Inhibition by Acetylcholine of Adrenergic Neurotransmission in Vascular Smooth Muscle

By Paul M. Vanhoutte

ABSTRACT

Changes in the isometric tension of isolated strips of cutaneous, femoral, mesenteric, pulmonary, and muscle arteries and veins were recorded at 37°C in an organ bath. Acetylcholine (5 x 10^{-8} and 10^{-7} g/ml) caused relaxation of strips from the saphenous veins, the femoral veins, and all of the arteries after contraction by norepinephrine released from nerve terminals by electrical stimulation (2–5 Hz); in the pulmonary and mesenteric veins, acetylcholine caused a further increase in tension. Pulmonary artery and mesenteric vein strips were incubated with [3H]norepinephrine and mounted for superfusion (3 ml/min) and isometric tension recording. Electrical stimulation increased the tension and the total radioactivity released in both preparations. Acetylcholine (2 x 10^{-7} g/ml) depressed the contractions of the pulmonary artery strips but augmented those of the mesenteric vein strips; it diminished the efflux of radioactivity in both, indicating that acetylcholine inhibits adrenergic neurotransmission. In the absence of sympathetic stimulation, acetylcholine (5 x 10^{-10}-10^{-5} g/ml) caused all vein strips to contract; the most common reaction in artery strips was a slight relaxation (at 10^{-9}-10^{-8} g/ml) followed by a contraction (at 5 x 10^{-8}-10^{-6} g/ml). During contractions caused by norepinephrine, acetylcholine caused a further increase in tension in vein strips but a relaxation in artery strips. Atropine abolished the effects of acetylcholine. The results of this study suggest the presence in vascular smooth muscle of both excitatory and inhibitory cholinergic receptors.

KEY WORDS

atropine  cholinergic dilation  dog
isolated veins and arteries  norepinephrine release  sympathetic nerve endings
tetrodotoxin  tyramine

In dog saphenous vein, acetylcholine, in doses that do not cause a contraction, augments contractions caused by norepinephrine but inhibits those caused by nerve stimulation; these paradoxical effects have been observed both in isolated preparations and in the intact dog (1). In the isolated saphenous vein, the depression of the reaction to nerve stimulation is due to inhibition of the release of adrenergic neurotransmitter (2). In the rat mesenteric artery (3) and the rabbit ear artery (4-6), acetylcholine depresses the reaction to nerve stimulation more than it depresses the reaction to norepinephrine.

To determine if acetylcholine is a general inhibitor of adrenergic neurotransmission in the vascular system, the following experiments were performed to examine the action of acetylcholine in ten different vascular smooth muscle preparations from the dog.

Methods

The experiments were performed on the following blood vessels taken from dogs (15-25 kg) anesthetized with sodium pentobarbital (30 mg/kg, iv): saphenous, anterior mesenteric, pulmonary, femoral, and gracilis muscle veins and anterior tibial, superior mesenteric, pulmonary, femoral, and gracilis muscle arteries. Helical strips were cut from all vessels except the mesenteric vein from which longitudinal strips were prepared. Each preparation was placed in a chamber filled with Krebs-Ringer’s bicarbonate solution of the following millimolar composition: NaCl 118.3, KCl 4.7, MgSO_4 1.2, KH_2PO_4 1.2, CaCl_2 2.5, NaHCO_3 25.0, calcium ethylenediaminetetraacetate (EDTA) 0.026, and glucose 11.1. The solution was 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 FT03) for isometric recording.

For electrical stimulation of the preparation, two rectangular platinum electrodes were placed parallel to the strips as described previously (7, 8). Electrical impulses consisted of square waves (8v, 2 msec) provided by a d-c power supply and switching transistor (RCA 2N3034) triggered by a Grass stimulator (model S4).
The following pharmacologic agents were used: acetylcholine chloride, l-norepinephrine bitartrate, isoproterenol hydrochloride, tetrodotoxin, and atropine sulfate. The dose of each drug was contained in 0.1 ml of Krebs-Ringer’s solution, and this same volume was added to the organ bath. All doses are expressed as final bath concentrations of the salts, except those for norepinephrine, which are expressed in terms of the free base. 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 which reacted to electrical stimulation were placed at the optimal point of their length-tension relationship (7); the other strips were progressively stretched to approximately 150% of their length at an isometric tension of 0 g.

[^3H]NOREPINEPHRINE EFFLUX

In some experiments, the preparations were incubated for 4 hours in Krebs-Ringer’s solution containing [7-[^3H]] norepinephrine at 5 X 10^-8 g/ml (specific activity 4.18 c/mmole, New England Nuclear). At the end of incubation the strips were rinsed in fresh Krebs-Ringer’s solution and mounted for superfusion as previously described (2).

The preparation was suspended in a moist tunnel-shaped chamber maintained at 37°C; the strip was superfused at 3 ml/min by a constant-flow roller pump with aerated Krebs-Ringer’s solution prewarmed to 37°C. The preparation was connected to a strain gauge (Grass FT03) for continuous isometric tension recording. The initial tension was set at 3 g; after this initial stretch, the tension decreased and stabilized within 30 minutes. At that time, sampling of the superfusate was begun. The superfusate was collected at 2-minute intervals for direct estimation of total radioactivity. For electrical stimulation of the preparation, two platinum wires (0.5 mm in diameter, 10 cm long) were placed parallel to the strip; both the vessel and the electrodes were continuously superfused. Acetylcholine chloride and tyramine hydrochloride were infused at a constant speed upstream from the roller pump. All doses are expressed as final concentrations of the salts in the superfusing fluid.

RADIOACTIVITY MEASUREMENTS

Samples (1 ml) of the superfusate were added to 10 ml of InstaGel (Packard), and radioactivity was measured in a liquid scintillation counter. Corrections for quenching were made with an external standard. The counting efficiency was 22.1%; the samples were counted for 10 minutes. Data are expressed as disintegrations per minute (dpm) per minute of superfusion.

Results

For each group of preparations, the number of strips reported is also the number of dogs used. The doses of the drugs and the frequencies of the electrical impulses were randomized. The data are expressed as means ± SE.

ELECTRICAL STIMULATION

Electrical stimulation caused contraction of the different blood vessel strips tested. Earlier experiments (8-11) have shown that electrical stimulation like that used in the present experiment causes contraction of saphenous, mesenteric, and femoral vein strips by activation of sympathetic nerve endings. To ascertain that this finding was also true in the other preparations, the effect of tetrodotoxin (10^-4 g/ml) on the reaction to electrical stimulation was investigated with tibial, mesenteric, femoral, and pulmonary artery strips and pulmonary vein strips. Tetrodotoxin inhibited the response of tibial, mesenteric, and pulmonary artery strips to supramaximal electrical stimulation (15 Hz) but not to the addition of norepinephrine (5 X 10^-8 g/ml) (Fig. 1); an identical inhibition by tetrodotoxin was obtained in femoral artery and pulmonary vein strips.

ACETYLCHOLINE AND VEIN STRIPS: UNSTIMULATED PREPARATIONS

With ten saphenous, nine mesenteric, six femoral, six pulmonary, and six gracilis muscle vein strips, increasing doses of acetylcholine (5 X 10^-10 to 10^-8 g/ml) caused a mean increase in tension in all preparations. The threshold acetylcholine concentrations were 10^-10, 10^-9, 5 X 10^-9, 10^-8, and 5 X 10^-8 g/ml for mesenteric, femoral, pulmonary, saphenous, and gracilis muscle vein strips, respectively.

ACETYLCHOLINE AND VEIN STRIPS: STIMULATED PREPARATIONS

Mesenteric Vein. — Figure 2 shows an experiment in which acetylcholine (5 X 10^-8 g/ml) was given during a sustained contraction caused by a 2-Hz electrical stimulation; this dose causes maximal relaxation in saphenous vein strips under similar conditions (1) (Fig. 2 bottom). Acetylcholine caused a marked increase in tension in the mesenteric strips; in 11 preparations the average response to electrical stimulation was augmented from 0.86 ± 0.29 g to 2.75 ± 0.40 g. In the absence of electrical stimulation, acetylcholine (5 X 10^-8 g/ml) caused a mean increase in tension of 2.00 ± 0.35 g. Figure 2 also shows that, in the same mesenteric strip, isoproterenol (10^-7 g/ml) depressed the reaction to electrical stimulation as it does in the saphenous vein. In six mesenteric strips, the depression caused by isoproterenol averaged 62.3 ± 10.7% of the 2-Hz response.

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In five other mesenteric strips, smaller doses of acetylcholine \((5 \times 10^{-10}, 10^{-9}, 5 \times 10^{-9}, \text{and} 10^{-8} \text{g/ml})\) were added to the bath solution during contractions obtained with electrical stimulation at 1 and 10 Hz. None of these doses caused relaxation; starting with \(10^{-9} \text{g/ml}\), all reactions to electrical stimulation were augmented. In five other preparations, both during electrical stimulation (2 and 5 Hz) and in the presence of norepinephrine \((10^{-8} \text{and} 2 \times 10^{-8} \text{g/ml})\), the addition of acetylcholine \((5 \times 10^{-8} \text{and} 10^{-7} \text{g/ml})\) caused a further increase in tension (Fig. 3).

Femoral Vein.—In six femoral vein strips, the effect of acetylcholine \((5 \times 10^{-8} \text{and} 10^{-7} \text{g/ml})\) was
Effect of two doses of acetylcholine (A $5 \times 10^{-8}$ g/ml and B $10^{-7}$ g/ml) on contractions of femoral vein, pulmonary vein, and pulmonary artery strips during electrical stimulation (5 Hz) and norepinephrine-induced (pulmonary vein $5 \times 10^{-9}$ g/ml, other vessels $10^{-8}$ g/ml) contractions. Acetylcholine was removed during continuous electrical stimulation but was present with norepinephrine (W₀). The dose of norepinephrine was adapted to obtain contractions comparable with those induced by a 5-Hz electrical stimulation.

Examined during contractions obtained by electrical stimulation (2 and 5 Hz) and by addition of norepinephrine ($10^{-8}$ and $2 \times 10^{-8}$ g/ml). In five preparations, weak contractions occurred in response to electrical stimulation. The lower dose of acetylcholine depressed the reaction to stimulation at 2 Hz in four strips and that to stimulation at 5 Hz in three of those four strips. At $5 \times 10^{-7}$ g/ml, acetylcholine caused an increase in tension in all but one strip during stimulation at both frequencies. The two doses of acetylcholine caused further increases in tension during responses to norepinephrine in all preparations (Figs. 3 and 4).

Pulmonary Vein.—In four of six pulmonary vein strips, weak contractions were evoked with electrical stimulation (2 and 5 Hz) and by addition of norepinephrine ($10^{-8}$ and $2 \times 10^{-8}$ g/ml). In five preparations, weak contractions occurred in response to electrical stimulation. The lower dose of acetylcholine depressed the reaction to stimulation at 2 Hz in four strips and that to stimulation at 5 Hz in three of those four strips. At $5 \times 10^{-7}$ g/ml, acetylcholine caused an increase in tension in all but one strip during stimulation at both frequencies. The two doses of acetylcholine caused further increases in tension during responses to norepinephrine in all preparations (Figs. 3 and 4).

**FIGURE 5**

Effect of increasing doses of acetylcholine on unstimulated mesenteric arteries of dogs. Data are expressed as means ± SE.
cal stimulation (2, 5, and 10 Hz). Acetylcholine (5 × 10⁻⁹-10⁻⁷ g/ml) caused a further increase in tension in the four strips at all frequencies. Acetylcholine also augmented the reaction to norepinephrine (10⁻⁸ and 2 × 10⁻⁸ g/ml) in the six strips (Figs. 3 and 4).

Gracilis Muscle Vein.—Only one of six gracilis muscle vein strips reacted slightly to electrical stimulation (10 Hz); this contraction (0.02 g) was decreased by acetylcholine (10⁻⁸ g/ml). Norepinephrine (10⁻⁸ and 2 × 10⁻⁸ g/ml) caused small increases in tension. These responses were not affected by acetylcholine (5 × 10⁻⁸ and 10⁻⁷ g/ml) in two preparations and were slightly decreased in the other four preparations.

Saphenous Vein.—In nine saphenous vein strips, acetylcholine depressed the responses to electrical stimulation but augmented those to norepinephrine (Fig. 3).

ACETYLCHOLINE AND ARTERY STRIPS: UNSTIMULATED PREPARATIONS

In six anterior tibial, seven mesenteric, six femoral, six pulmonary, and six gracilis muscle artery strips, the effect of increasing doses of acetylcholine (5 × 10⁻¹¹-10⁻⁵ g/ml) was investigated. In some preparations, acetylcholine had no effect or caused either slight relaxations or weak contractions. However, the most common reaction was an initial relaxation with the lower doses of acetylcholine followed by an increase in tension. The threshold concentration for relaxation ranged from 5 × 10⁻¹⁰ to 5 × 10⁻⁹ g/ml and that for increased tension ranged from 10⁻⁸ to 10⁻⁶ g/ml (Fig. 5).

TABLE 1

Effect of Acetylcholine on Tension and [³H]Norepinephrine Release during Responses of Vessel Strips to Electrical Stimulation and Tyramine

<table>
<thead>
<tr>
<th>Condition</th>
<th>Mesenteric vein strips</th>
<th>Pulmonary artery strips</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Electrical stimulation (2 Hz)</td>
<td>Electrical stimulation (2 Hz)</td>
</tr>
<tr>
<td></td>
<td>Tension (g)</td>
<td>[³H] (dpm/min)</td>
</tr>
<tr>
<td>Control</td>
<td>2.310 ± 0.318</td>
<td>21.1 ± 2.7</td>
</tr>
<tr>
<td>Stimulation</td>
<td>5.500 ± 0.875</td>
<td>58.5 ± 11.5</td>
</tr>
<tr>
<td>Stimulation and acetylcholine*</td>
<td>6.000 ± 1.040</td>
<td>34.0 ± 5.6</td>
</tr>
<tr>
<td>Stimulation</td>
<td>3.640 ± 0.614</td>
<td>44.7 ± 7.9</td>
</tr>
</tbody>
</table>

All values are means ± SE, and six strips were studied in each experiment. Experimental values were obtained after stabilization of reactions for 8 minutes.

*Acetylcholine concentration was 2 × 10⁻⁷ g/ml.
noted with 5-Hz electrical stimulation. In these three strips, acetylcholine caused a relaxation. Norepinephrine caused all of the strips to contract; acetylcholine (5 \times 10^{-8} \text{ and } 10^{-7}\text{ g/ml}) caused an increase in tension in two preparations and a slight relaxation in two others.

ATROPINE

During electrical stimulation, atropine (10^{-8} \text{ g/ml}) abolished the acetylcholine-induced inhibition in saphenous and femoral vein strips and in mesenteric, pulmonary, tibial, and femoral artery strips; it also inhibited the increase in tension observed with acetylcholine in mesenteric and pulmonary vein strips. In higher concentrations (10^{-7} \text{ g/ml}), atropine abolished the relaxation obtained with acetylcholine during responses to norepinephrine in femoral, mesenteric, tibial, and pulmonary artery strips.

[3 H] NOREPINEPHRINE EFFLUX

Mesenteric Vein.—Six mesenteric vein strips were incubated in a solution containing tritiated norepinephrine; they were then studied in the superfusion apparatus. Electrical stimulation resulted in augmentation of the efflux of tritiated compounds into the superfusate (Fig. 8). Acetylcholine (2 \times 10^{-7} \text{ g/ml for 8 minutes}) given during a contraction induced by electrical stimulation at 2 Hz caused a further increase in tension but a marked decrease in the radioactivity in the superfusate. These effects of acetylcholine could be rapidly reversed (Table 1). In four strips, the same dose of acetylcholine was infused in the absence of electrical stimulation. This procedure produced an increase in tension but did not influence the efflux of tritiated compounds.

Pulmonary Artery.—With six pulmonary artery

Comparison of the effects of the same dose of acetylcholine on tension and total 3H efflux induced by electrical stimulation or by tyramine in the same pulmonary artery strip.
strips, electrical stimulation (2 Hz) augmented the efflux of tritiated compounds into the superfusate (Fig. 9). Acetylcholine (2 × 10^{-7} g/ml for 8 minutes) given during a contraction induced by electrical stimulation caused a marked relaxation of the strip. This relaxation was paralleled by a decrease in radioactivity in the superfusate (Table 1). In the absence of electrical stimulation the same dose of acetylcholine caused a slight relaxation in one strip, an increase in tension in another, and no effect in the remaining strips; it did not affect the basal efflux curve for tritiated compounds. When the same six pulmonary artery strips were caused to contract by tyramine (4 × 10^{-6} g/ml), the efflux of tritiated compounds into the superfusate was augmented (Fig. 9). With the addition of acetylcholine (2 × 10^{-7} g/ml) the increase in tension caused by tyramine was slightly depressed in three strips, but the radioactivity level of the superfusate was unchanged (Table 1).

Discussion

The interpretation of the results depends on the evidence that the electrical stimulation caused contraction of the strips by stimulation of sympathetic nerve endings rather than by activation of the smooth muscle cells. Contraction of isolated dog saphenous vein strips in response to electric field stimulation, as used in the present experiments, is abolished by blockade of postganglionic adrenergic transmission with bretylium tosylate (9, 11) or tetrodotoxin (1), by treatment with reserpine before stimulation (9), by chronic sympathectomy (10, 11), and by α-receptor blockade (9, 11). Bretylium tosylate and α-receptor blockade also inhibit the reaction to electric field stimulation in mesenteric (9) and femoral veins (8). In the present experiments, tetrodotoxin inhibited the reactions of pulmonary vein and artery strips and of femoral, mesenteric, and tibial artery strips to electrical stimulation but not to exogenously applied norepinephrine. Thus, it can be concluded that the contractions of the isolated vascular preparations reported in the present study with electric field stimulation are caused mainly by nerve-mediated release of catecholamines from the adrenergic nerve terminals.

In the experiments performed on mesenteric vein and pulmonary artery strips previously incubated in solutions containing [3H]norepinephrine, electrical stimulation caused an increase in the total radioactivity of the superfusate. Similar increases have been described for the saphenous vein of the dog (2), the portal-mesenteric veins of the rat (12-14) and the rabbit (15), and the pulmonary artery of the rabbit (16, 17). In the studies in which intact [3H]norepinephrine was separated chemically from its metabolites, the increase in total radioactivity of the superfusate during electrical stimulation was partly due to a substantial increase in intact [3H]norepinephrine, indicating an overflow of neurotransmitter (2, 12, 14-17). Hence, in the present experiments, the washout of tritiated compounds provided a qualitative measure of the release of adrenergic neurotransmitter.

INHIBITION OF ADRENERGIC NEUROTRANSMISSION BY ACETYLCHOLINE

The depression of the reactions to electrical stimulation and the diminished output of [3H]norepinephrine during such stimulation provide evidence that acetylcholine inhibits adrenergic neurotransmission in dog saphenous vein (1, 2). In the present experiments, this inhibitory action of acetylcholine was demonstrated in arterial and venous smooth muscle from many different vascular beds.

Considering first the changes in isometric tension, the depression by acetylcholine of the reaction to electrical stimulation was confirmed in the saphenous vein and shown to occur also in femoral and muscle veins and in femoral, mesenteric, muscle, pulmonary, and tibial arteries but not in mesenteric and pulmonary veins. In these last two, acetylcholine caused a further increase in tension during electrical stimulation.

In the saphenous vein strip contracted by norepinephrine, doses of acetylcholine that depressed the reactions to electrical stimulation caused a further increase in tension; this finding confirmed previous observations (1). This paradoxical effect of acetylcholine also was observed in the present experiments in strips from femoral veins and several arteries. In most arteries, however, acetylcholine caused relaxation during contractions induced by norepinephrine; in each case, for comparable contractions, the reaction to electrical stimulation was depressed to a greater extent for a given dose of acetylcholine. From similar observations in rat mesenteric and in rabbit ear arteries, other investigators (3-6) have also concluded that acetylcholine inhibits the release of norepinephrine during nerve activation. This conclusion, based on a difference in the degree of relaxation, was strengthened by the experiments in which [3H]norepinephrine efflux from pulmonary artery
strips was followed. These experiments demonstrated that in arterial preparations, which relax with acetylcholine more during electrical stimulation than they do during norepinephrine-induced responses, acetylcholine causes a marked inhibition of the release of adrenergic neurotransmitter by electrical stimulation.

In pulmonary and mesenteric vein strips, acetylcholine even in small doses caused a further increase in tension during electrical stimulation. This observation does not seem to support the hypothesis that acetylcholine inhibits the release of catecholamines during sympathetic nerve activation in all vascular smooth muscle. However, the direct effect of acetylcholine on pulmonary and mesenteric veins could possibly mask the inhibitory effect on the sympathetic nerves. This interpretation was validated by the experiments which showed that, during electrical stimulation of mesenteric vein strips, the efflux of [3H]norepinephrine decreased with acetylcholine even though the tension increased.

In several superfused pulmonary artery strips, acetylcholine caused small relaxations during tyramine-induced contractions. These relaxations, similar to those observed during norepinephrine-induced contractions in the organ bath experiments, were much smaller than those obtained during electrical stimulation. In none of the pulmonary artery strips was a decrease in radioactivity of the superfusate observed with acetylcholine during contractions caused by tyramine. These experiments confirm the conclusion from studies in the isolated saphenous vein that acetylcholine inhibits the release of norepinephrine by nerve impulses but not its pharmacological displacement by tyramine (2). In vascular smooth muscle, blockers of adrenergic postganglionic conduction, such as bretylium tosylate, inhibit the contractions caused by nerve stimulation but not those caused by tyramine (18, 19); hence the experiments in saphenous vein and pulmonary artery strips suggest that acetylcholine causes a rapid, reversible interruption of adrenergic postganglionic conduction rather than an inhibition of in and out movements of neurotransmitter.

The inhibition of the reactions to nerve stimulation cannot be explained by the cholinergic link hypothesis of Burn and Rand (20-21), since the initial effect of acetylcholine is not induction of a transient increase in the release of norepinephrine (2) and since the acetylcholine inhibition is not blocked by nicotinic antagonists (1) but is abolished by low doses of atropine. These findings confirm the presence of a muscarinic inhibitory receptor on the sympathetic nerve endings in the blood vessel wall (1, 3-6). Loffelholz and Muscholl (22) have demonstrated the presence of similar inhibitory muscarinic receptors on the sympathetic nerve endings to the heart; those receptors are activated not only during exogenous acetylcholine administration but also during vagal nerve stimulation (23), implying that they have physiological importance.

Taken in conjunction with previous work on dog saphenous vein (1, 2) and with the experiments on isolated arteries of other species (3-6), the present data indicate that acetylcholine can inhibit the release of the adrenergic transmitter throughout the vascular system. In the intact dog, acetylcholine inhibits the reaction of the cutaneous vein to sympathetic nerve activation, showing that the depression of adrenergic neurotransmission can occur in vivo (1). This action of acetylcholine explains part of its potent dilator effect in the intact organism, including the phenomenon of cholinergic neurogenic vasodilation in which acetylcholine is released in the vicinity of the adrenergic vasoconstrictor nerve endings.

**DIRECT ACTION OF ACETYLCOLINE ON VASCULAR SMOOTH MUSCLE CELLS**

In the absence of electrical stimulation or norepinephrine, acetylcholine in higher doses causes contraction of isolated cutaneous (1, 9-11, 24-28), jugular (26), mesenteric-portal (29-35), pulmonary (36, 37), and umbilical vein strips (38, 39). This effect has been confirmed in the different veins tested in the present experiments; however, in the mesenteric vein strips, as previously shown for the saphenous vein (2), the contraction is not accompanied by an increase in [3H]norepinephrine efflux. Contractions of cutaneous and mesenteric veins caused by acetylcholine are not inhibited by blockers of postganglionic adrenergic conduction, by ganglionic blocking agents, or by α-receptor inhibitors (1, 9, 11, 26, 27, 35). These contractions are present in denervated (11) and nerve-free venous tissue (38, 39) and can be explained by a direct action of the drug on the smooth muscle cells. The threshold concentration for this direct effect of acetylcholine differed in the different venous preparations used in the present study.

The most common reaction to acetylcholine in the isolated artery strips in the absence of electrical stimulation was a slight relaxation; with larger doses this relaxation was followed by a contraction.
In most artery strips the direct relaxing effect of acetylcholine could be exaggerated by preconstricting the strips with norepinephrine. Depression of the reactions to norepinephrine by acetylcholine has also been reported in rabbit aorta (40) and ear artery (4, 5, 41) and in rat mesenteric artery (3).

The transition from relaxation to constriction observed with increasing doses of acetylcholine illustrates the dual action of acetylcholine on some vascular smooth muscle cells. Both the inhibitory and the excitatory components are blocked by atropine. Thus, they both involve a muscarinic action of acetylcholine, and they cannot be separated according to the known classes of cholinergic receptors. Excitatory and inhibitory actions of acetylcholine, both of which are atropine-sensitive, have also been demonstrated in sympathetic ganglion cells (42, 43). The present data indicate that most venous smooth muscle is only sensitive to the excitatory action of acetylcholine; arteries are sensitive to both, with the inhibitory component having a lower threshold.

The dual action of acetylcholine on the smooth muscle cells, the differences in sensitivity to the inhibitory and the excitatory components among vascular preparations, and the action of acetylcholine on the sympathetic nerve endings may help reconcile the apparent discrepancy between the in vivo vasodilator and the in vitro vasoconstrictor properties of acetylcholine.

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**References**


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