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THE EFFECTS OF ACETYLCHOLINE ON THE MEMBRANE AND CONTRACTILE PROPERTIES OF SMOOTH MUSCLE CELLS OF THE RABBIT SUPERIOR MESENTERIC ARTERY

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1 Effects of acetylcholine (ACh) on the membrane potential and mechanical properties of rabbit superior mesenteric artery were investigated by the use of microelectrode and isometric tension recording methods. The membrane potential was -62.5 ± 3.0 mV (s.d.). The maximum slope of the membrane depolarization produced by tenfold increase in $[K]_o$ plotted on a log scale was 48 mV. Excess $[K]_o$ and low $[K]_o$ depolarized the membrane and produced contraction (contracture). The minimum depolarization to produce contraction was 10 mV.

2 Low concentrations (10 and 100 ng/ml) of ACh hyperpolarized the membrane. Increased concentrations of ACh (1 and 10 μg/ml) hyperpolarized the membrane further in adult rabbit, while increased concentrations of ACh produced a smaller hyperpolarization in young rabbit. These potential changes produced by ACh in immature and adult rabbits were suppressed by treatment with atropine (0.1 μg/ml).

3 ACh (10 ng to 1 μg/ml) consistently generated contraction in Krebs solution. However, ACh relaxed the contraction induced by either K^+ or noradrenaline in the adult rabbit, and it enhanced contraction produced by this treatment in the immature rabbit. In Ca-free EGTA solution, the action of ACh on the mechanical response was markedly suppressed, although high concentrations of ACh still evoked contraction. However, treatment with atropine (1 μg/ml) completely prevented these actions of ACh.

4 ACh-induced relaxation during either K^+-induced or noradrenaline-induced contraction was not caused by the hyperpolarization of the membrane.

5 It is concluded that ACh possesses dual actions on smooth muscle cells of the rabbit superior mesenteric artery in Krebs solution, i.e. ACh hyperpolarizes the membrane, while it consistently generates contraction. These ACh actions on the muscle cells were modified by aging.

Introduction

Electrophysiological evidence concerning the functions of arterial smooth muscles have been reviewed by many authors (Somlyo & Somlyo, 1968a; Holman, 1969; Speden, 1970; Bevan & Su, 1973; Somlyo, 1975). Most of the electrophysiological studies of vascular smooth muscles were done on large elastic arteries such as aorta or pulmonary artery, but the membrane properties and the mechanisms of actions of drugs at a cellular level in the smooth muscle of small arterial resistance vessels are not yet fully understood.

Acetylcholine (ACh) is known to produce an excitatory action in many smooth muscles. Most of the isolated vascular smooth muscles develop contraction on treatment with ACh, accompanied either by increase in spike discharges (Funaki & Bohr, 1964; Nakajima & Horn, 1967) or by slow depolarization of the membrane (Su & Bevan, 1965; Keatinge, 1966). However, ACh-induced vasodilatation has been reported in the intact organism or in isolated vascular smooth muscles which are constricted either by nerve stimulation or by administration of vasoactive agents such as noradrenaline and KCl (Burn & Rand, 1965; Rice & Long, 1966; Malik & Ling, 1969; Rand & Varma, 1970; Hume, de la Lande & Waterson, 1972; Steinsland, Furchgott & Kirpekar, 1973; Vanhoutte, Lorenz & Tyce, 1973). Some of the vasodilator actions of ACh are thought to be due to a decrease in transmitter release from the sympathetic nerve endings in the wall of the blood vessel (Vanhoutte *et al.*, 1973; Vanhoutte, 1976). The inhibitory action of ACh on adrenergic transmission was suggested to

account for the suppression of the reactivity of isolated arteries to sympathetic nerve stimulation (Malik & Ling, 1969; Rand & Varma, 1970; Hume *et al.*, 1972) as well as the decrease in release of transmitter. However, ACh caused a contraction of non-innervated veins after α-adrenoceptor blockade. Therefore, a direct action of ACh was also postulated (Ehinger, Gennser, Owman, Persson & Sjöberg, 1968; Vanhoutte & Lorenz, 1970; Loh, 1971; Altura, Malaviya, Reich & Orkin, 1972).

The present experiments were designed to study the effects of ACh on membrane potential and contractile activities of isolated rabbit mesenteric artery to clarify the postsynaptic actions of ACh.

Methods

Albino rabbits, immature (about 3 months old) and adult (more than one year old) were used. A branch of the superior mesenteric artery, 200 μm to 500 μm in diameter, was taken from the animal which had been killed by injection of air into the ear artery. All the side branches from the artery were cut off. To measure the membrane potential, connective tissues surrounding the vessel were removed under a microscope. The tissue was mounted in an organ bath having a volume of 2 ml, without opening the vessel, and each end was pinned in position. In some experiments, the tissues were cut in a helical direction to give a width of about 1.0 mm and a length of about 10 mm. A glass microelectrode filled with 3 M KCl, having a resistance of more than 50 MΩ, was inserted into the cells from the serosal side of the vessel. Mechanical responses were recorded from helical strips of the mesenteric artery (1 mm wide, 10 mm long). Two pieces of the tissue were mounted in parallel in the same organ bath which had a vertical tubular shape and a volume of 2 ml. One end of the tissue was fixed at the bottom of the bath and the other end was connected to an isometric tension recorder by a thread. Owing to the use of the above procedure to prepare the muscle strip, it was difficult to define whether the contraction was mainly generated from the longitudinal muscle layer or from the circular muscle layer.

Modified Krebs solution which had the following ionic composition (mM) was used; Na^+ 137.4, K^+ 5.9, Mg^{2+} 1.2, Ca^{2+} 2.5, HCO_3^- 15.5, $H_2PO_4^-$ 1.2, Cl^- 134.0 and glucose, 11.5. The solution was aerated with 97% O_2 and 3% CO_2, and pH was adjusted to 7.2. In the excess $[K]_o$ solution NaCl was replaced with KCl. In the Na-deficient solution NaCl was replaced with an equimolar concentration of sucrose. Ca-free solution contained 1 mM EGTA (1,2,bis,2-aminoethoxyethane-*NNN'N'*-tetra-acetic acid, Dozin Kagaku Pharm. Co.). The temperature of the perfusing solution was kept at 35°C.

Drugs used in these experiments were acetylcholine chloride (Daiichi Pharm. Co.), (±)-noradrenaline (Sankyo Pharm. Co.), atropine sulphate (Tanabe Pharm. Co.), ouabain (G-strophanthin, Takeda Chem. Ind.), phentolamine (Takeda Chem. Ind.), and tetrodotoxin (Sankyo Pharm. Co.). Drugs were dissolved in the perfusing solution and final concentrations of the drugs are expressed as g/ml.

Results

Membrane properties

Smooth muscle cells of the superior mesenteric artery of the rabbit remained electrically quiescent and did not show any spontaneous electrical activity. The membrane potential of the mesenteric artery was -62.5 ± 3.0 mV (mean ± s.d., $n = 55$) under unstretched conditions. The membrane potential was sensitive to stretch, and when the tissue was stretched severely the membrane depolarized by several mV. Measurement of the passive electrical properties of the membrane was not possible due to technical difficulties.

Changes in membrane potential and tension development induced by various extracellular potassium concentrations ($[K]_o$) are shown in Figure 1. Increased $[K]_o$ depolarized the membrane from the resting potential of -62.3 ± 2.3 mV (±s.d., $n = 42$) and produced a contracture. In Figure 1a the depolarization was plotted against $[K]_o$ on a logarithmic scale, the maximum slope of the line represents a depolarization of 48 mV for a tenfold increase in $[K]_o$. Decrease in $[K]_o$ to 3 mM hyperpolarized the membrane, but further decrease in $[K]_o$ depolarized the membrane again. An increase in tension was generated by depolarization of the membrane, induced by either increasing or decreasing $[K]_o$ (Figure 1b). The minimum depolarization to produce contraction was 10 mV at 15.8 mM $[K]_o$ which was almost a 3 times higher concentration than the control value (5.9 mM). In K-free solution, the membrane depolarized (about 10 mV) and tension developed. Following pretreatment with K-free solution, normal Krebs solution hyperpolarized the membrane from -51 mV to -74 mV within 10 min (Figure 2a).

Depolarization of the membrane was also produced by treatment with ouabain (0.5 μg/ml) (Figure 2b). The amplitude of the membrane depolarization induced by 0.5 μg/ml ouabain was nearly the same as that induced by K-free solution. Differences between ouabain treatment and K-free solution were observed in the recovery process, i.e., transient hyperpolarization was observed after K-free induced depolar-

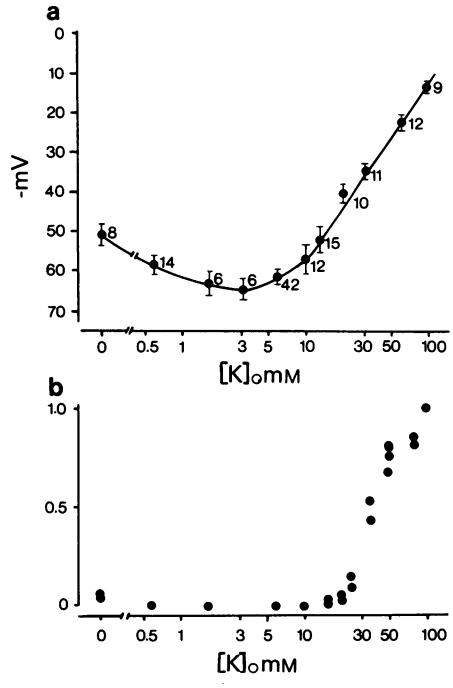

Figure 1 Changes in membrane potential (a) and tension development (b) of 12 months old rabbit at various concentrations of external potassium ion. (a) Membrane potentials (mean, vertical lines show s.d.) were plotted against log $[K]_o$ (mM). Number of observations are shown beside each point. (b) Relative values of tension development induced by various $[K]_o$ are plotted against log $[K]_o$ (mM). The amplitude of contracture induced by 98 mM $[K]_o$ is shown as 1.0.

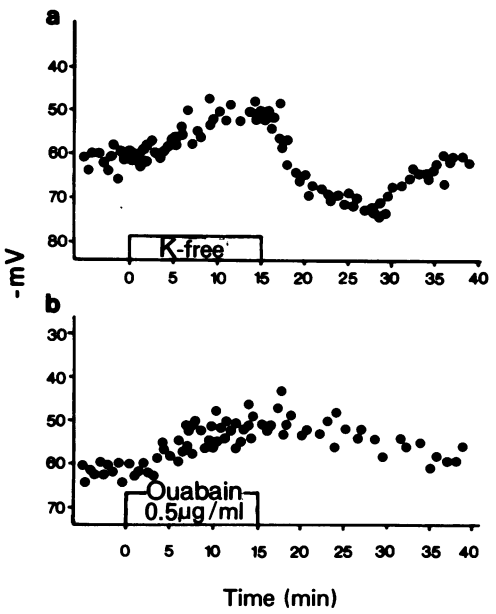

Figure 2 (a) Time course of changes in membrane potential of 12 months old rabbit superior mesenteric artery in K-free solution. Krebs solution was changed to K-free solution for 15 min. (b) Effect of ouabain (0.5 µg/ml) on membrane potential.

ization but it was not observed after treatment with ouabain.

Effects of noradrenaline

Noradrenaline is known as a vasoconstrictor, but its action differs according to the concentration; for example in the rabbit main pulmonary artery lower concentrations of noradrenaline evoked contraction without any changes in the membrane potential and ion fluxes, and higher concentrations evoked contraction with depolarization of the membrane (Su, Bevan & Ursillo, 1964; Somlyo & Somlyo, 1968b; Casteels, Kitamura, Kuriyama & Suzuki, 1977).

The effects of noradrenaline on membrane potential and contractile activity of the superior mesenteric artery are shown in Figure 3. In concentrations of more than 10 ng/ml, noradrenaline evoked contraction (Figure 3a). Increasing the concentration induced larger and irregular contraction.

Figure 3b shows the changes in membrane potential produced by various concentrations of noradrenaline. Higher concentrations of noradrenaline (100 ng/ml) depolarized the membranes. However, the threshold concentration of noradrenaline (10 ng/ml) for evoking a mechanical response, did not depolarize the membrane, as was observed in the rabbit pulmonary artery (Casteels, *et al.*, 1977).

Effects of acetylcholine

In most of the vascular smooth muscles, treatment with ACh depolarized the membrane and produced a contraction (Speden, 1970). On the other hand, suppression of spontaneous electrical activity by ACh was observed in rat mesenteric artery (Steedman, 1966).

In the present experiments the membrane potentials were measured by successive impalements with the microelectrode. Acetylcholine induced a hyperpolarization, but the amplitude differed from animal to animal, with the time after application of ACh and also with the age of the animal. The time course of changes in membrane potential of the mesenteric artery of the immature rabbit (less than 3 months old) induced by different concentrations of ACh (0.1 µg/ml and 10 µg/ml) is shown in Figure 4. When 0.1

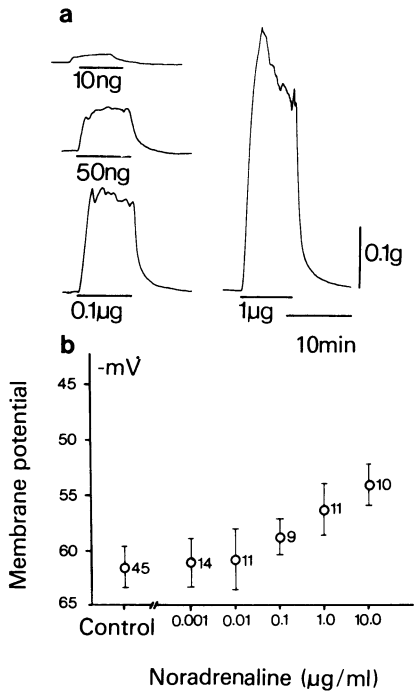

Figure 3 Effects of noradrenaline on tension development (a) and changes in membrane potential (b) of 6 months old rabbit. Membrane potentials (mean, vertical lines show s.d.) obtained between 5 and 30 min after application of noradrenaline are plotted against logarithmic concentration of noradrenaline (µg/ml). Number of observations are given beside each point.

µg/ml ACh was added, approximately the same amplitude of hyperpolarization was maintained throughout the application, and the membrane showed large rebound excitation (depolarization) when ACh was washed out. On the other hand, 10 µg/ml ACh induced a large initial hyperpolarization which declined to nearly the same level as the resting membrane potential, although ACh was still in the bath. However, in the adult rabbit (more than one year old) almost the same amplitude of hyperpolarization was induced by 10 µg/ml ACh and was maintained for more than 30 min. As a result of these observations, the effects of various concentrations of ACh on the membrane potential were mainly observed between 5 and 15 min after the application of ACh. Tissues obtained from the adult rabbit showed a smaller hyperpolarization than those from the immature rabbit. The membrane of the mesenteric artery of the adult rabbit hyperpolarized in step with the increase in concentrations of ACh between 10 ng and 1 µg/ml (Figure 5b). However, mesenteric artery

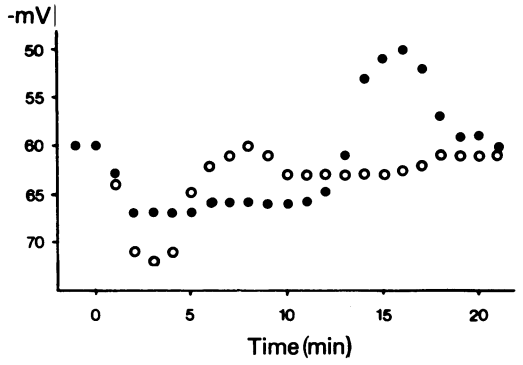

Figure 4 Time course of changes in membrane potential induced by different concentrations of acetylcholine (●, 0.1 µg/ml; ○, 10 µg/ml). The membrane potential was recorded from a single smooth muscle cell of mesenteric artery of an immature rabbit (3 months old).

from the immature rabbit showed different responses to ACh with differing concentrations; i.e., low concentrations of ACh (10 ng to 100 ng/ml) hyperpolarized the membrane, but the amplitude of the hyperpolarization decreased with an increase in ACh concentration to 1 µg/ml, and a further increased concentration (10 µg/ml) did not change the membrane potential from the control value (Figure 5a).

The amplitude of the hyperpolarization induced by ACh depended on the external K ion concentration (Figure 6). Decrease in $[K]_o$ to one tenth the normal concentration (0.59 mM) depolarized the membrane by about 8 mV, and application of ACh produced a larger hyperpolarization than that observed in Krebs solution (7 mV in Krebs, 20 mV in 1/10 $[K]_o$ solution). Increase in $[K]_o$ from 5.9 mM to 25 mM depolarized the membrane from −61 mV to −40 mV, and ACh induced only 2 mV hyperpolarization of the membrane. Decrease in $[Na]_o$ to 1/9 the normal concentration did not affect the hyperpolarizing action of ACh. These results indicate that hyperpolarization by ACh is due to an increase in the K-conductance of the membrane. The larger hyperpolarization produced by ACh in 1/10 the the normal $[K]_o$ solution may be due to shift of K-equilibrium potential (E_k) in a more negative direction, while the smaller hyperpolarization in excess-K ion solution may be due to a decrease in E_k.

The hyperpolarization of the membrane induced by ACh may be due to a muscarinic action of ACh on the mesenteric artery, since treatment with atropine suppressed this hyperpolarization.

A vasoconstrictor action of ACh in small arterial vessels has been reported by several investigators (Nielsen & Owman, 1971; Altura *et al.*, 1972). ACh

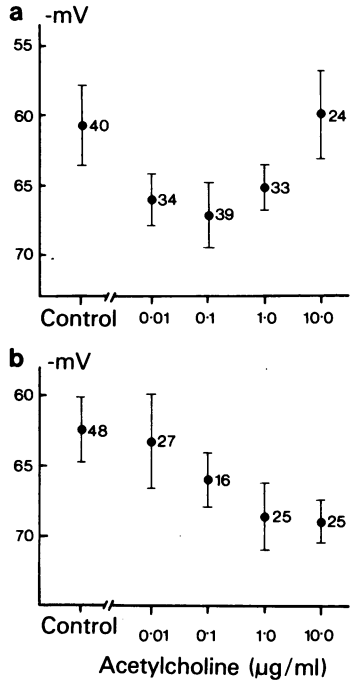

Figure 5 Effects of acetylcholine (ACh) on membrane potential of superior mesenteric artery obtained from immature (3 months old) (a) and from adult (12 months old) (b) rabbits. Membrane potentials (mean, vertical lines show s.d.) are plotted against logarithmic scale of ACh concentration. Number of observations are shown beside each point. Membrane potentials were measured at 5 and 30 min during application of ACh by successive penetrations of the microelectrode into the cells.

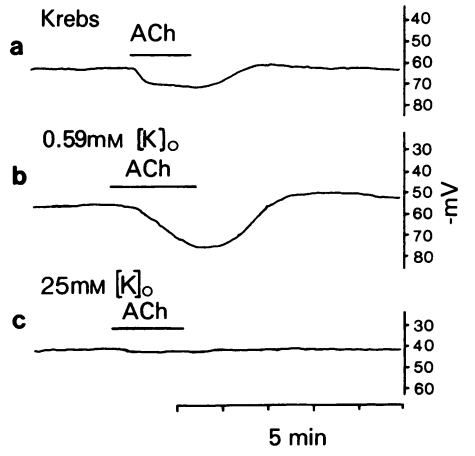

Figure 6 Effects of different external potassium concentrations ($[K]_o$) on hyperpolarizing action of acetylcholine (ACh) on the mesenteric artery of 6 months old rabbit. These records were obtained from the same tissue but different cells. Membrane potentials in Krebs (a), 0.59 mM $[K]_o$ (b), and 25 mM $[K]_o$ (c) solutions were −61 mV, −52 mV, and −40 mV, respectively. ACh in a concentration of 0.1 μg/ml was applied at bar shown in each record. Amplitude of the hyperpolarization induced by ACh was 7 mV, 20 mV, and 2 mV in Krebs, 0.59 mM $[K]_o$, and 25 mM $[K]_o$ solutions, respectively.

(1 ng to 10 μg/ml) consistently generated contraction in the mesenteric artery in immature and adult rabbits and these actions were not affected by pretreatment with tetrodotoxin (0.1 μg/ml) or with phentolamine (1 μg/ml).

The effects of two different concentrations of ACh (10 ng and 10 μg/ml) on the contractile response of the mesenteric arteries excised from immature and adult rabbits were observed in Ca-free EGTA (1 mM) solution. Each concentration of ACh induced contraction in Krebs solution. When the tissues were bathed in Ca-free (EGTA 1 mM) solution, 10 ng/ml ACh could not evoke contraction. However, the high concentration of ACh (10 μg/ml) evoked contraction, although the amplitude was reduced compared to that in Krebs solution. This suggests that ACh may release stored calcium and produced the contraction without influx of Ca^{2+}.

When the effects of ACh on K-induced contraction of mesenteric artery were observed, partial relaxation of K-induced contraction occurred as has been reported in the other vascular systems by Toda (1974) and Vanhoutte (1976). However, ACh showed different actions on K-induced contraction depending on the age of the rabbits.

To investigate these differences, solutions with excess K^+ up to 71.0 mM were prepared as described previously, by replacing potassium with sodium. Control experiments using solutions where Na was replaced by sucrose, had shown that the equivalent reduction of Na^+ to 72.3 mM did not produce a contraction of the mesenteric artery.

Figure 7 shows the effects of ACh on the contractile response of muscle from a 3 months old rabbit. A low concentration of ACh (10 ng/ml) produced a minute change in contractile response. Increased concentrations of ACh increased the tension development (Figure 7a). However, when muscles were contracted by 27.3 mM $[K]_o$ solution, ACh (10 ng/ml) induced partial relaxation, increase in ACh concentration up to 0.1 μg/ml increased the relaxation. Further increase in ACh concentration (1 μg/ml) resulted in the appearance of initial relaxation which was followed by contraction. When contraction was induced by 48.7 mM potassium, relaxation was produced by all concentrations of ACh (10 ng to 1 μg/ml). However,

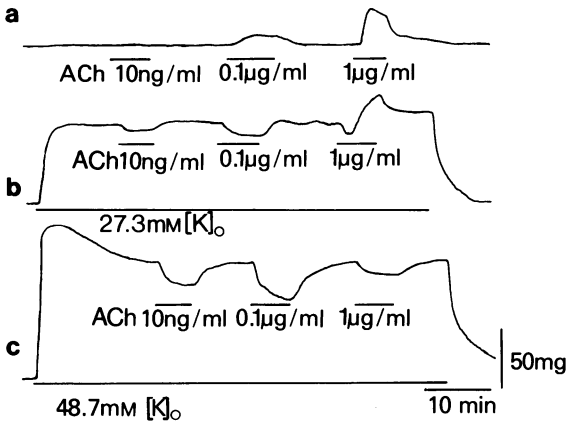

Figure 7 Effects of acetylcholine (ACh) on K-induced contraction of superior mesenteric artery of 3 months old rabbit. Three different concentrations of ACh (10 ng, 0.1 μg, 1 μg/ml) were applied at the bar under each record. (a) ACh-induced contraction in Krebs solution. ACh concentrations exceeding 0.1 μg/ml induced contraction. (b) Contraction induced by 27.3 mM $[K]_o$ was partially relaxed by 10 ng and 0.1 μg/ml ACh. Treatment with 1 μg/ml ACh induced initial relaxation followed by augmentation of contraction. (c) Contraction induced by 48.7 mM $[K]_o$ was suppressed partially by ACh in concentrations between 10 ng and 1 μg/ml. Amplitude of relaxation was marked when 0.1 μg/ml ACh was applied.

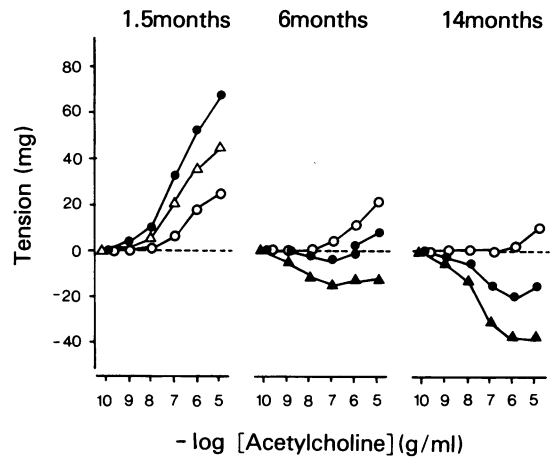

Figure 8 Rabbit mesenteric artery: dose-response relationships of tension development induced by acetylcholine (ACh) in Krebs and in high $[K]_o$ solution at different ages. (○) Krebs solution; (●) 23.8 mM $[K]_o$; (▲) 48.7 mM $[K]_o$; (△) 71.0 mM $[K]_o$. The resting tension of these tissues in Krebs solution was kept at 10 to 20 mg. In 1.5 months rabbit (40–50 days after birth), ACh-induced contractions were augmented by the increase in $[K]_o$. Artery from rabbit aged 6 months showed biphasic response to 1 μg/ml ACh in 23.8 mM $[K]_o$. The maximum relaxation and maximum contraction were plotted on the same scale. At 14 months old, mesenteric artery consistently showed partial relaxation by ACh during K-induced contraction. Dotted lines show the tension levels before application of ACh. This means that the zero tension levels are varied by applied $[K]_o$.

the maximum relaxation was observed on treatment with 0.1 μg/ml ACh.

Figure 8 shows typical examples of ACh-induced contraction in rabbits of different ages. The tissue of 1.5 months old rabbit showed a greater sensitivity to ACh (10 ng/ml), for the production of contraction, than that of adult rabbit (14 months old). The threshold concentration to generate contraction in Krebs solution increased from 10 ng/ml (1.5 months old) to 1 μg/ml (14 months old), and the amplitude of contraction produced by any given concentration of ACh decreased in proportion to the age of the rabbits. In the immature rabbit (1.5 months old) the contractions induced by all concentrations of ACh were greater in the presence of high $[K]_o$ solution (23.8 mM to 71.0 mM) than in normal Krebs solution. On the other hand, in the adult rabbit (14 months old), ACh caused partial relaxation of the contraction produced by excess $[K]_o$ solution (23.8 mM and 48.7 mM). In the immature rabbit, the potentiation of the contractions induced by acetylcholine was greatest in 23.8 mM $[K]_o$ and less in 71.0 mM $[K]_o$, while the relaxant effects of ACh on K-induced contraction in the adult rabbit were proportionally increased with increasing $[K]_o$. In the 6 months old rabbit, 1 μg/ml ACh induced a biphasic response as has been observed in 3 months old rabbit, i.e., initial relaxation was followed by contraction as shown in Figure 7b.

The effects of phentolamine and atropine on the relaxation produced by ACh (100 ng/ml) during K-induced contraction in the adult rabbit (15 months old) were observed; phentolamine (0.1 μg/ml) was without effect but the relaxant action of ACh was suppressed by pretreatment with atropine (0.1 μg/ml).

Figure 9 shows the effects of ACh on noradrenaline-induced contraction in immature (3 months old) and in adult (12 months old) rabbits. As observed in Figure 3, noradrenaline generated contraction with or without depolarization of the membrane i.e., in both immature and adult rabbits, the membrane was depolarized by 10 μg/ml but not by 10 ng/ml. A low concentration of ACh (10 ng/ml) suppressed the contraction induced by either low (10 ng/ml) or high (1 μg/ml) concentrations of noradrenaline in immature and adult rabbits. However, a high concentration of ACh (1 μg/ml) enhanced the noradrenaline-induced

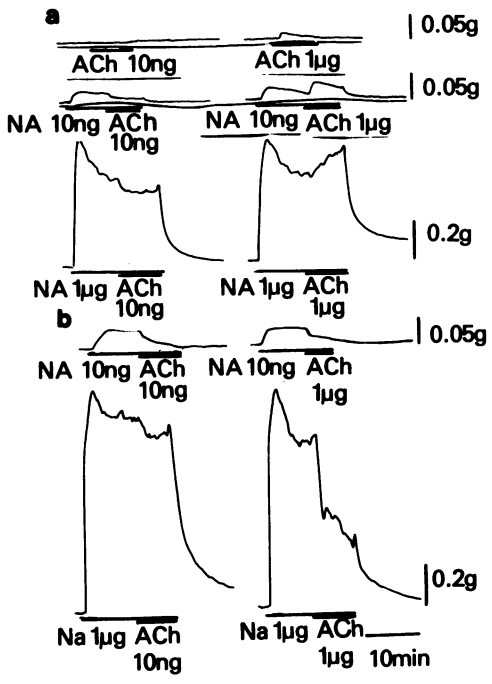

Figure 9 Effects of acetylcholine (ACh) on noradrenaline-induced contraction of rabbit superior mesenteric artery. In Krebs solution, 1 μg/ml ACh did but 10 ng/ml ACh did not evoke the contraction in both young and adult rabbits. (a) In young rabbit (3 months old) contractions induced by 10 ng/ml and 1 μg/ml noradrenaline (NA) were suppressed by simultaneous application of 10 ng/ml ACh, but they were augmented by 1 μg/ml ACh. (b) Contractions induced by noradrenaline were consistently suppressed by 10 ng/ml or 1 μg/ml ACh in adult rabbit (12 months old).

contraction in the immature rabbit but suppressed it in the adult rabbit.

Discussion

Most of the venous and some of the small arterial muscles generate spontaneous electrical activity (slow waves or action potentials, Funaki, 1961; Cuthbert & Sutter, 1964; Nakajima & Horn, 1967), whereas large arteries such as aorta, pulmonary artery, carotid artery, and some of the small arteries are electrically quiescent (Su *et al.*, 1964; Somlyo & Somlyo, 1968a; Holman, 1969; Speden, 1970; Casteels *et al.*, 1977).

The electrical activity of the mesenteric artery differs from one species to another. For example, the mesenteric artery of rat (Speden, 1969; Chernukh & Timkina, 1976) and guinea-pig (Speden, 1964) generate spontaneous electrical discharges. Spikes are also evoked by nerve stimulation (Steedman, 1966). The first branch of the posterior mesenteric artery of the rabbit shows neither spontaneous nor evoked discharges (Speden, 1967). The main tract of the superior mesenteric artery of rabbit used in this experiment also failed to show any spontaneous activity of the membrane.

In the mesenteric artery of rabbit, contraction was developed in 15 mM $[K]_o$, a concentration about three times higher than that in the normal solution, and the membrane was depolarized by about 10 mV. The threshold membrane potential for tension development in the mesenteric artery was higher than in other quiescent tissues such as dog tracheal muscle (4.5 mV; Suzuki, Morita & Kuriyama, 1976) and rabbit pulmonary artery (5 mV; Kitamura, Kuriyama & Suzuki, 1976; Casteels *et al.*, 1977).

Maintenance of resting membrane potential in the mesenteric artery is partly due to an active Na-pump, since treatment with ouabain or K-free solution caused depolarization of the membrane by about 10 mV. The amplitude of the depolarization induced by suppression of Na-K ATPase by ouabain was nearly the same as that obtained in other visceral smooth muscles (Casteels & Kuriyama, 1966; Casteels, 1969; Kuriyama, Ohshima & Sakamoto, 1971; Casteels, Droogmans & Hendrickx, 1971; Hendrickx & Casteels, 1974).

The mesenteric artery of rabbit was contracted by noradrenaline at concentrations of more than 10 ng/ml. Depolarization of the membrane was, on the other hand, observed at concentrations of more than 100 ng/ml, i.e., noradrenaline showed two actions on smooth muscles of the mesenteric artery as described on the pulmonary artery by Su *et al.* (1964), Somlyo & Somlyo (1968b), Kitamura *et al.* (1976) and Casteels *et al.* (1977). Low concentrations of noradrenaline induced contraction without depolarizing the membrane, while high concentrations induced contraction with depolarization of the membrane. The former was referred to as pharmacochemical coupling (Somlyo & Somlyo, 1968b) or non-electrogenic response (Casteels *et al.*, 1977), and the latter as electrochemical coupling (Somlyo & Somlyo, 1968b) or electrogenic response (Casteels *et al.*, 1977).

The hyperpolarizing action of ACh on the rabbit mesenteric artery differed from its actions observed in other vascular systems, because the arterial blood vessels such as a sheep carotid (Keatinge, 1966) and rabbit pulmonary artery (Su & Bevan, 1965) are depolarized by treatment with ACh. Despite the hyperpolarizing action of ACh, the muscle contracted, although the amplitude of contraction was very small compared to noradrenaline- or K-induced contraction. Treatment with tetrodotoxin or phentolamine had no effect, but atropine prevented the ACh-

induced contraction. In Ca-free solution, contraction induced by low concentrations of ACh disappeared, while higher concentrations still evoked contraction. These observations suggest that ACh has dual actions on smooth muscle of rabbit mesenteric artery; one is to hyperpolarize the membrane by stimulation of muscarinic receptors, and the other is to release intracellularly sequestered Ca directly by stimulation of muscarinic receptors, since atropine suppressed both ACh-induced hyperpolarization and the mechanical response.

Vanhoutte (1976) described two kinds of muscarinic receptors, inhibitory and excitatory, on the sympathetic nerve endings in the blood vessel wall. The former had a lower threshold to ACh than the latter. Low concentrations of ACh stimulated inhibitory muscarinic receptors, which caused reduction in transmitter release from the sympathetic nerve endings. High concentrations of ACh stimulated excitatory muscarinic receptors, which induced an increment in transmitter (noradrenaline) release, thus causing contraction of the muscle. However, in the rabbit mesenteric artery, ACh-induced contraction was not suppressed by pretreatment with phentolamine but was suppressed by atropine. The relaxation of high-K-induced contraction by ACh in the adult rabbit and enhancement of K-induced contraction by ACh in the immature rabbit is not to be explained by suppression of transmitter release from the nerve terminal, because treatment with phentolamine neither suppressed K-induced contraction nor suppressed the ACh-induced relaxation. In high-K solution, ACh did not hyperpolarize the membrane (mainly due to reduction in E_k), yet the K-induced contraction was relaxed. This means that relaxation induced by ACh was not only due to hyperpolarization of the membrane but increased K-permeability might also contribute to reduce the intracellular free Ca ion concentration. However, the underlying mechanism is still open for further investigation.

The hyperpolarizing action of ACh on the mesenteric artery was also dependent on the age of the rabbit. Hyperpolarization induced by low concentrations of ACh was large in amplitude in the immature rabbit compared with that in the adult rabbit, and increase in ACh concentration induced larger contractions in the immature than in the adult rabbit. Furthermore immature rabbits showed greater sensitivity to ACh in producing the contraction than the adult ones. In the immature rabbits, a high concentration of ACh induced a large initial hyperpolarization which declined to almost the same level as the resting membrane potential. This phenomenon may be partly due to desensitization of the membrane but may also be due to suppression of K-permeability by increased mobilization of Ca^{2+} from the membrane. It is probable that quantitative differences in muscarinic receptors to ACh at the surface membrane and the muscarinic excitatory receptors for Ca-release from the storage sites in smooth muscle cells of the mesenteric artery may develop between the immature and adult stage in rabbits. Presumably numbers of muscarinic receptors at the membrane and at Ca-storage sites are gradually reduced with age.

It is possible to conclude that ACh has dual actions on rabbit mesenteric artery: an inhibitory action of the membrane by increasing membrane permeability to K ion, which is mediated by stimulation of muscarinic receptors, and an excitatory action on Ca-releasing mechanism which generates contraction, also mediated by activation of muscarinic receptors. The latter action seems to be less dependent on the changes in membrane potential.

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