Electrical Responses of Smooth Muscle Cells
During Cholinergic Vasodilation in the Rabbit Saphenous Artery

Kimihiro Komori and Hikaru Suzuki

Isolated smooth muscle tissues of the rabbit saphenous artery precontracted with noradrenaline (NE) were relaxed by acetylcholine (ACh, greater than 10^-7 M) or oxotremorine (greater than 10^-7 M), through the activation of muscarinic receptors, only when the endothelial cells were intact. ACh (greater than 10^-7 M) transiently hyperpolarized the membrane (1-4 minutes) with an associated decrease in the membrane resistance, either in the presence or absence of NE, and these changes ceased during the continuous application of ACh. The ACh-induced transient hyperpolarization was not generated after mechanically removing the endothelium or by treatment with atropine. Oxotremorine (up to 10^-5 M) did not alter the membrane potential in the presence or absence of the endothelium. NE (10^-6 M) depolarized the smooth muscle membrane, which remained unchanged by additional application of ACh or oxotremorine for more than 5 minutes, or after removal of the endothelium.

The excitation junction potential generated by perivascular nerve stimulation was inhibited by ACh (greater than 10^-8 M) or oxotremorine (greater than 10^-8 M) in a concentration-dependent manner. These inhibitory actions of ACh or oxotremorine were blocked by atropine but were not affected by removal of the endothelial cells. These results suggest that the inhibitory actions of muscarinic agonists on electrical responses of smooth muscle cells of the rabbit saphenous artery were mainly indirect, i.e., a release of inhibitory substances from the endothelial cells and the inhibition of adrenergic transmission. The former required higher concentrations of ACh or oxotremorine, thereby suggesting that the latter may be more important for vasodilation related to cholinergic mechanisms. (Circulation Research 1987;61:586-593)

In isolated arteries, acetylcholine (ACh) produces endothelium-dependent relaxation; that is, arterial smooth muscles that are precontracted by noradrenaline (NE), high potassium or other vasoactive agents are relaxed by ACh, and this ACh-induced relaxation disappears after removal of the endothelium. ACh also reduces the release of NE from vascular tissues during transmural nerve stimulation.

Electrophysiologic studies have revealed that in arterial smooth muscles, ACh hyperpolarizes the membrane and inhibits generation of the excitatory junction potential (EJP) evoked by stimulation of perivascular adrenergic nerves.

However, cellular mechanisms related to cholinergic vasodilation are not well understood. In the guinea pig mesenteric artery, stimulation of the muscarinic receptor by carbachol hyperpolarizes the membrane, and this hyperpolarization is converted to depolarization after removal of the endothelium. Thus, substances released from the endothelial cells during activation of the muscarinic receptors may be the central factor related to cholinergic vasodilation.

We investigated the effects of muscarinic agonists, ACh or oxotremorine, on smooth muscle cells and on adrenergic transmission in the rabbit saphenous artery by recording electrical responses of the smooth muscle cell membrane. The effects of these muscarinic agonists on adrenergic transmission were estimated from changes in the amplitude of EJP evoked by perivascular nerve stimulation. Involvement of the endothelium-derived substances on the actions of these muscarinic agonists was studied following mechanical removal of the endothelial cells.

Materials and Methods

Young albino rabbits (2-3 months old) of either sex, weighing 1.8-2.5 kg, were anesthetized with sodium pentobarbital (40 mg/kg) and exsanguinated. Segments of the saphenous artery (1-2 cm long) were excised, and the connective tissues were removed in Krebs solution at room temperature.

Helically cut strips of the artery (about 0.2 cm wide, 2 cm long, 0.15-0.2 mm thick) were suspended in a recording chamber and superfused with Krebs solution (35.5° C). The recording chamber was cylindrical (about 5 mm diameter) with a volume of about 0.4 ml. Both ends of the tissue were connected by silk thread; one end was fixed at the bottom of the chamber and the other end to a mechno-transducer (FD pick-up, TB-612T, Nihon-Kohden, Tokyo) for isometric tension recordings. The mechanical responses were displayed on a pen-writing recorder (VP-6521A, National Electric, Tokyo).

To record electrical responses of smooth muscle cells of the rabbit saphenous artery, tissues (about 0.2 cm wide, 2 cm long) were mounted in an organ bath that was made from Lucite plate (Figure 1). The organ bath of about 2 ml capacity was warmed to 35° C by water circulating under the chamber. The bath was separated...
into three compartments; the second compartment was separated from the other two by two silver plates (0.5 mm thick) at a 1-cm-wide distance. Each silver plate was coated with silver chloride on the side facing the center through which Krebs solutions were allowed to flow in directions from the first to the third compartments. A silicon rubber plate (1.5–2 mm thick; KE-66, Shin-etsu Kagaku, Tokyo) was fixed at the bottom of the second and third compartments. The tissue segment was mounted on the rubber plate so as to cross these two compartments through the slit on the silver plate and was immobilized by tiny pins. The tissue was mounted so that the adventitial layer was facing upwards and was superfused with Krebs solutions that were warmed by passing through the 35°C circulating water at a flow rate of 2–3 ml/min. Glass capillary microelectrodes filled with 3 M KCl (tip resistance, 50–80 MΩ) were inserted through the adventitial layer of the vessel to record electrical responses. Electrotonic potentials were produced by applying current pulses (1–2 seconds duration) between two silver plates (i.e., the partition stimulating method, Abe and Tomita), and the intensity of the current was measured as the voltage difference between these two silver plates (as V/cm). Perivascular nerves were stimulated by two silver wires (0.5 mm diameter) as described by Suzuki and Fujiwara. Briefly, the cathodal electrode was coated with enamel, except for the cut end, which was allowed to gently touch the surface of the vessel wall, and the anodal electrode was placed at the wall of the third compartment (the point stimulating method, Figure 1). The cathodal electrode was placed near the silver plate (distance, about 0.5–1.0 mm). Current pulses of 0.02–0.05 msec duration and 20–50 V intensity were applied through these two wires. Electrical responses of the smooth muscle membrane were displayed on a pen-writing recorder (Recticorder RJG4024, Nihon-Kohden, Tokyo).

The endothelium was removed by gentle rubbing with a cotton ball moistened with Krebs solution according to Furchgott and Zawadzki. After experiments, all the tissues were placed in neutralized 10% formaldehyde solution for histological examination, the objective being to confirm the removal of the endothelium. The samples were dehydrated using ethylalcohol and xylene and then were embedded in paraffin. Cross or vertical sections (20 μM thick) were prepared and stained with hematoxylin and eosin.

In both the electrical and mechanical experiments, 1–2 hours were needed to mount tissues in the chamber. The tissues were then allowed to equilibrate for 1–2 hours before starting the experiments, and all experiments were carried out within 2.5–10 hours after removal from the animal. During this time, the responses of the smooth muscle cells to drugs or perivascular nerve stimulation remained constant.

The Krebs solution had the following ionic composition (mM): Na+ 137.4, K+ 5.9, Mg2+ 1.2, Ca2+ 2.5, H2PO4− 1.2, HCO3− 15.5, Cl− 134, glucose 11.5. The solution was aerated with 97% O2-3% CO2, and the pH was maintained at 7.3–7.4. This solution was replaced with the one containing drugs just before it was allowed to enter the organ bath.

Drugs used were acetylcholine chloride, atropine sulphate, oxotremorine sesquisulphamate salt, noradrenaline hydrochloride (Sigma Chemical Co., St. Louis, Mo.), guanethidine sulphate (Tokyo Kasei Kogyo, Tokyo) and tetrodotoxin (Sankyo, Tokyo). Drugs were dissolved in Krebs solution to give the final concentrations described in “Results.”

The obtained values were expressed as mean ± SD. Differences between means within each experiment were evaluated by an analysis of variance. If the analysis of variance showed a significant difference among the means, Student’s t test or Sheffe’s test was used to determine which pairs of means were significantly different. Probabilities of less than 5% (p < 0.05) were considered significant.

**Results**

**Effects of ACh or Oxotremorine on the NE-Induced Contraction**

Experiments were first demonstrated to determine the involvement of the endothelium in the muscarinic agonists-induced relaxation of smooth muscle of the rabbit saphenous artery. As shown in Figure 2A, application of ACh (10−3 M) or oxotremorine (10−5 M) inhibited the NE (10−4 M)-induced contraction only when the endothelium was intact. This inhibition continued as long as these muscarinic agonists were present in the superfusate (up to 20 minutes). As the effects of ACh or oxotremorine were completely blocked by treatment (3 minutes) with atropine (10−4 M), these effects were considered to be mediated through activation of the muscarinic receptors.

Figure 2C shows the concentration-response relation of the ACh- or oxotremorine-induced relaxation in tissues that were contracted by NE (10−6 M). The tissues were relaxed by ACh (>10−7 M) or oxotremorine...
(>10^{-7} M), in a concentration-dependent manner. ACh was more potent than oxotremorine in reducing the NE-induced contraction up to 10^{-6} M; however, at 10^{-5} M both agents relaxed the tissue to a similar extent. The ACh- or oxotremorine-induced relaxation was abolished after mechanical removal of the endothelial cells (Figures 2B and 2C), thereby indicating that the relaxation was mainly related to these cells.

Electrical Responses of Smooth Muscle Membrane to ACh or Oxotremorine

Electrical responses of smooth muscle cells of the rabbit saphenous artery were recorded by impaling the microelectrode in cells located within the electrical length constant of this artery (about 1.1 mm, Holman and Surprenant) from the stimulating electrode. The criteria for a successful impalement were that the potentials obtained were stable and that electrotonic potentials with the time constant of 200–300 msec could be produced by inward current pulses of 1–1.5 sec duration and 0.1–0.3 V/cm intensity. If the recorded potentials were stable but electrotonic potentials could not be produced, then the effects of muscarinic agonists on such cells were not tested.

The effects of ACh or oxotremorine were observed in the presence of guanethidine (5 × 10^{-6} M) and tetrodotoxin (3 × 10^{-7} M) to eliminate the possible involvement of transmitter substances released from perivascular adrenergic nerves. The smooth muscle membrane of the rabbit saphenous artery was electrically quiescent, and the resting membrane potential ranged between -65 and -75 mV, as has been reported by Holman and Surprenant. Application of ACh (10^{-5}–10^{-4} M) transiently hyperpolarized the membrane, and with continued application of ACh, the membrane potential reverted to the resting potential level within 1–4 min (mean ± SD, 122 ± 38 sec, n = 20). The amplitude of the ACh (10^{-5} M)-induced transient hyperpolarization varied between tissues in the range of 2.5–8.0 mV; however, in the same tissue, the variation was slight. For example, the ACh responses recorded in 6 different cells from the same tissue were 4–5 mV in amplitude (4.6 ± 0.3 mV) and 150–180 seconds in duration (163 ± 10 seconds). These same findings were apparent in all 15 tissues studied. Therefore, we considered that ACh produced a transient hyperpolarization of the membrane, nearly homogeneously, in all smooth muscle cells of the tissue.

The transient hyperpolarization seen in the case of ACh was not produced by oxotremorine. The effects of oxotremorine were tested in cells that were hyperpolarized by application of ACh, the membrane potential reverted to the resting potential level within 1–4 min (mean ± SD, 122 ± 38 sec, n = 20). The amplitude of the ACh (10^{-5} M)-induced transient hyperpolarization varied between tissues in the range of 2.5–8.0 mV; however, in the same tissue, the variation was slight. For example, the ACh responses recorded in 6 different cells from the same tissue were 4–5 mV in amplitude (4.6 ± 0.3 mV) and 150–180 seconds in duration (163 ± 10 seconds). These same findings were apparent in all 15 tissues studied. Therefore, we considered that ACh produced a transient hyperpolarization of the membrane, nearly homogeneously, in all smooth muscle cells of the tissue.

The transient hyperpolarization seen in the case of ACh was not produced by oxotremorine. The effects of oxotremorine were tested in cells that were hyperpolarized by application of ACh. The ACh-induced transient hyperpolarization was reproducible when the intervals were sufficiently long (usually more than 15
minutes washing was required to produce a similar amplitude of the response); however, oxotremorine never produced a transient or sustained change in the membrane potential (Figure 3A).

Application of NE (>3 x 10^{-7} M) depolarized the smooth muscle cell membrane in a concentration-dependent manner. With 10^{-6} M NE, the membrane was depolarized to about -62 mV (Table 1), and additional application of ACh but not oxotremorine again produced a transient hyperpolarization (Figure 3B). The duration of the ACh-induced transient hyperpolarization in the presence of NE was 129 ± 52 seconds (n = 18), this value being insignificantly different compared with the control (p > 0.6).

Mechanical removal of the endothelial cells did not alter the resting membrane potential of the smooth muscle cells (Table 1), and in such tissues, ACh or oxotremorine failed to produce the transient hyperpolarization (Figure 3C). The NE-induced depolarization was also not affected by removal of the endothelial cells (Table 1).

Figure 4 summarizes the concentration-response relation of ACh or oxotremorine on the membrane potential in which the amplitude of the transient hyperpolarization (Figure 4A) and the membrane potential at a steady state (more than 5 minutes application of ACh or oxotremorine, Figure 4B) are shown in different scales. ACh (>10^{-6} M) produced a transient hyperpolarization only in tissues with an intact endothelium, and the maximum amplitude occurred at 10^{-5} M. Oxotremorine (up to 10^{-4} M) did not produce a transient hyperpolarization. Mechanical removal of the endothelium inhibited generation of the ACh-induced transient hyperpolarization.

The membrane potential at the steady state remained unchanged during application of ACh or oxotremorine in both the presence or absence of the endothelial cells (Figure 4B, Table 1).

Figure 5A shows the effects of ACh (10^{-3} M) on electrotonic potentials produced by alternate applications of the constant intensity of inward and outward current pulse in the rabbit saphenous artery. During the ACh-induced transient hyperpolarization, the amplitude of electrotonic potentials was decreased, and this amplitude was restored with recovery of the membrane potential.

The relation between the intensity of the current pulse and amplitude of the resulting electrotonic potential (Figure 5B) showed that in the absence of ACh, it was linear to the inward current and to outward currents with a weaker intensity. Stronger intensities of the outward current (>0.4 V/cm) showed a rectification of the membrane. After ACh had been applied for more than 5 minutes, the current-voltage relation observed in the same cell was much the same as that seen before the application of ACh.

**Modulation of Adrenergic Transmission by ACh or Oxotremorine**

The effects of ACh or oxotremorine on adrenergic transmission in the rabbit saphenous artery were

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### Table 1. Membrane potentials of smooth muscle cells of rabbit saphenous artery during application of norepinephrine, acetylcholine, or oxotremorine in tissues with or without endothelial cells

<table>
<thead>
<tr>
<th>Condition</th>
<th>Membrane potential (mV) ± SD</th>
<th>Number of observations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Endothelium intact</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>-69.5 ± 2.3</td>
<td>46</td>
</tr>
<tr>
<td>Oxo 10^{-5} M</td>
<td>-68.9 ± 1.8</td>
<td>16*</td>
</tr>
<tr>
<td>NE 10^{-6} M</td>
<td>-62.0 ± 2.9</td>
<td>15†</td>
</tr>
<tr>
<td>NE 10^{-5} M + Oxo 10^{-5} M</td>
<td>-61.4 ± 1.6</td>
<td>15†</td>
</tr>
<tr>
<td>NE 10^{-6} M + ACh 10^{-5} M</td>
<td>-62.2 ± 2.3</td>
<td>16†</td>
</tr>
<tr>
<td><strong>Endothelium removed</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>-68.7 ± 2.2</td>
<td>23</td>
</tr>
<tr>
<td>Oxo 10^{-5} M</td>
<td>-68.0 ± 1.3</td>
<td>14*</td>
</tr>
<tr>
<td>NE 10^{-6} M</td>
<td>-61.5 ± 2.0</td>
<td>13†</td>
</tr>
<tr>
<td>NE 10^{-5} M + Oxo 10^{-5} M</td>
<td>-61.3 ± 2.2</td>
<td>14†</td>
</tr>
<tr>
<td>NE 10^{-6} M + ACh 10^{-5} M</td>
<td>-62.1 ± 2.5</td>
<td>17†</td>
</tr>
</tbody>
</table>

Membrane potentials were measured by impaling the microelectrode into different cells from 3 to 5 different tissues, while norepinephrine (NE), acetylcholine (ACh), or oxotremorine (Oxo) was applied for 5 to 20 minutes. Mean ± SD (number of observations is in parenthesis).

*Control significance is p < 0.05. *Not significant from control; † not significant from NE-induced depolarization; ‡ significant from control.
ACh10^{-5}M

5mV

ACh10^{-5}M

0.5V/cm

5mV

1min

FIGURE 5. Effects of ACh on electrical properties of smooth muscle membrane. A: Effects of ACh on electrotonic potentials produced by alternate application of a constant intensity (0.3 V/cm) of inward and outward current pulses (1.5 sec duration) at a constant rate (0.1 sec^-1) in the presence of guanethidine (5 x 10^{-4} M) and tetrodotoxin (3 x 10^{-7} M). ACh (10^{-5} M) was applied at arrow and was present throughout the record shown. Upper trace, current monitor in which upward and downward deflections indicate outward and inward currents, respectively. Lower trace, membrane potential change.

B: Current-voltage relation obtained before (O) application of 10^{-5} M ACh and after (*) ACh had been applied over 5 minutes.

estimated from changes in the amplitude of the excitatory junction potentials (EJPs) evoked by perivascular nerve stimulation. In preliminary experiments, application of a brief current pulse (0.05 msec duration, 10–50 V intensity) evoked an EJP that was reversibly blocked by tetrodotoxin (3 x 10^{-5} M) or irreversibly by guanethidine (5 x 10^{-4} M, more than 20 minutes). Thus, the EJP was confirmed to be generated as a result of perivascular nerve excitation.

Perivascular nerves were stimulated every 30 seconds while ACh (10^{-4} M) or oxotremorine (10^{-5} M) was applied to the superfusate. ACh transiently hyperpolarized the membrane and blocked the generation of EJP; this blockade continued after the membrane potential had been restored (Figure 6A). Oxotremorine also blocked the EJP without generation of the transient hyperpolarization (Figure 6B). In tissues without endothelium, ACh (10^{-5} M) inhibited the EJP without generation of the transient hyperpolarization (Figure 6C). These effects of ACh or oxotremorine were absent in the presence of atropine (10^{-6} M, Figure 6D).

The effects of ACh or oxotremorine on the EJP amplitude are shown in Figure 7. The amplitude of EJPs evoked by single stimuli was expressed relative to the control. The EJP was inhibited by ACh or oxotremorine (>10^{-9} M) in a concentration-dependent manner, and there was no causal relation to the presence or absence of the endothelial cells. Figure 7 also shows that in the presence of atropine (10^{-6} M), ACh or oxotremorine did not inhibit EJPs in concentrations up to 10^{-3} M.

The EJP decayed exponentially in the rabbit saphenous artery, and the potential plotted on a logarithmic scale against time decayed linearly up to 500 msec. The time constant of the falling phase of the EJP calculated from the time when the potential decayed to 1/e was 256 ± 26 msec (n = 18) under control conditions. ACh at a concentration of 10^{-4} M reduced the amplitude of the EJP to about 70% of the control; however, the time constant of the falling phase of the EJP did not significantly change (249 ± 30 msec, n = 16, p>0.3).

Reduction of the EJP amplitude by oxotremorine (10^{-4} M) to about 70% of the control was not accompanied by changes in the time constant of the decay of EJP (248 ± 15 msec, n = 7, p>0.4).

The inhibitory effects of ACh or oxotremorine were examined on EJPs evoked by repetitive stimulation of perivascular nerves at rates that were expected to be within the physiologic limits, i.e., below a rate of 8 sec^{-1}. With repetitive stimulation of perivascular nerves at rates of 0.2–1 sec^{-1}, the amplitudes of EJPs were increased successively in the initial 3–5 stimuli (facilitation phenomenon), then reached a steady amplitude 2–5 times larger than the first, or sometimes...
Muscarinic Vasodilation

A. Concentration-response relation of effects of ACh (A) or oxotremorine (B) on EJP amplitude. Amplitude of EJPs induced by single stimuli (0.03–0.05 msec duration and 30–50 V intensity) was expressed relative to control. O, in the presence of endothelium; •, after removal; ▲, in the presence of atropine (10⁻⁶ M). Each point shows mean ± SD of 8–10 observations obtained from 3–5 different tissues.

B. Decreased again to 70–80% of the maximum amplitude during the continued stimulation. Figure 8A shows that with application of 10⁻⁸ M ACh, the amplitudes of EJPs evoked by 10 stimuli at a rate of 0.5 sec⁻¹ were decreased, but the facilitation phenomenon remained intact. Such effects of ACh on EJPs were also observed in tissues from which the endothelium had been mechanically removed (Figure 8B).

Figure 8C shows the effects of oxotremorine (10⁻⁸ and 10⁻⁶ M) on EJPs evoked by a train of perivascular nerve stimulation at a rate of 0.5 sec⁻¹ in the frequency. Oxotremorine reduced the amplitudes of EJPs, but the facilitation of EJPs remained intact as in the case of ACh (Figure 8A).

Histologic Examination

All the tissues used in the experiments were examined histologically. Light microscopy revealed that the endothelial cells were intact in the tissues used as the controls, but in tissues in which the internal lumen was removed, the endothelial layers were all but completely absent.

Discussion

In some arteries, both adrenergic and cholinergic nerves are located in close apposition to their terminals at the vessel wall,¹³,¹⁴ and in general, their main functions are vasoconstriction and vasodilation, respectively.¹⁵ The neurogenic cholinergic mechanisms that may contribute to vasodilation are either “active” vasodilation or withdrawal of the adrenergic constrictor tone.¹⁶ In the present experiments, we investigated the cellular mechanisms related to each of these two possibilities using the isolated rabbit saphenous artery by recording electrical responses of the smooth muscle cells. As substances released from endothelial cells are largely related to the cholinergic vasodilation,¹³,¹⁶ the possible involvement of the endothelial cells on the actions of muscarinic agonists was also examined.

Experiments were carried out by recording electrical responses from single cells; therefore, these responses may not be equal to those for all populations of cells in the tissue. We recorded the responses from several cells in each tissue and found that within the same tissue, the variation of the responses between cells was minute compared with those obtained from different tissues. When considering electrical coupling between cells, the wall thickness (up to 200 μm) was less than

Figure 8. Effects of ACh (A and B) or oxotremorine (C) on EJPs elicited by perivascular nerve stimulation (0.03 msec duration and 30 V intensity). A train of 10 pulses at a rate of 0.5 sec⁻¹ was applied in the presence of different concentrations of ACh or oxotremorine. A and C, endothelium intact; B, no endothelium. Each group of responses was obtained from single cells in different tissues.
the electrical length constant of this artery. Therefore, electrical responses generated in cells located near the endothelium could propagate to the recorded cells located near the surface of the vessel, with a decrease of less than 17% the original response [the value being calculated by the cable equation: \( V_x = V_0 \exp \left( -x/\lambda \right) \), where \( V_0 \) and \( V_x \) equal amplitude of responses evoked in cells located at the inner and the outer regions of the vessel, respectively; \( x \) is the distance between cells, equal to 0.2 mm; and \( \lambda \) is the length constant, equal to 1.1 mm]. Such would allow for a valid estimation of the responses of all the cells when recording from single smooth muscle cells.

The smooth muscle membrane of the rabbit saphenous artery was transiently hyperpolarized by ACh but not by oxotremorine in the presence or absence of NE, but both of these muscarinic agonists relaxed the tissues precontracted by NE. Because the ACh-induced transient hyperpolarization was observed only when the endothelial cells were intact, this hyperpolarization may be generated by substances released from the endothelial cells. The differences between ACh and oxotremorine with regard to electrical and mechanical responses could be explained by postulating that stimulation of muscarinic receptors releases two different substances from the endothelial cells; one relaxes the smooth muscle, and the other hyperpolarizes the smooth muscle membrane. Muscarinic receptors are classified into two subtypes (M<sub>1</sub> and M<sub>2</sub>, see Goyal and Rattan). Although the present experiment did not determine the subtype of muscarinic receptors located in the endothelial cells, it would be of interest if each subtype of muscarinic receptor is functionally different as related to the cholinergic vasodilation.

In the rabbit saphenous artery, the ACh-induced hyperpolarization was associated with a decrease in membrane resistance as estimated from the decrease in amplitude of the electrotonic potential. This hyperpolarization was presumably produced by an increase in permeability of the membrane to potassium ions as has been suggested in other arteries. After the removal of the endothelium, ACh no longer produced the transient hyperpolarization, i.e., ACh had no direct excitatory or inhibitory actions on electrical properties of smooth muscle cell membrane in this artery. This implies that the cessation of the hyperpolarization during continued application of ACh is not due to simultaneously developing depolarization of the membrane. Because the membrane potential and the membrane resistance were not altered during prolonged exposure to ACh, this concept is given support. However, these actions of ACh on the rabbit saphenous artery are in contrast to the actions of carbachol on the guinea pig mesenteric artery in which stimulation of the muscarinic receptor by carbachol hyperpolarized the membrane and, after deendothelialization, depolarized the membrane, thereby suggesting that carbachol has direct depolarizing actions and indirect hyperpolarizing actions on smooth muscle cells. It remains to be determined whether the differences between ACh and carbachol are due to different agonists, as in the case of ACh and oxotremorine, or to regional variations that are frequent in vascular tissues.

The relaxation of smooth muscle of the rabbit saphenous artery induced by muscarinic agonists was independent of the membrane potential, i.e., the NE-induced depolarization of the membrane remained unchanged during application of ACh or oxotremorine. The release of the endothelium-derived relaxing factor (EDRF) may be stimulated by NE, as deduced from evidence that the NE-induced contraction is enhanced by removing the endothelial cells or by blocking the release of EDRF using hydroquinone. The amplitude of the NE-induced depolarization was not affected by removing the endothelium, thereby indicating that this depolarization was not affected by EDRF as in the case of the guinea pig mesenteric artery.

Evidence suggests that the EJP evoked in smooth muscle of blood vessels or vas deferens may be produced by substances other than NE, possibly by ATP released together with NE from adrenergic nerves. The cellular responses of vascular smooth muscles to perivascular nerve stimulation are an EJP, or a slow depolarization, or both. Among these, the slow depolarization is generated by NE released from adrenergic nerves, as deduced from evidence that \( \alpha \)-adrenoceptor antagonists can block the slow depolarization but not the EJP and that depletion of NE from nerve terminals leads to cessation of the generation of the slow depolarization but not the EJP. The EJP is related to the slow depolarization, and there is a linear relation between amplitudes of the EJP and the slow depolarization; that is, the amount of NE released could be estimated from the amplitude of EJP.

In the rabbit saphenous artery, the EJP was generated as a result of perivascular nerve excitation because the EJP was blocked reversibly by tetrodotoxin or irreversibly by guanethidine. The generation of EJPs was inhibited by ACh or oxotremorine. This may be related to the inhibition by ACh or oxotremorine of the transmitter release from adrenergic nerves through activation of muscarinic receptors located at the nerve terminal. At the adrenergic nerve terminals, two types of ACh-receptor are identified, and stimulation of nicotinic receptors facilitates, while muscarinic receptors inhibit, the release of NE from nerve terminals during nerve excitation. In the rabbit saphenous artery, and also in the guinea pig mesenteric artery, ACh or oxotremorine applied after atropinization did not alter the amplitude of EJP. Thus, these muscarinic agonists probably do not have nicotinic excitatory effects on transmitter release from perivascular adrenergic nerves.
The ACh- or oxotremorine-induced inhibition of the EJP may be mainly due to activation of the presynaptic muscarinic receptors and endothelium-derived substances or changes in properties of the postsynaptic membrane that may not be involved because ACh- or oxotremorine-induced inhibition of EJP remained unchanged after removal of the endothelium. The threshold concentration of ACh or oxotremorine required to inhibit the EJP was over 100 times lower than that which inhibited the NE-induced contraction. All these observations suggest that in the rabbit saphenous artery, substances released from the endothelium by ACh or oxotremorine are not involved in the inhibition of adrenergic transmission.

We observed that ACh or oxotremorine inhibits the smooth muscle of the rabbit saphenous artery mainly by an indirect action, i.e., release of endothelium-derived substances and inhibition of adrenergic transmission. Direct actions of ACh or oxotremorine on the smooth muscle may be weak in this artery. Because ACh or oxotremorine inhibited the EJP at concentrations that were over 100 times lower than those required for the inhibition of the NE-induced contraction, withdrawal of the adrenergic constrictor tone may be more important for the physiologically occurring vasodilation induced by muscarinic agonists.

Acknowledgments
The authors are grateful to Professor H. Kuriyama for helpful discussions and critical comments and to M. Ohara for reading the manuscript.

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**Key Words** • vascular smooth muscle • acetylcholine • oxotremorine • vasodilation • excitatory junction potential • endothelium • adrenergic transmission
Electrical responses of smooth muscle cells during cholinergic vasodilation in the rabbit saphenous artery.
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Circ Res. 1987;61:586-593
doi: 10.1161/01.RES.61.4.586

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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