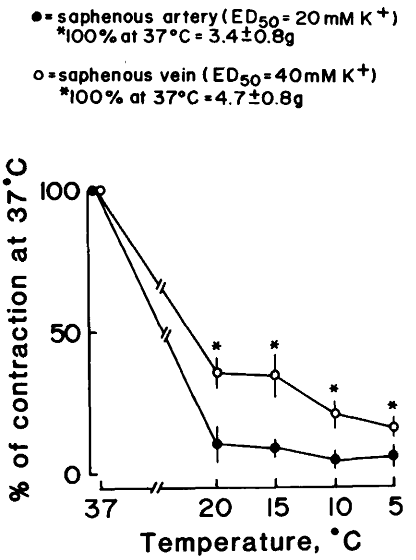

FIG. 4. The effect of cold on contractions caused by high K^+ solutions. ED_{50} concentrations of 20 and 40 mM were used to contract the saphenous artery (●) and vein (O), respectively. Progressive cooling (20, 15, 10, and 5°C) depressed the K^+-induced contraction initially obtained at 37°C in the artery, as has been noted previously in the vein (3). However, this depression was significantly greater in the artery than the vein at all temperatures studied. Data shown as means ± SEM (* denotes significant difference between values for artery and vein).

Release of norepinephrine and metabolites. To investigate if the release of norepinephrine from adrenergic nerve endings in arterial and venous muscle had a different sensitivity to cold, in six saphenous arteries and four saphenous veins previously incubated with [^{3}H]norepinephrine the superfusate was cooled to 5°C and continuous electrical stimulation (5 Hz) started. Stepwise increases in temperature from 5°C (to 10, 15, 20, 25, 30, and 37°C) caused comparable, progressive increases in the total fractional release of tritiated compounds in the arteries and the veins (Fig. 5). Warming increased the overflow of all ^{3}H metabolites of norepinephrine in the artery and the vein when temperature was changed from 5 to 20°C (Table 1). The overflow of intact [^{3}H]norepinephrine also was progressively higher with stepwise warming from 5° to 20°C. Even at the coldest temperature studied (5°C), electrical stimulation of the adrenergic nerve endings caused release of norepinephrine, although it was vastly reduced.

Discussion. The present studies show that the canine saphenous artery, like the saphenous vein (3), can contract to norepinephrine at temperatures of 10°C. However, at this temperature sympathetic nerve stimulation causes contractions in the saphenous vein but no response in the corresponding artery. This differential effect of cold on neurotransmission in arteries and veins was not caused by affecting the release or the metabolism of norepinephrine differently in the artery than in the vein. Indeed, although cold inhibits transmitter release and slows its metabolism (7), and progressive cooling reduced norepinephrine release and the overflow of its metabolites in both blood vessels, transmitter release still continued at very cold temperatures in the artery and the vein. Likewise since the artery, as well as the vein, contracted to exogenous norepinephrine at 10°C, the poor response to sympathetic

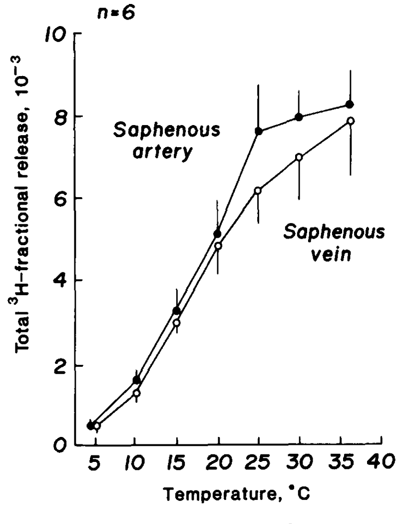

FIG. 5. Total fractional release of ^{3}H compounds in canine saphenous arteries (n = 6) and veins (n = 4) previously incubated with [^{3}H]norepinephrine. The preparations were cooled to 5°C before electrical stimulation (5 Hz) of the adrenergic nerve endings was begun. The temperature was then increased progressively; each step was maintained for 20 min and three 2-min samples were taken at the end of each 20-minute step for chromatographic analysis. Data shown as mean ± SEM. There was no significant difference between the arteries and the veins.

TABLE I. Progressive Rewarming and Overflow of [^{3}H]Norepinephrine and Its Metabolites during Electrical Stimulation of Saphenous Arteries and Veins[a]

	Overflow of metabolites (10^3 dpm)			
	5°C	10°C	15°C	20°C
Norepinephrine				
Arteries (6)	0.13 ± 0.08	2.46 ± 1.04	4.7 ± 1.67	4.81 ± 1.47
Veins (4)	0.74 ± 0.33	3.54 ± 0.93	10.38 ± 1.07	13.77 ± 2.49
3,4-Dihydroxyphenolglycol (DOPEG)				
Arteries (6)	0.16 ± 0.07	0.85 ± 0.24	2.35 ± 0.54	4.03 ± 0.72
Veins (4)	1.14 ± 0.35	2.47 ± 0.51	5.09 ± 0.95	6.92 ± 1.30
3,4-Dihydroxymandelic acid (DOMA)				
Arteries (6)	0.02 ± 0.01	0.72 ± 0.28	1.58 ± 0.44	3.17 ± 0.77
Veins (4)	0.60 ± 0.22	1.55 ± 0.36	3.88 ± 0.55	5.99 ± 1.58
Normetanephrine (NMN)				
Arteries (6)	0	0.34 ± 0.20	0.91 ± 0.40	1.95 ± 1.70
Veins (4)	0	0.45 ± 0.12	2.12 ± 0.43	4.63 ± 0.65
3-Methoxy-4-hydroxyphenolglycol (MOPEG)				
Arteries (6)	0.02 ± 0.02	0.62 ± 0.23	2.70 ± 0.80	5.16 ± 1.23
Veins (4)	0.43 ± 0.33	1.60 ± 0.34	5.21 ± 1.70	9.73 ± 1.90
3-Methoxy-4-hydroxymandelic acid (VMA)				
Arteries (6)	0	0.04 ± 0.04	0.28 ± 0.15	0.88 ± 0.35
Veins (4)	0.31 ± 0.17	0.69 ± 0.45	1.29 ± 0.46	2.33 ± 1.21

[a] Data shown as means ± SEM and expressed in absolute values. Numbers in parentheses indicate the number of vessels studied. The preparations were maintained at 5°C for 30 min before continuous electrical stimulation (5 Hz) began. The temperature of the superfusate was increased every 20 min in 5° increments from 5 to 20°C. Samples for chromatographic analysis were obtained by pooling three 2-min samples taken at the end of each 20-min period at each temperature.

nerve stimulation at this temperature in the saphenous artery cannot be attributed to an inability of the arterial muscle to contract to norepinephrine.

The data suggest that the greater inhibition by cold of neurotransmission in the artery is due to a greater sensitivity of the arterial muscle to the direct depressant effect of cold on the contractile processes (4–6). This interpretation is supported by the observation that cold depressed the contraction to high K^+ solution more in the saphenous artery than in the vein. It appears that in the artery at 10°C, the diminished amount of transmitter available to the smooth muscle during sympathetic nerve stimulation is insufficient to elicit a contraction, because of the direct depressant action of the cold. At warmer temperatures, when the direct depressant effect of cold is less and transmitter release increases, the contractile response resumes.

The present results imply that in the intact circulation during progressive cooling, sympathetically mediated constriction will be interrupted at a higher temperature in the cutaneous arteries than in the corresponding veins. In the cold-exposed hand of a comfortably warm subject, this would permit dilatation of resistance vessels to provide warm blood to maintain tissue function, and concurrent venoconstriction to reduce heat loss to the cold environment by shunting the venous return through deeper channels. In the subject whose whole body is exposed to cold, both pre- and postcapillary vessels in the digits remain constricted when, in addition, the hand is exposed to severe local cold (2). This maintained constriction presumably is due to circulating catecholamines, which can induce constriction of the vascular smooth muscle even at temperatures as low as 10°C. In these circumstances the body elects to maintain the core temperature at the expense of tissue perfusion.

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