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THE ROLE OF HYPERPOLARIZATION IN THE RELAXATION OF SMOOTH MUSCLE OF MONKEY CORONARY ARTERY

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SUMMARY

1. In monkey coronary arteries, outer and inner muscle had a similar resting potential (−39·5 and −40·0 mV). Both showed strong outward-going rectification, with no regenerative depolarization, on injection of depolarizing current. The depolarization spread electrotonically in all directions, particularly around the vessel wall.

2. Hyperpolarization up to −45 mV by injection of constant current caused relaxation. Depolarization caused contraction.

3. Pulses of field stimulation caused a brief depolarization which was reduced by tetrodotoxin or by stripping of the adventitia. They also caused a prolonged hyperpolarization which was not prevented by either, but was prevented by rubbing of the endothelium.

4. The hyperpolarization in response to field stimulation therefore appears to result from electrically stimulated release of a substance from endothelial cells.

5. Relaxation accompanied this hyperpolarization. It was twice the size of the relaxation produced by a similar hyperpolarization due to constant injection.

6. Isoprenaline also produced hyperpolarization, and relaxation five times that seen with a similar hyperpolarization induced by direct current.

7. Hyperpolarization appears to be an important, but not the only, mediator of relaxation induced in this artery both by endothelial cells and by β-adrenergic stimulation.

INTRODUCTION

In spontaneously active smooth muscle, relaxation is usually accompanied by hyperpolarization and a reduction in the frequency, or cessation, of action potentials. Hyperpolarization may also be important in the relaxation of some quiescent smooth muscles, since amyl nitrite and inorganic sodium nitrite hyperpolarized and relaxed sheep carotid arteries which had been depolarized and contracted by noradrenaline (Keatinge, 1966), though relaxation could also be obtained without electrical changes in the artery after depolarization by potassium-rich solution. Therefore there was a non-electrical component in the drug-induced relaxation. In the smooth muscle of carotid artery, hyperpolarization evoked by current application failed to induce

muscle relaxation (Mekata, 1984). Recently the endothelial cells of blood vessels have been shown to mediate some types of relaxation (Furchgott & Zawadzki, 1980; DeMey & Vanhoutte, 1982; Cocks & Angus, 1983). In several vascular tissues, there is evidence that the vasodilators, acetylcholine, substance P and ATP, and even the powerful vasoconstrictors, noradrenaline and serotonin, appear to release a vasodilator substance from endothelial cells which in turn relaxes the underlying smooth muscle. Such evidence was usually obtained from the measurement of the mechanical responses of the arteries. The present study describes evidence that field stimulation, by brief pulses, produced muscle relaxation and hyperpolarization of coronary arteries of monkeys, as well as brief depolarizing excitatory junction potentials (e.j.p.s). The ability of hyperpolarization to relax this smooth muscle and the role of nerves and endothelial cells in its response to field stimulation, were investigated.

METHODS

Thirty-eight adult monkeys of either sex, 6–12 years old weighing 4–14 kg were used. Muscle strips were removed from circumflex coronary artery, about 3 mm in outside diameter, after exsanguination from the carotid artery. Strips, 10–15 mm in length and about 2 mm in width, were spirally cut from the artery and mounted in the organ bath used in previous experiments (Mekata, 1984). The end of the preparation in the recording bath was attached by a fine silk thread to a strain gauge and the other end was fixed in the stimulating bath. Membrane potential of the smooth muscle cells was intracellularly recorded by inserting micro-electrodes into the tissues, from the adventitial side in most experiments and from the intimal surface in other experiments. All recordings by micro-electrodes were made from the outer muscle, except when otherwise stated. In experiments studying the relationship between tension and membrane potential, or comparing isoprenaline-induced relaxation with prolonged direct current-induced relaxation, all the tissue in the recording chamber, except within 0·8 mm of the stimulating partition, was crushed and the stimulating compartment was irrigated with solution two times normal tonicity by injecting hypertonic Krebs solution containing 300 mM-sucrose at rates of 4 ml/min in addition to normal perfusion at a rate of 4 ml/min. This was done to permit fairly uniform polarization of the membrane by prolonged direct current, and to prevent interference by tension changes generated in other parts of the artery by the direct current or isoprenaline. Taking 1·0 mm as the space constant (see Results) the decay of electrotonic potential at the edge of the contracting area will only be to 0·82 of the value at the partition. For experiments comparing relaxation evoked by brief pulses with prolonged direct inward current-induced relaxation, intact preparations, without the crushing procedure, were used, although the stimulating compartment was still irrigated with the hypertonic solution, since the hyperpolarization evoked by brief pulses was extremely reduced by the crushing procedure. When the effect of brief pulses only was investigated, normal Krebs solution alone was perfused through the organ bath. Irrigation of the solutions through the organ bath and the arrangement for stimulating and recording of mechanical and electrical responses were the same as those described by Mekata (1984).

The endothelium was removed from some muscle strips by gentle and repeated rubbing of the intimal surface of the spiral strips with the side of a pair of forceps. This procedure removed entirely the endothelial cells as observed from silver-stained whole-mount sections by light microscopy.

Standard Krebs solution of the following composition was used (mM): Na^+, 137·4; K^+, 5·9; Mg^{2+}, 1·2; Ca^{2+}, 2·5; Cl^-, 134·0; HCO_3^-, 15·5; $H_2PO_4^-$, 1·2; glucose, 11·5.

RESULTS

Passive electrical properties of the outer and inner muscles

When micro-electrodes were inserted from the endothelial or adventitial side, a quiescent membrane potential was recorded from both outer and inner smooth muscle cells. Mean values of membrane potential were $-40{\cdot}0 \pm 5{\cdot}2$ mV ($n = 265$) (mean,

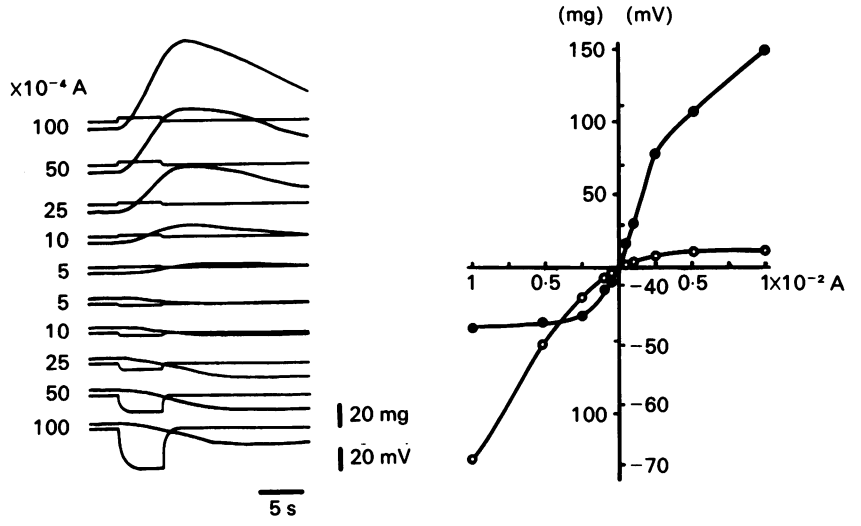

Fig. 1. Electrical and mechanical responses of a preparation with low membrane potential (−37 mV) to prolonged direct currents (5 s pulse duration). Left: depolarization (top) or hyperpolarization (bottom) of the membrane and contraction (top) or relaxation (bottom) produced by outward or inward current pulses respectively in normal Krebs solution. Figures on the left-hand side of the traces indicate applied current in amperes. The fraction entering the cells is uncertain. Right: steady-state current–voltage (○) and current–tension (●) relationships obtained from the records. Upward: depolarization and contraction. Downward: hyperpolarization and relaxation; abscissa. Right: depolarizing current, left: hyperpolarizing current.

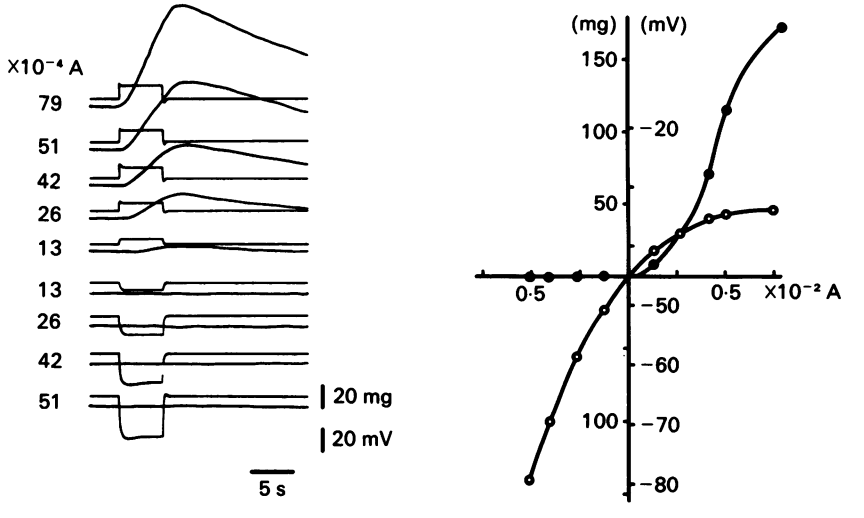

Fig. 2. Electrical and mechanical responses of a preparation with a higher membrane potential (−45 mV) to prolonged direct current. Explanation of the Figure is the same as for Fig. 1. Note that inward current does not evoke muscle relaxation, while it is produced in the case of Fig. 1.

standard deviation, number of penetrations) for outer muscle and $-39{\cdot}5 \pm 5{\cdot}1$ mV ($n = 153$) for inner muscle. There was no marked difference in voltage–current relations between the outer and inner muscles. The membrane resistance increased progressively when weak hyperpolarizing currents were applied. When the membrane was hyperpolarized beyond -50 to -55 mV, the membrane resistance became fairly constant. In the depolarizing direction, the resistance was greatly decreased with increase in intensity of outward current (Figs. 1 and 2). Strong depolarizing currents

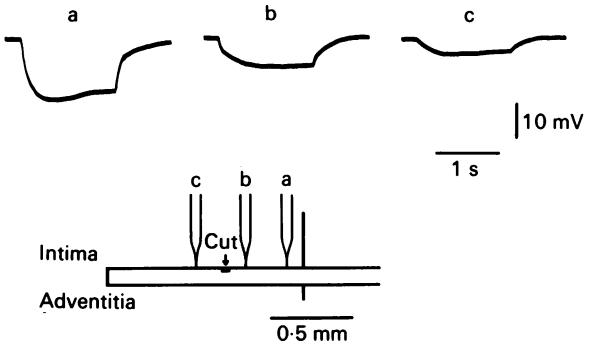

Fig. 3. Measurement of electrotonic spread of current between outer and inner muscle. The traces show that electrotonic potentials measured in inner muscle (a, b and c in upper traces), evoked by a stimulating electrode, decay exponentially with the distance along the artery (a, b and c in lower illustration), even though inner muscle was cut between points b and c.

failed to evoke an action potential from the outer or inner muscle, and no anodal break excitation was recorded in these muscles. In order to assess electrical transmission between smooth muscle cells in the direction (longitudinal direction) of the long axis of the cells and in the direction (transverse direction) transverse to the long axis, the decay of electrotonic potentials in each direction was measured. Space constants were calculated (see Mekata, 1974) from current–voltage (I–V) curves obtained at different distances from the partition; the gradient of the I–V curve at zero current was measured for each recording point, and the distance at which this gradient decayed to 1/e of its value is taken as the space constant. The space constants in the longitudinal direction (around the vessel wall) were 1·02 mm (S.D., $\pm 0{\cdot}35$ mm, $n = 8$) for the inner muscle and 0·99 mm (S.D., $\pm 0{\cdot}48$ mm, $n = 7$) for the outer muscle. The space constant in the transverse direction was measured by using long strips cut in the direction of the long axis of the vessel. The measurement was made only in outer muscles and the value was 0·44 mm (S.D., $\pm 0{\cdot}06$, $n = 3$). In order to see whether electrotonic current can flow from outer to inner muscle cells, a preparation was made as shown in Fig. 3, in which a thin muscle layer on the intimal surface was transversely cut to a depth of approximately 20 μm, at a distance of about 0·5 mm from the stimulating partition. Before and after the cut was made, electrotonic potentials were recorded intracellularly from intimal surfaces at distances between 0·2 and 1·0 mm from the stimulating partition. Amplitude recorded at the same distance showed a similar value before and after the cut and exponential spatial decay of electrotonic

potential was observed. These results suggest that electrical connexions are present between smooth muscle cells in the directions along, around and through the vessel wall.

Tension change evoked by direct current

Preparations with small or large membrane potentials showed different mechanical responses to outward or inward direct current.

In preparations with membrane potential more positive than −40 mV, where the *I–V* curve has largely flattened out, contraction was initiated by even a very small intensity of outward current (Fig. 1). The amplitude of tension development

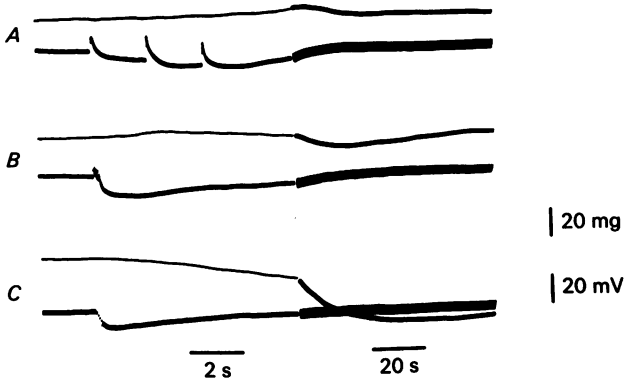

Fig. 4. Three typical patterns of electrical (lower traces) and mechanical (upper traces) responses recorded from outer muscle of the different coronary arteries during repetitive brief pulse stimulation (0·1 ms pulse duration and 10 V); (*A*) 0·5 Hz and 3 pulses (*B* and *C*) 20 Hz and 5 pulses.

increased with increase in outward current intensity. Inward current caused muscle relaxation, which increased with increase in current intensity to a maximum value when the membrane potential was hyperpolarized to approximately −45 to −50 mV. Increasing the duration of outward or inward current pulses increased contraction or relaxation respectively. In most preparations, the contraction was initiated with a short time lag (0·3–1 s) or even by a very short pulse (30 ms minimum). Initiation of relaxation needed current duration as long as 2 s or longer with minimum current.

In preparations with membrane potential more negative than −45 mV, where the *I–V* curve was linear or had just started to flatten out, outward currents initiated a contraction only when the membrane was depolarized beyond about −40 mV (Fig. 2). Inward current caused little or no relaxation in these preparations, even with hyperpolarization to −80 mV.

Effect of brief field stimulation

Field stimulation with either a large single pulse or repetitive pulses (0·1–0·2 ms duration, 30–100 V) caused either slow hyperpolarization with a time-to-peak of some 1–2 s (Fig. 4*C*) or fast depolarization with a time to peak of some 20–50 ms or both (Fig. 4*A*, *B*). Repetitive stimulation at frequencies over 0·1 Hz could produce facilitation of both depolarizing and hyperpolarizing responses. The amplitudes of

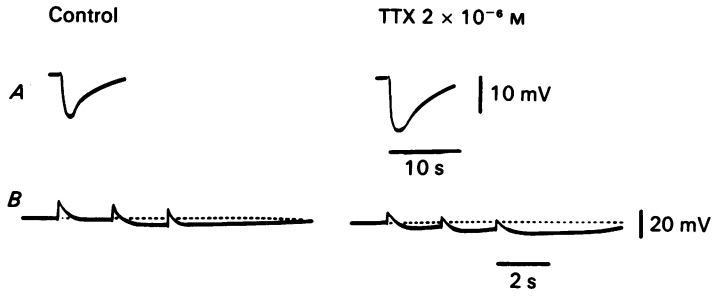

Fig. 5. Effect of tetrodotoxin (2×10^{-6} M) on the e.j.p.s and the hyperpolarizing response evoked by field stimulation (0·1 ms pulse duration and 50 V; *A*, 20 Hz and 5 pulses; *B*, 0·5 Hz and 3 pulses). These records were made from the outer muscle of two different preparations of the coronary arteries generating a hyperpolarizing response (*A*) and depolarizing response (*B*).

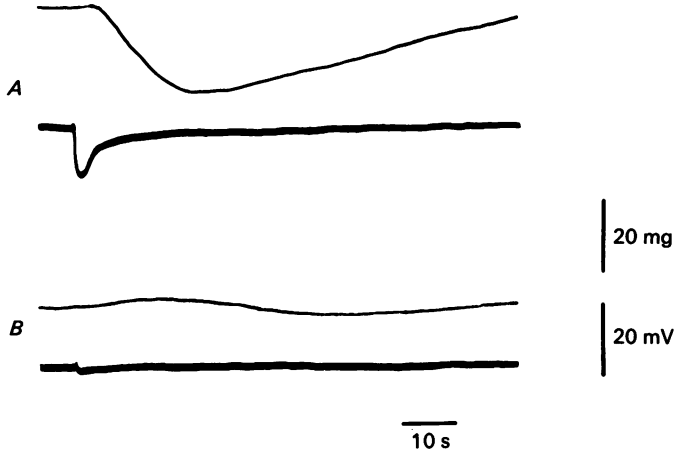

Fig. 6. Effects of damage of the internal layer on hyperpolarizing responses (lower traces), recorded from the outer muscle, and relaxation (upper traces) evoked by field stimulation (0·1 ms pulse duration, 50 V, 20 Hz and 5 pulses). *A*, the control response; *B*, the responses after damage of internal surface. *A* and *B* were recorded from the same preparation of the coronary artery.

both depolarizing and hyperpolarizing responses were graded with the number and frequency of stimulating pulses. Their maximum values were 10 mV for the former and 15 mV for the latter. Preparations with smaller membrane potentials generated generally larger amplitudes of hyperpolarizing response. In preparations with membrane potential more negative than -50 mV ($n = 8$), repetitive brief pulses (0·2 ms duration, 20 pulses, 20 Hz and 100 V) caused little or no hyperpolarizing responses. No action potentials following e.j.p.s, like those which have been observed in many visceral and vascular smooth muscles, were ever recorded.

Tetrodotoxin (TTX) (2×10^{-6} M) suppressed the depolarizing response to some extent ($n = 10$), but potentiated the hyperpolarizing response ($n = 9$) (Fig. 5). The effect of field stimulation was examined in arteries stored in cold Krebs solution (at

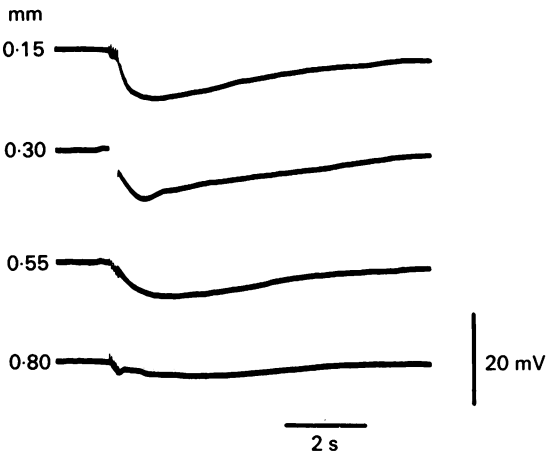

Fig. 7. Spatial decay of the amplitude of the hyperpolarization evoked by brief pulse stimulation (0·1 ms pulse duration, 50 V, 5 pulses and 20 Hz). Figures on the left-hand side of the traces show distances between the recording and stimulating electrodes.

0 °C) for 48 h, which depresses the function of adrenergic nerves (Bevan, Bevan & Duckles, 1980). In such arteries strong, repetitive, brief pulses (maximum, 30 pulses, 20 Hz, 0·2 ms duration and 100 V) failed to initiate e.j.p.s, but in ten of fifteen such preparations large hyperpolarizing responses were observed.

Damage of the inner surface of the vessel wall by forceps reduced the amplitude of the hyperpolarizing response ($n = 10$) (Fig. 6), while the depolarizing response and passive electrical properties were normally maintained ($n = 6$) and isoprenaline (10^{-5} M) caused a similar amplitude of relaxation to that seen in intact muscle ($n = 5$). Stripping of connective tissue from the outer surface of the vessel wall, had no effect on the hyperpolarizing response (119–80 % of control in the amplitude, mean; 99 %, $n = 5$) but suppressed depolarizing response (90–23 % of control in the amplitude, mean; 40 %, $n = 5$). These results suggest that the depolarizing response is an e.j.p. evoked by transmitter released from nerve terminals, and the hyperpolarizing response may be initiated by some chemical substances from other cells.

Spatial decay of the hyperpolarizing response was recorded at four different distances from the stimulating partition ($n = 3$). The amplitude decreased exponentially with the distance from the stimulating partition except for near the partition, and the value of the space constant (1·20 mm $\pm$ 0·21 mm, $n = 3$) was similar to that calculated from the spatial decay of electrotonic potential evoked by rectangular current pulses (Fig. 7). It suggests that only cells at or near the stimulating partition were stimulated by the hyperpolarizing transmitter. Bülbring & Tomita (1967) reported that the spatial decay of e.j.p. in guinea-pig taenia coli is much smaller than that of passive electrotonic potential. Thus, the present results support the idea that the hyperpolarizing response seen in monkey coronary artery is caused by some substance less diffusible than transmitters released by nerves.

Field stimulation with brief pulses (0·1–0·2 ms pulse duration, 20 Hz and 30–100 V) caused three different patterns of tension changes, i.e. (1) contraction (Fig. 4*A*), (2) early contraction and later relaxation (Fig. 4*B*), (3) relaxation (Fig. 4*C*). In

preparations showing obvious e.j.p.s. ($n = 18$) type (1) was seen frequently ($n = 11$), type (2) less often, and type (3) was not observed. In preparations in which field stimulation evoked hyperpolarizing responses only, type (1), (2) and (3) responses occurred in approximately equal numbers. TTX (2×10^{-6} M) caused a decrease in the component of contraction and an increase in the component of relaxation in such responses. In arteries showing only a hyperpolarizing response, damage to the inner surface of the artery induced decrease or disappearance of both the hyperpolarization and relaxation (Fig. 6) ($n = 7$). To determine whether the hyperpolarization evoked by field stimulation was sufficient to account fully for the relaxation, the amplitude of the brief pulse-induced relaxation was compared with that of the relaxation produced by prolonged direct current. Direct inward current which evoked similar amplitude and duration to the pulse-induced hyperpolarization was applied to preparations of type (3). The amplitude of this relaxation was between 20 and 80% (mean, 46%, $n = 5$) of the size of the pulse-induced relaxation. These results suggest that the pulse-induced relaxation contained both an electrical and non-electrical component. Similar studies were made on relaxations evoked by isoprenaline. Isoprenaline (10^{-7} and 10^{-5} M) caused large muscle relaxations in all preparations used, and hyperpolarization in some preparations (0 to -12 mV, mean, 6 mV, $n = 8$ for 10^{-7} M and 0 to -15 mV, mean, 8 mV, $n = 10$ for 10^{-5} M). The intensity of direct current (30 s) needed to produce the same amplitude of hyperpolarization that was produced by 20 s application of isoprenaline was determined. These durations of stimulation produced comparable durations of electrical response, but the relaxation produced by direct current was 0–80% (mean 22%, $n = 10$) of the isoprenaline-induced relaxation, and was less prolonged.

DISCUSSION

One of the interesting findings of the present studies was that hyperpolarization of the membrane by application of prolonged inward current caused relaxation of resting monkey coronary artery in normal physiological solution. This has not been observed in any other electrically quiescent smooth muscle. Quiescent smooth muscle of blood vessels usually shows strong outward going rectification, which is an important factor in preventing action potentials (Mekata, 1971), and rectification appears to be related to tension. In the rabbit aorta for example (Mekata, 1981), the membrane potential at which the extreme decrease in membrane resistance started (about -40 mV) coincided with the level at which contraction was initiated. No change in tension was produced by changes in potential more negative than this threshold. In the monkey coronary artery, in the present studies, there was no effect of membrane potential on tension at membrane potentials more negative than -45 mV but many preparations had a membrane potential of about -40 mV, and these could contract or relax with either prolonged outward or inward currents respectively. Rabbit aorta (resting potential, -55 mV (Mekata, 1974)) and coronary arteries of animals other than monkey (resting potential, -46 to -52 mV (Kitamura & Kuriyama, 1979; Ito, Kitamura & Kuriyama, 1979, 1980)) as well as some monkey coronary arteries with large membrane potentials in the present experiments, have resting potentials more negative than the threshold potential for contraction.

Although hyperpolarization was an important factor in relaxations of the monkey coronary artery, isoprenaline- or brief pulse-induced relaxations clearly also depended on non-electrical means to a considerable degree, since the hyperpolarization was not large enough to explain the relaxation. The evidence of the present study that smooth muscle cells of the monkey coronary artery were connected by a low resistance pathway in three dimensions contrasts with rabbit carotid artery (Mekata, 1984) in which current cannot spread from the outer to the inner muscle layer.

Another interesting finding of the present study is that hyperpolarizing, as well as depolarizing, e.j.p.s could be produced by brief pulse stimulations. Depolarizing e.j.p.s, and sometimes slower depolarization, have been recorded after field stimulation in rat tail (Cheung, 1982), rabbit carotid (F. Mekata, unpublished observation) and rabbit saphenous (Holman & Surprenant, 1979) arteries, among others. Slow hyperpolarization of the kind recorded in monkey coronary arteries has not apparently been reported in other arteries. The chemical substance which may have mediated this response in monkey coronary arteries was not investigated, but it appears to have been released by extraneuronal cells and probably by endothelial cells.

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