Venous Relaxation Caused by Acetylcholine Acting on the Sympathetic Nerves

By Paul M. Vanhoutte and John T. Shepherd

ABSTRACT

Experiments were performed to determine whether acetylcholine affects the sympathetic activation of the cutaneous veins of the dog. Changes in isometric tension of saphenous vein strips were recorded at 37°C in an organ bath. Addition of acetylcholine at 10^{-11} to 10^{-8} g/ml did not affect basal tension, but larger doses (5 \times 10^{-8} to 5 \times 10^{-7} g/ml) caused a contraction of the strips which varied from slight to marked. Acetylcholine at 10^{-8} to 10^{-7} g/ml caused a further increase in tension when it was added to strips already contracted by norepinephrine, tyramine, KCl, or BaCl_2; in contrast, similar doses of acetylcholine caused relaxation of strips contracted by liberation of norepinephrine from the nerve terminals by electrical stimulation (1-10 cps). This relaxation was not influenced by propranolol or hexamethonium but was abolished by atropine (10^{-8} g/ml). In intact dogs, the lateral saphenous vein was perfused with autologous blood at constant flow. A sustained venoconstriction was induced either by electrical stimulation of the lumbar sympathetic chain or by a continuous infusion of norepinephrine. An infusion of acetylcholine (10^{-7} to 10^{-6} g/ml min^{-1}) relaxed veins constricted by sympathetic stimulation but not those constricted by norepinephrine. Thus, acetylcholine, in doses smaller than those known to have a direct constrictor effect, causes relaxation of cutaneous veins, probably by inhibiting the release of norepinephrine from nerve terminals.

KEY WORDS: cutaneous veins, cholinergic dilatation, dog, vascular smooth muscle, inhibition of adrenergic transmission, norepinephrine, tyramine, atropine, tetrodotoxin

• Acetylcholine, a potent vasodilator in vivo, causes contraction in most isolated vascular smooth muscle preparations (1, 2). In the venous system, this direct constrictor effect has been observed in isolated cutaneous (3-8), jugular (9), mesenteric (9-12), portal (9, 13-16), pulmonary (17, 18), and umbilical (19) veins. In the intact animal, application of acetylcholine causes splanchnic (20, 21) and cutaneous (4, 22, 23) veins to contract.

Following monamine-induced constrictions, rabbit aorta (24) and ear artery (25) are relaxed by acetylcholine, but arterioles from skin and mesentery are not (26). Reportedly, acetylcholine can also depress the reaction of rat mesenteric (27) and rabbit ear (28, 29) arteries to sympathetic stimulation.

Clement et al. (6) showed that, following norepinephrine-induced contraction, the cutaneous vein of the dog is not relaxed by acetylcholine. The present experiments were designed to determine if acetylcholine modifies the response of this vein to sympathetic stimulation.

Methods

IN VITRO STUDIES

The experiments were performed on helical strips of lateral saphenous veins isolated from dogs (15-25 kg) anesthetized with sodium pentobarbital (30 mg/kg, iv). The preparation was placed in a chamber filled with Krebs-Ringer's bicarbonate solution (NaCl 118.2 mM, KCl 4.7 mM, MgSO_4 1.2 mM, KH_2PO_4 1.2 mM, CaCl_2 2.5 mM, NaHCO_3 25.0 mM, calcium disodium ethylenediaminetetraacetate, 0.028 mM, and glucose 11.1 mM) maintained at 37°C and aerated with a 20% O_2-75% N_2-5% CO_2 mixture. The strips were connected to a strain gauge (Grass FT-03) for isometric tension recording.

For electrical stimulation of the preparation, two rectangular platinum electrodes were placed parallel to the strips, as described previously (30, 31). Impulses (1-10 cps) consisted of square waves (8 v, 2 msec) provided by a d-c power supply and switching transistor (RCA 2N-3034) triggered by a Grass S4 stimulator.

The following pharmacologic agents were used: acetylcholine chloride, L-norepinephrine bitartrate, tyramine hydrochloride, isoproterenol hydrochloride, propranolol hydrochloride, hexamethonium bromide, tetrodotoxin, and atropine sulfate. Each drug was dissolved...
in 0.1 ml of Krebs-Ringer's solution, which was the volume added to the organ bath. The doses, except those for norepinephrine and hexamethonium which are given in terms of the free base, are expressed as final bath concentrations of the salts. The drugs were removed from the bath solution by overflowing the preparations with aerated Krebs-Ringer's solution at 37°C.

Before the experiments were begun, the preparations were placed at the optimal point of their length-tension relationship (30). In each group of preparations, only one strip was obtained from any one dog. The doses of the drugs and the frequencies of the electrical impulses were randomized. For the statistical analysis of the data, Student's t-test for paired observations was used.

The data are expressed as means ± SE.

**IN VIVO STUDIES**

The method used in this study has been described (32). Dogs (16–20 kg) were anesthetized with sodium thiopental (15 mg/kg, iv) and chloralose (80 mg/kg, iv). Additional amounts of chloralose were given as required to maintain the proper level of anesthesia. The left lateral saphenous vein was cannulated at the ankle and perfused at constant flow (100 ml/min) with autologous blood from the terminal aorta. The perfusion circuit consisted of a roller pump, a depulsator, and a heat exchanger (37°C). The aortic pressure, the saphenous vein perfusion pressure, and the femoral vein pressure were continuously recorded. Since the blood flow through the saphenous vein and the femoral vein pressure were constant, the saphenous vein perfusion pressure was a measure of the tone in the vein wall.

Sympathetic Stimulation.—The lumbar sympathetic trunk was exposed, severed, and stimulated (Grass SD5 stimulator) via a platinum bipolar electrode placed between the fifth and the sixth lumbar ganglion. Square-wave impulses (10 v, 2 msec) were applied at frequencies of 1–10 cps.

Drugs.—Norepinephrine bitartrate and acetylcholine chloride were infused at constant speed upstream from the roller pump and the cannula, respectively.

**Results**

**IN VITRO STUDIES**

Unstimulated Preparations.—In nine saphenous vein strips, the effect of increasing doses of acetylcholine (5 × 10⁻¹¹ to 5 × 10⁻² g/ml) was investigated. The lower doses (5 × 10⁻¹¹ to 10⁻⁸ g/ml) had no effect on base-line tension (0.845 ± 0.480 g). A dose of 5 × 10⁻⁸ g/ml caused a slight contraction in seven of the nine strips (mean increase in tension, 0.030 ± 0.010 g); 10⁻⁷ g/ml caused all of the strips to contract slightly (mean increase in tension, 0.070 ± 0.18 g). With the highest dose tested, a marked increase in tension (1.63 ± 0.380 g) was obtained in all preparations.

Stimulated Preparations.—Figure 1 shows the original record of an experiment in which a single dose of acetylcholine (5 × 10⁻⁸ g/ml) was given during a sustained response to a 2-cps electrical stimulation. Acetylcholine caused a marked relaxation of the strip, and this effect could be rapidly reversed by washing out the drug. In identical experiments on 15 preparations from different dogs, the average response to electrical stimulation was depressed to 9.1 ± 1.6% of the original reaction (mean increase in tension, 1.540 ± 0.238 g).

In six saphenous vein strips, sustained contractions (mean increase in tension, 1.050 ± 0.140 g) were obtained by stimulation at 1 cps. While the stimulation was continued, doses of acetylcholine (5 × 10⁻⁹ to 5 × 10⁻² g/ml) were added to the bath solution (Fig. 2, top). As the dose of acetylcholine was increased, there was a progressive decrease in the reaction to electrical stimulation; in five of the six strips the decrease was maximal at a dose of 5 × 10⁻⁸ g/ml. At this dose the reaction to electrical stimulation was decreased to 11.6 ± 3.9% of the control contraction. Increasing the acetylcholine concentration to 5 × 10⁻⁷ g/ml caused all of the strips to contract.

Although acetylcholine decreased the contractions caused by electrical stimulation, it augmented
VENOUS RELAXATION BY ACETYLCHOLINE

Typical experiment showing the effect of increasing doses of acetylcholine (A = 5 \times 10^{-9}, B = 10^{-8}, C = 5 \times 10^{-8}, D = 10^{-7}, and E = 5 \times 10^{-7} g/ml) on reactions of a saphenous vein strip to electrical stimulation (top) and norepinephrine (bottom). Acetylcholine was removed at the time electrical stimulation ceased or together with the norepinephrine.

In six strips, a comparison was made of the effect of increasing doses of acetylcholine (10^{-9}, 5 \times 10^{-9}, 10^{-8}, 5 \times 10^{-8}, and 10^{-7} g/ml) on contractions induced by electrical stimulation and by addition of norepinephrine. Five stimulation frequencies (0.5, 1, 2, 5, and 10 cps) and four norepinephrine concentrations (5 \times 10^{-9}, 10^{-8}, 5 \times 10^{-8}, and 10^{-7} g/ml) were selected to cause comparable changes in tension (Fig. 3). During electrical stimulation, the addition of acetylcholine decreased the contractions. When expressed as a percent of the control increase in tension, this relaxation was directly related to the dose of acetylcholine. In the presence of norepinephrine, the addition of acetylcholine caused a further increase in tension, and this augmentation was also directly related to the dose of acetylcholine.

Six strips were made to contract by applying norepinephrine, tyramine, or electrical stimulation. The frequency of stimulation and the dose of each agent were selected to give comparable contractions. With acetylcholine (Fig. 4), the increases in tension caused by norepinephrine (0.790 ± 0.085 g) and by tyramine (0.730 ± 0.100 g) were augmented to 117 ± 4.19% and 124 ± 7.45%, respectively, and the increase caused by electrical stimulation (0.850 ± 0.115 g) was decreased to 11.4 ± 2.71%.

Six strips were made to contract by bathing them in a solution containing 25 mM KCl (mean increase

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**FIGURE 2**

Typical experiment showing the effect of increasing doses of acetylcholine (A = 5 \times 10^{-9}, B = 10^{-8}, C = 5 \times 10^{-8}, D = 10^{-7}, and E = 5 \times 10^{-7} g/ml) on reactions of a saphenous vein strip to electrical stimulation (top) and norepinephrine (bottom). Acetylcholine was removed at the time electrical stimulation ceased or together with the norepinephrine.

**FIGURE 3**

Effect of increasing doses of acetylcholine on the reactions of dog saphenous vein strips to electrical stimulation at five different frequencies (left) and to norepinephrine at four different concentrations (right). Data are expressed as means for six strips. ø = difference from control value is statistically significant (P < 0.05), ø = difference from control value and from next lower acetylcholine dose is statistically significant (P < 0.05), and ø = difference from control value is not significant.

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Effects of acetylcholine on contractions of the same saphenous vein strip caused by norepinephrine (top), electrical stimulation (middle), and tyramine (bottom). After stabilization of the effect, both acetylcholine and the agonist were removed simultaneously. Tyramine was administered in stepwise increases until the tension was similar to that with electrical stimulation and norepinephrine.

in tension, 0.630 ± 0.182 g); acetylcholine (5 × 10⁻⁸ g/ml) caused an additional increase in tension to 1.000 ± 0.275 g. Six other strips were made to contract by adding BaCl₂ at 0.25 mg/ml (mean increase in tension, 0.542 ± 0.042 g); acetylcholine caused a further increase in tension to 0.983 ± 0.172 g. In these 12 strips, the typical depression by acetylcholine of the reaction to electrical stimulation was observed.

Pharmacologic Inhibitors.—With six strips, studies were made to determine if the relaxation caused by acetylcholine during electrical stimulation was affected by beta-receptor blockade. Propranolol (10⁻⁶ g/ml) prevented the relaxation caused by isoproterenol (10⁻⁷ g/ml) given during sustained 2-cps electrical stimulation.

The strips were made to contract by electrical stimulation (2 cps), and acetylcholine (5 × 10⁻⁸ g/ml) was added to cause relaxation. Then the experiment was repeated in the presence of propranolol at 10⁻⁶ g/ml. The acetylcholine-induced relaxation was not influenced by beta-receptor blockade.

With six strips, acetylcholine (5 × 10⁻⁸ g/ml) was added to the bath solution during a sustained contraction caused by electrical stimulation (2 cps). After stabilization of the relaxation induced by acetylcholine, increasing amounts of hexamethonium were added (10⁻⁸ to 10⁻⁵ g/ml). Hexamethonium was essentially without effect, although higher doses did result in a slight increase in tension (Fig. 5, left).
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In the same strips, after the hexamethonium was washed out, a second sustained contraction was induced by electrical stimulation (2 cps), and then relaxation was induced by acetylcholine (5 \times 10^{-8} g/ml); the relaxation was allowed to stabilize. Atropine (10^{-8} g/ml) was added, and this drug caused a rapid abolition of the relaxation due to acetylcholine (Fig. 5, right). Atropine also caused an abolition of the contraction due to acetylcholine during norepinephrine-induced contractions (Fig. 6) and during contractions caused by KCl and BaCl₂.

In five other strips, acetylcholine at 5 \times 10^{-8} g/ml caused a slight increase in tension (0.062 ± 0.025 g) in resting preparations and depressed the reaction to electrical stimulation (2 cps) to 13.5 ± 5.9\% of the original contractions (mean increase in tension, 0.518 ± 0.118 g). The same dose of acetylcholine augmented the reactions to norepinephrine at 2 \times 10^{-6} g/ml (mean increase in tension, 0.799 ± 0.071 g) by 0.100 ± 0.020 g and the reactions to BaCl₂ at 0.25 mg/ml (mean increase in tension, 0.515 ± 0.035 g) by 0.286 ± 0.186 g. The addition of tetrodotoxin (10^{-6} g/ml) abolished the reactions to electrical stimulation (1–15 cps) but did not inhibit the reactions to norepinephrine or BaCl₂ (mean increase in tension, 0.670 ± 0.065 g). In the presence of tetrodotoxin, acetylcholine still caused an increase in tension (0.073 ± 0.033 g) in resting preparations and further increased the reactions to norepinephrine and BaCl₂ by 0.120 ± 0.020 g and 0.395 ± 0.254 g, respectively (Fig. 7).

IN VIVO STUDIES

Unstimulated Preparations.—In the five dogs studied, acetylcholine infused for 2 minutes at a dose of 10^{-7} g/ml min^{-1} had little or no effect on the perfusion pressure in the saphenous vein. With a higher dose (4 \times 10^{-7} g/ml min^{-1}), the vein constricted in four of the five dogs (mean increase in perfusion pressure, 9.4 ± 5.7 mm Hg).

Lumbar Sympathetic Stimulation and Norepinephrine.—The infusion of acetylcholine during venoconstriction induced by lumbar sympathetic chain stimulation caused a decrease in perfusion pressure. The decrease could be obtained with 10^{-7} g/ml min^{-1}, and the maximal effects were obtained with four times this dose (Fig. 8). The results in the five dogs are summarized in Table 1. In every dog, acetylcholine (4 \times 10^{-7} g/ml min^{-1}) caused a marked inhibition of the response to each frequency of stimulation. When the infusion stopped, the perfusion pressure increased to the control level. In two of these five dogs an additional stimulation at 10 cps was used. The acetylcholine infusion decreased the perfusion pressure from 130 to 55 mm Hg and from 115 to 60 mm Hg, respectively; the pressure returned to control levels when the infusion was stopped.

In the same dogs, the effects of infusing acetylcholine (4 \times 10^{-7} g/ml min^{-1}) were investigated during a sustained venoconstriction caused by the infusion of norepinephrine at 2 \times 10^{-7} and 4 \times 10^{-7} g/ml min^{-1} (Table 1). Acetylcholine caused either an additional increase or no change in perfusion pressure; in no case was there a decrease in perfusion pressure. After the infusion, the perfusion pressure usually decreased, but in three dogs it did not decrease to the level observed before acetylcholine infusion; recirculation of the infused norepinephrine may explain this finding.

Discussion

The purpose of these experiments was to examine the influence of acetylcholine on veins constricted by sympathetic nerve activity. A convenient approach to the problem was to study the changes in isometric tension in the isolated dog saphenous vein subjected to electrical stimulation. The interpretation of the results depends on the evidence that the electrical stimulation caused contraction of the vein.
strips by stimulation of the sympathetic nerve endings and not by direct activation of the smooth muscle cells. The contractions of isolated dog saphenous veins in response to electrical field stimulation (identical to that used in the present experiments) are abolished by blockage of post-ganglionic adrenergic transmission with bretylium tosylate (5, 8), by reserpine pretreatment (5), by chronic sympathectomy (7, 8), and by alpha-receptor blockade (5, 8). Thus, in the present experiments, it can be concluded that the contractions of the isolated saphenous vein strips during electrical stimulation are caused mainly by the release of catecholamines from the adrenergic nerve terminals; that tetrodotoxin abolished the response to electrical stimulation supports this conclusion.
VENOUS RELAXATION BY ACETYLCHOLINE

TABLE 1
Effect of Acetylcholine on Venoconstrictions Induced by Lumbar Sympathetic Stimulation or Norepinephrine Infusion in Intact Dogs

<table>
<thead>
<tr>
<th></th>
<th>Increases in driving pressure (mm Hg)</th>
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<tbody>
<tr>
<td></td>
<td>Before ACh infusion</td>
</tr>
<tr>
<td>Lumbar Sympathetic Stimulation</td>
<td></td>
</tr>
<tr>
<td>1 cps</td>
<td>18.1 ± 4.5</td>
</tr>
<tr>
<td>2 cps</td>
<td>40.7 ± 6.5</td>
</tr>
<tr>
<td>5 cps</td>
<td>80.9 ± 8.2</td>
</tr>
<tr>
<td>Norepinephrine Infusion</td>
<td></td>
</tr>
<tr>
<td>2 × 10⁻¹⁷ g/ml min⁻¹</td>
<td>49.8 ± 14.3</td>
</tr>
<tr>
<td>4 × 10⁻¹⁷ g/ml min⁻¹</td>
<td>76.3 ± 14.2</td>
</tr>
</tbody>
</table>

Acetylcholine (ACh) at 4 × 10⁻¹⁷ g/ml min⁻¹ was infused for 2 minutes.

The appropriate control for the reactions obtained with electrical stimulation was the vein constricted by exogenous norepinephrine. Strikingly, acetylcholine decreased the response to electrical stimulation, but it augmented the reaction to exogenous catecholamines. This finding strongly suggests that acetylcholine has a dual action: at the presynaptic level it inhibits the release of norepinephrine from the tissue stores leading to relaxation of smooth muscle constricted by sympathetic impulses, and at the smooth muscle cells acetylcholine causes contraction. Acetylcholine did not inhibit the contractions caused by tyramine, suggesting that it cannot prevent the mobilization of norepinephrine by that drug. These responses to acetylcholine resemble those noted in isolated arterial smooth muscle with guanethidine and bretylium tosylate, because the latter drugs block the reactions to nerve activation but not the responses to norepinephrine and tyramine (27, 33, 34).

Since the relaxation caused by acetylcholine is not influenced by beta-receptor blockade and hexamethonium but is abolished by atropine, it must be due to the muscarinic action of acetylcholine.

Our results in venous smooth muscle substantiate the findings of Malik and Ling (27), Rand and Varma (28), and Hume et al. (29), who worked on isolated rat mesenteric and rabbit ear arteries and reached similar conclusions on the mode of action of acetylcholine.

In contrast, when contractions similar to those caused by electrical stimulation were evoked by norepinephrine, acetylcholine did not cause relaxation; in fact, a further increase in tension usually resulted. An increase in tension was also obtained with acetylcholine in the vein strips made to contract by addition of tyramine, KCl, or BaCl₂. These effects of acetylcholine were not affected by tetrodotoxin and were blocked by atropine. They can be explained by a direct action on the smooth muscle cells, and this direct action can be obtained in the resting strips (3, 5, 6, 8–10, 13, 16) but usually only with larger doses than those necessary to increase the tension in a strip already contracted.

Doses of acetylcholine which have no effect on the tension of resting strips or strips contracted by norepinephrine can cause marked relaxation of strips contracted by electrical stimulation. This finding suggests that the action on the sympathetic nerve endings may occur with concentrations lower than those necessary for the direct contracting action of the drug.

In the in vivo studies, in which the electrical stimulation was applied directly to the sympathetic nerves, a striking difference was seen between the depression by acetylcholine of the vasoconstrictions caused by lumbar sympathetic stimulation and the augmentation by acetylcholine of the contractions caused by norepinephrine. The similarity of these findings with the in vitro observations strengthens the conclusion that, in the isolated venous strips, acetylcholine is inhibiting the release of norepinephrine. In the arterial bed of the rabbit's ear, the dilatation caused by acetylcholine is decreased by reserpine (35). This effect, considered in conjunction with the present experiments, suggests that the vasodilatation caused by acetylcholine in vivo may be explained, at least in part, by the action of the drug on sympathetic nerve endings.

Cholinergic vasodilator fibers in association with sympathetic nerves have been described in different species (36, 37). The transmitter released on
stimulation of these fibers could act on a receptor in the smooth muscle cells to cause the dilatation. Another possibility, suggested by the present experiments and by studies on isolated arteries (27–29, 38, 39) and on the heart (40, 41), is that the cholinergic transmitter acts by inhibiting sympathetic adrenergic transmission.

References


Vanhoutte, Shepherd
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