Mechanical and Electrical Properties of Smooth Muscle Cells and Their Regulations by Endothelium-Derived Factors in the Guinea Pig Coronary Artery

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Abstract—Effects of high K+, acetylcholine (ACh), 9,11-epithio-11,12-methanotrans thromboxane A2 (STA2), thrombin and endothelin were investigated on smooth muscles of the guinea pig coronary artery in intact and endothelium-denuded tissues. In intact tissues, ACh transiently inhibited but ATP produced maintained inhibition of the STA2-induced contraction. However, in the endothelium-denuded tissue, ACh produced contraction and ATP inhibited the STA2-induced contraction. In intact tissues, thrombin produced dual actions on the STA2-induced contraction with an initial relaxation followed by contraction. In endothelium-denuded tissues, thrombin enlarged the STA2-induced contraction without transient relaxation. In intact tissues prepared from both proximal and distal regions, endothelin showed the same dual action as observed with thrombin, whereas higher concentrations of endothelin showed only contraction. In endothelium-denuded tissues, endothelin consistently produced contraction. In intact tissues prepared from proximal and distal regions, ACh produced a biphasic response (initial hyperpolarization and subsequently generated depolarization). The amplitudes of both potential changes occurred in a membrane potential-dependent manner. In endothelium-denuded tissues, ACh depolarized the membrane in both tissues. In intact and endothelium-denuded tissues, ATP hyperpolarized the membrane in inverse proportion to the membrane potential level, whereas thrombin and endothelin consistently depolarized the membrane. The results indicate that ACh acts on endothelial and smooth muscle cells, and the former releases both EDRF and EDHF. ATP only acts on smooth muscle cells and hyperpolarizes the membrane. STA2, thrombin and endothelin act on both endothelial and smooth muscle cells. STA2 and endothelin may release EDRF but not EDHF, and thrombin may release EDRF and endothelin.

Endothelium-derived factors, such as endothelium-derived relaxing factor (EDRF; 1), endothelium-derived hyperpolarizing factor (EDHF; 2, 3) and endothelin (4), regulate muscle tone of vascular beds. EDRF and EDHF relax pre-contracted vascular tissues with (EDHF) or without (EDRF) hyperpolarization of the membrane (5, 6). Endothelin contracts vascular tissues (4) and also releases EDRF which produces relaxation of vascular beds. This relaxation occurs with much lower concentrations of endothelin than that necessary to produce the contraction (7).

Contributions of EDRF and EDHF released from endothelial cells differ according to the region of the vascular bed, e.g., in the rat main pulmonary artery, the contribution of EDHF on the ACh-induced relaxation is small in comparison to that of EDRF, as estimated from the actions of methylene blue on the relaxation and hyperpolarization of the membrane (2, 3). However, in the guinea pig basilar artery, the initial part of the ACh-induced relaxation is mainly due to release of EDHF and the sustained relaxation is due to release of EDRF, as estimated from the actions of hemoglobin on the relaxation and hyperpolarization of the membrane (8). In
vascular tissues, ACh produces either contraction (9–12) or relaxation (13–18). Kuriyama and Suzuki reported that ACh causes hyperpolarization of the membrane yet produces contraction of the rabbit mesenteric artery, and the degree of hyperpolarization and contraction are dependent on age (19). The response to purine compounds also differs with the region or the species. For example, ATP and ADP produce contraction of the dog and rat portal veins, although ATP, ADP and AMP relax the rabbit portal vein (20–22). Furthermore, ATP released from nerve terminals as a co-transmitter of noradrenaline (NAD) contributes to the generation of the excitatory junction potential (23, 24).

When ACh (25) or ATP (26) was applied to smooth muscle tissues of the guinea pig coronary artery, these agents caused hyperpolarization of smooth muscle membranes, and ACh produced contraction and ATP relaxation of the tissue. However, these experiments did not consider the role of endothelial cells on the regulation of the vascular tone.

The present experiments were intended to re-examine the effects of spasmogenic agents on electrical and mechanical responses of the guinea pig coronary artery, in the presence or absence of endothelial cells.

**Materials and Methods**

**Materials:** Guinea pigs of either sex, weighing 300–350 g, were stunned and bled. The heart with aorta attached was carefully removed into oxygenated Krebs solution. Since the responses of different regions of the coronary artery to stimulants differed (27), we used two regions of the anterior descending branch of the left coronary artery (proximal region: diameter about 200 µm and distal region: diameter is about 100 µm as shown in Fig. 1). Connective tissue was removed under a dissecting microscope. For the tension recording, a small piece of muscle was prepared by cutting the tissue in a circular direction (60–80 µm in diameter and 200–250 µm in length) from each region. To record the membrane potential, the tissue of both regions was cut in a longitudinal direction (2–2.5 mm in length). The endothelium-denuded tissue was prepared by rubbing endothelial cells off with a moistened cotton ball (1) or the inside of the vessel was rubbed with knives made from small pieces of razor blade (8, 27, 28).

**Tension recording:** Mechanical responses were measured by attaching a circular strip to a strain gauge (UL-2, Shinko Co., Tokyo). The tissue was superfused in a chamber with a capacity of 1.0 ml filled with Krebs solution. The superfusate was changed rapidly from one end, while the solution already present was simultaneously aspirated off with a water pump from the other end. The resting tension was adjusted to obtain the maximum contraction in 128 mM K+ and was not greater than 15 μN. All experiments were performed at 25 °C to prevent deterioration of the fine tissues over several hours.

**Membrane potential recording:** A glass capillary microelectrode filled with 3 M KCl (the resistance being 40–80 megohm) was inserted into smooth muscle cells from the adventitial side of the muscle strip mounted on a rubber plate. To measure the membrane potential, the warmed Krebs solution (30–31 °C) was superfused over the tissue at a flow rate of about 3 ml min⁻¹. The membrane potential measured at 25 °C was several mV lower than that measured at 31–35 °C. Therefore, to measure the membrane potential over several hours, 31 °C was used.
Solution: The ionic composition of the Krebs solution was as follows: 137.4 mM Na⁺, 5.9 mM K⁺, 1.2 mM Mg²⁺, 2.5 mM Ca²⁺, 15.5 mM HCO₃⁻, 1.2 mM H₂PO₄⁻, 134.0 mM Cl⁻ and 11.5 mM glucose. The solution was aerated with 97% O₂ and 3% CO₂, and the pH of the solution was 7.2–7.4.

Drugs: Drugs used were acetylcholine-CI (Daiichi Pharm. Co.), adenosine triphosphate (ATP, Sigma), thrombin (Sigma), 9,11-epithio-11,12-methano-thromboxane A₂ (STA₂, a derivative of thromboxane A₂; Ono Pharm. Co.), hemoglobin (Sigma) and endothelin (procine endothelin, kindly provided by Prof. T. Masaki, Tsukuba Univ.). Hemoglobin was used as oxyhemoglobin (8, 29).

Statistics: The values recorded are expressed as the mean±S.D., and the statistical significances were determined with Student's t-test. Probabilities of less than 5% (P<0.05) were considered significant.

Results

Regional differences in mechanical responses induced by various substances in intact and endothelium-denuded tissues: Figure 2 shows the effects of high K⁺ (128 mM), ACh (10 μM), ATP (10 μM), thrombin (1 U/ml) and STA₂ (10 nM) on mechanical responses in the intact and endothelium-denuded tissues recorded from distal (A) and proximal (B) regions of the left anterior de-
cesnding coronary artery. The above concentrations of stimulants were selected from previous experiments as adequate concentrations (the minimum concentration to produce the maximum amplitude of contraction) to investigate the drug action on these tissues (8, 28, 30). In intact tissues prepared from the distal region, high K+ produced larger contraction than other stimulants. ACh (10 μM) but not ATP (10 μM) produced a small contraction (Fig. 2Aa). After removal of endothelial cells, the phasic component of K+-induced contraction remained unchanged but the tonic component was markedly enhanced, whereas the ACh-, thrombin- and STA2-induced contractions were markedly enhanced. Especially in the case of ACh, the amplitude exceeded the K+-induced contraction (Fig. 2Ab). ATP, however, did not produce contraction after removal of endothelial cells (Fig. 2Ab).

In intact tissues prepared from the proximal region, the above spasmogenic substances produced contraction except for ATP. However, regional differences were observed on the actions of STA2 and ACh (Fig. 2Aa vs. 2Ba), i.e., in intact tissues of the distal region, STA2 produced the largest contraction but not in those of the proximal region. Following removal of endothelial cells, the response induced by ACh (10 μM) was enhanced but the amplitude was much smaller than that observed in the distal region. With application of thrombin (1 U/ml), a distinct difference

![Graph A: Distal region](image)

**Fig. 3.** Statistical observations made on the effects of ACh, ATP, thrombin and STA2 before and after removal of endothelial cells. A: Distal region. B: Proximal region. Open and shaded columns indicate before and after removal of endothelial cells, respectively. The amplitudes of contraction evoked by 128 mM K in intact and endothelium-denuded tissues in distal and proximal regions were normalized as a relative tension of 1.0 (n=4–5). Vertical bars: S.D. *: P<0.05, **: P<0.01.
was observed on mechanical responses in the presence or absence of endothelial cells in both regions. Relaxation of the tissue after removal of thrombin was rapid in the endothelial denuded tissue, but actions were long lasting in the intact tissue (Fig. 2Aa vs. 2Ab and Fig. 2Ba vs. 2Bb). ATP (10 nM) did not produce any mechanical response in the presence or absence of endothelial cells as observed in the distal region. Figure 3 summarizes the effects of K+ (128 mM), ACh (10 \mu M), ATP (10 \mu M), thrombin (1 U/ml) and STA2 (10 nM) on mechanical responses recorded from the distal (A) and proximal (B) regions. Since the amplitude of the phasic component of the K+-induced contraction recorded from the intact and endothelium-denuded tissues was almost the same, the phasic component of the K+-induced contraction was normalized as a relative tension of 1.0. After removal of endothelial cells, the responses induced by ACh, thrombin and STA2 are consistently enhanced. ATP (10 nM) was unable to produce a mechanical response in the intact or endothelium-denuded tissues.

Effects of ACh or ATP on the pre-contracted tissue by STA2: In intact tissues prepared from the distal region, ACh (10 \mu M) and ATP (10 \mu M) completely relaxed tissue contracted by STA2 (10 nM). After removal of endothelial cells, ACh produced a contraction, but ATP relaxed the contracted tissue (Fig. 4A a and b). Figure 4B shows the effects of various concentrations of ATP or ACh on mechanical responses in the intact and endothelium-denuded tissues excised from the distal region. The phasic components of the K+-induced contraction in the intact and endothelium-denuded tissues were normalized as 1.0, respectively. To produce complete relaxation of the 10 nM STA2-induced contracted tissues, 1 \mu M ACh or 10 \mu M ATP was required. However, in endothelium-denuded tissues, ATP in concentrations over 0.3 \mu M consistently inhibited the contraction evoked by STA2, but 10 \mu M ATP did not completely relax the tissue. On the other hand, ACh in concentrations over 3 nM produced contraction and generated maximum amplitude of contraction at 1 \mu M.

To investigate the action of ATP further, the effects of ATP were observed on the STA2-induced contraction in intact or endothelium-denuded tissues. In the presence of endothelial cells in the distal region as shown in Fig. 5A, STA2 (over 1 nM) produced contraction, in a concentration-dependent manner. ATP (1 \mu M) consistently inhibited the STA2-induced contraction. In endothelium-denuded tissues, STA2 produced contraction at concentrations over 30 pM. ATP (1 \mu M) consistently inhibited this contraction, but did not completely relax tissues contracted by STA2 at over 50 pM (Fig. 5A). The effects of ATP on the same amplitude of STA2-induced contraction in intact (3 nM) and endothelium-denuded (40 pM) tissues were compared (Fig. 5B). In the intact tissue, ATP (1 \mu M) did not completely inhibit the 3 nM STA2-induced contraction, but ATP completely relaxed the 40 pM STA2-induced contraction in the endothelium-denuded tissue (Fig. 5B). When the effects of ATP on the matched amplitude of contractions evoked by 1 nM STA2 in intact tissues and by 30 pM STA2 in endothelium-denuded tissues or by 3 nM STA2 in intact tissues and 40 pM STA2 in endothelium-denuded tissues were compared, ATP (1 \mu M) completely relaxed the tissues in the endothelium-denuded tissue but not completely in the intact tissue (Fig. 5C).

Effects of ACh or ATP on the STA2-induced contraction following application of hemoglobin: In intact tissues excised from the distal region (Fig. 6), ATP (10 \mu M) or ACh (10 \mu M) completely relaxed the pre-contracted tissue evoked by 10 nM STA2. When the tissue was pretreated with 10 \mu M hemoglobin for 10 min, the STA2-induced contraction was markedly enhanced. ACh (10 \mu M) transiently relaxed the tissue to the resting level, and then gradually contraction developed due to attenuation of the relaxation, whereas ATP (10 \mu M) completely relaxed the tissue.

Figure 7 shows the effects of 0.3 \mu M ACh and 1 U/ml thrombin on the endothelium-denuded tissue prepared from the distal region. When ACh was successively applied every 15 min, the ACh-induced contraction (0.3 \mu M for 6 min) had a constant amplitude (Fig. 7a). When thrombin (1 U/ml) was ap-
Fig. 4. Effects of ATP and ACh on the mechanical response induced by STA2 or high K⁺ in the presence or absence of endothelial cells.  

Aa: Effects of 10 μM ACh and 10 μM ATP on the contraction evoked by 10 nM STA2 (tissues were prepared from distal region of the coronary artery). As the control, the K⁺ induced contraction (128 mM) was recorded.  

Ab: Effects of high K⁺ and ACh on the mechanical response and the effects of ATP on the STA2-induced contraction in endothelium-denuded tissues. B: The concentration-response relationship for ACh and ATP observed in intact and endothelium-denuded tissues. The K⁺-induced contraction evoked by 128 mM K⁺ in the presence or absence of endothelial cells was normalized as a relative tension of 1.0. Filled circles: ACh after removal of endothelial cells; Filled triangles: effects of ATP on the STA2-induced contraction (10 nM) after removal of endothelial cells; Open circles and open triangles: ACh and ATP on the STA2-induced contraction (10 nM) in the intact tissue, respectively. Vertical bars: S.D., n=4.

Applied under the same conditions, the second and subsequent application did not produce contraction. Once thrombin had been applied, contraction could not be evoked again for over 3 hr (Fig. 7b). Furthermore, if ACh and thrombin were applied alternately, tachyphylaxis of mechanical responses occurred only thrombin (Fig. 7c). Much the same effects were observed on application of thrombin to the intact tissue.

In the proximal (Fig. 8) and distal regions, STA2 (10 nM) produced contraction in the presence of endothelial cells. When the tissue was rinsed with Krebs solution following application of STA2, the tissue produced a rebound mechanical response. A second application of STA2 (10 nM) relaxed the tissue but not completely (Fig. 8a and b). Again
when the tissue was rinsed with Krebs solution, a rebound contraction developed, and ACh (10 μM) and ATP (10 μM) relaxed the tissues. When thrombin (1 U/ml) was applied during the rebound contraction, a dual action on mechanical responses occurred: the initial
Fig. 6. Effects of hemoglobin (10 μM) on the ACh- (a) or ATP-induced (b) relaxation of intact tissues precontracted by 10 nM STA₂ (distal region). Hemoglobin was applied 10 min before the second application of STA₂. LAD: left anterior descending branch.

Fig. 7. Effects of repetitive application of ACh (0.3 μM) or thrombin (1 U/ml) on smooth muscle cells of endothelium-denuded tissues (distal region). The drugs were applied for 6 min at 15 min intervals. c shows alternate applications of ACh 1st, and thrombin.

transient relaxation was followed by contraction (Fig. 8c).

Effects of endothelin on mechanical responses: In intact tissues of the distal region, endothelin (10 nM) produced contraction and in endothelium-denuded tissues, the amplitude of contraction was further enhanced. Repetitive rinses of the tissue with
Krebs solution did not completely relax the tissues for over 30 min (Fig. 9A). In intact tissues excised from both distal and proximal regions, application of endothelin (0.5 nM), during the STA$_2$-induced contraction (10 nM), produced a dual mechanical response, i.e., an initial relaxation which was followed by contraction. When endothelin (0.5 nM) was applied during the rebound contraction following the application of STA$_2$, the dual response (initial relaxation and subsequently generated contraction) was again observed (Fig. 9Ba); whereas in the intact tissue, a high concentration of endothelin (10 nM) produced only contraction. A second application of endothelin (10 nM) did not modify the rebound contraction (Fig. 9Bb). When the effects of endothelin were compared in both distal proximal regions, the proximal region produced a larger response than the distal region (not shown).

Effects of ACh, ATP, STA$_2$, thrombin and endothelin on the membrane potential of smooth muscle cells in intact and endothelium-denuded tissues: The membrane potential of smooth muscle cells in intact tissues excised from the distal region 2 hr after superfusion in an organ bath was $-55.3\pm2.4$ mV ($n=3$ preparations and 18 impalements), but after 5–7 hours superfusion with Krebs solution, it had hyperpolarized by over 10 mV to $-67.7\pm2.0$ mV ($n=3$ preparations and 19 impalements). In contrast, in endothelium-denuded tissues, the membrane potential measured up to 7 hr remained unchanged ($-53.4\pm2.1$ mV, $n=20$ after 1–2 hr and $-54.2\pm2.6$ mV, $n=24$ after 6–7 hours superfusion). Therefore, the hyperpolarization that occurred during long superfusion in intact tissues is likely to be related to the presence of endothelial cells. The response of smooth muscle cells of the intact tissue to individual agents may differ with exposure time to Krebs solution. Therefore, the effects of various agents were observed at different membrane potentials.
Fig. 9. Effects of endothelin on the mechanical response in the distal and proximal regions of the guinea pig coronary artery. A: Distal region. High K (128 mM) and endothelin (10 nM) were applied to intact (a) and endothelium-denuded tissues (b). B: Proximal region. a: in the presence of endothelial cells (intact tissues), 0.5 nM endothelin was applied in the presence of STA2 (10 nM) or during rebound contraction following treatment with STA2. b: under the same conditions as a, 10 nM endothelin was applied. w: rinsed with Krebs solution.

Figure 10A shows the effects of ACh on the membrane potential of smooth muscle cells in the intact endothelium-denuded tissues. When the membrane potential was low (−50 mV), ACh (10 μM) hyperpolarized the membrane by 21 mV with gradual reduction in amplitude (a), whereas at a membrane potential of −62 mV, a biphasic response occurred, i.e., the initial hyperpolarization was followed by depolarization (b) and at −69 mV, only depolarization occurred. In the endothelium-denuded tissue, the membrane potential was −53 mV after 3 hours superfusion and 10 μM ACh depolarized the membrane to −45 mV (c). Figure 10B shows the effects of ACh at different membrane potential levels in the presence or absence of endothelial cells. When the membrane potential was
Fig. 10. Effects of ACh (A and B), ATP, thrombin and endothelin (C and D) on the membrane potential of smooth muscle cells of the distal region. E(+) and E(−) indicate the presence or absence of endothelial cells in tissues. B and D: Membrane potential changes induced by ACh, ATP, endothelin or thrombin at −53 mV, −60 mV, −65 mV and −70 mV (horizontal bars indicate the mean value of membrane potential with S.D. and vertical bars indicate the mean value of hyperpolarization or depolarization with S.D. n=4–8 impalements in 2–4 different tissues). In B: filled and open circles indicate amplitudes of the ACh-hyperpolarization and depolarization, respectively; and in D: open circles, triangles and quadrates indicate the effects of various agents on membrane potentials measured from smooth muscle cells in the presence of endothelial cells; and closed circles indicate the measurements of membrane potentials in the endothelium-denuded tissues.

more negative, the hyperpolarization induced by ACh was small and depolarization was larger, in a membrane potential-dependent manner (Fig. 10B).

The effects of ATP (10 μM) on the membrane potential were observed in smooth muscle cells of the intact and endothelium-denuded tissues excised from the distal region (Fig. 10C a and b, and D). ATP hyperpolarized the membrane in the presence or absence of endothelial cells, but this hyperpolarization depended on the membrane potential level: e.g., in the intact tissue, ATP (10 μM) hyperpolarized the membrane to −57 mV from a membrane potential of −51 mV (Fig. 10Ca), but at a membrane potential of −69 mV ATP hyperpolarized the membrane by only 2 mV (Fig. 10Cb). When ATP was applied to smooth muscle cells in the endothelium-denuded tissue, the membrane was hyperpolarized from −53±2.5 mV to −57.2±2.3 mV (after 3 hours superfusion in Fig. 10D). This ATP-induced hyperpolarization in intact tissue was not modified by hemoglobin (10 μM).

Thrombin (1 U/ml) depolarized the membrane in inverse proportion to the membrane potential level in the presence or absence of endothelial cells, but the absolute value of the depolarization was small (Fig. 10Cc and D); and once thrombin was applied, a second application of thrombin after 1 hr did not depolarize the membrane. With application of 10 nM STA2, the membrane was depolarized by 6.4±1.4 mV from the membrane potential
of $-61.2 \pm 1.4$ mV in intact tissues and by $7.3 \pm 1.6$ mV from the membrane potential of $-53.5 \pm 1.4$ mV in the endothelium-denuded tissues (3 preparations and 15 impalements each). In addition, when 10 nM endothelin was applied, the membrane potential ($-60 \pm 2.5$ mV) was lowered by $8.2 \pm 1.8$ mV in intact tissues and by $8.3 \pm 2.3$ mV from the resting membrane potential of $-53.4 \pm 2.1$ mV in endothelium-denuded tissues ($n=3$ preparations and 12 impalements each).

**Discussion**

The proximal and distal regions of the anterior descending branch of the left coronary artery showed some different response to various stimulants before and after removal of endothelial cells (Figs. 2 and 3). Namely, the muscarinic response occurred more potently in the distal region than in the proximal region. In intact tissues, STA$_2$ produced a larger contraction in the distal region than that in the proximal region. Endothelial regulations on the STA$_2$-induced contraction but not the thrombin-induced contraction occurred more dominantly on the proximal region rather than the distal region.

Following removal of endothelial cells, the phasic component of high K$^+$-induced contraction remained unchanged in comparison to that of the intact tissues, but the tonic component was markedly enhanced. Thus the role of EDRF released by high K$^+$ should be considered during generation of the tonic response. Application of ACh to tissues contracted by STA$_2$ relaxed and hyperpolarized the membrane in intact tissues, while ACh produced contraction and depolarized the smooth muscle membrane in endothelium-denuded tissues. Following application of hemoglobin, the ACh-induced relaxation was transient, thus ACh may release both EDRF and EDHF from the endothelial cell. As reported by Komori and Suzuki (6), ACh may activate the M$_1$-subtype and M$_2$-subtype in the endothelial cell which then release EDHF and EDRF, respectively. In the rat main pulmonary artery, the role of EDHF was small in comparison to EDRF to produce relaxation (2, 3), whereas in the guinea pig basilar artery, the initial relaxation induced by ACh was completely due to release of EDHF estimated from the action of hemoglobin (8), because, hemoglobin and methylene blue are known to inhibit the action of EDRF through inhibition of synthesis of cyclic GMP (31–34). Therefore, release and contribution of EDHF may differ with the region and also with the species. In the guinea pig coronary artery, ACh produced only a minute contraction in the intact tissues. When the membrane potential of smooth muscles was below $-60$ mV in intact tissues, ACh produced a biphasic membrane response, i.e., a transient hyperpolarization which was followed by a depolarization. In addition, in the denuded tissues excised from the distal region, ACh produced larger contraction than the K$^+$-induced contraction, but in the proximal region, the amplitude of the ACh-induced contraction was much smaller than the K$^+$-induced contraction. Therefore, direct excitatory actions of ACh on smooth muscle tissues may be masked by strong relaxing actions of EDRF and EDHF, and heterogenous distributions of the muscarinic receptor in smooth muscle cells of the coronary artery are apparent.

ATP did not produce contraction in either the intact or endothelium-denuded tissues. However, ATP relaxed tissue precontracted by STA$_2$ with hyperpolarization of the membrane in the presence or absence of endothelial cells. This ATP-induced relaxation and hyperpolarization were not modified by hemoglobin. Therefore, it seems that ATP acts solely on the purinergic receptor (P1$_1$-receptor) distributed on smooth muscle cells. In other regions of the vascular bed, ATP is reported to release EDRF through activation of the P1$_1$-receptor (35, 36) in the endothelial cell and relaxed vascular tissues. The regional and species differences in distribution of receptors on the endothelial cell should be considered. During the matched experiments of mechanical responses, ATP relaxed the STA$_2$-induced contraction in the endothelium-denuded tissue more than that observed in the intact tissue.

STA$_2$ produced contraction in intact tissue and much larger contraction in the endothelium-denuded tissues. When the tissue was rinsed with Krebs solution following application of STA$_2$, a rebound contraction
occurred with much higher amplitude than that observed in the presence of STA$_2$. During the generation of rebound contraction, re-applied STA$_2$ relaxed the tissue. On the other hand, STA$_2$ consistently depolarized the membrane in the presence or absence of endothelial cells. Therefore, STA$_2$ may release EDRF but not EDHF, and the rebound contraction provoked after application of STA$_2$ may indicate stronger binding of this substance to smooth muscle cells than endothelial cells.

Thrombin is known to release endothelin from endothelial cells (4). Thrombin enhanced the mechanical response after removal of endothelial cells. During the rebound contraction after removal of STA$_2$, thrombin transiently relaxed the tissues and subsequently contraction developed. During the application of thrombin, the smooth muscle cell membrane was depolarized. This means that thrombin may release EDRF from the endothelial cells and also act directly on smooth muscles, thus causing the contraction. However, the direct action of thrombin on smooth muscle was masked partly by the action of EDRF as observed in the case of ACh. However, thrombin may release the contracting substance from the endothelial cells, because after the thrombin-containing solution was replaced with Krebs solution, the tissue relaxed quickly in the endothelium-denuded tissue but was long lasting in the intact tissue. From the mechanical response in the present experiments, it was difficult to detect the release of endothelin that would be caused by application of thrombin (4). Furthermore, thrombin, once applied, produced desensitization of smooth muscle cells in the presence or absence of endothelial cells for several hours. This desensitization did not affect the action of ACh on the muscarinic receptor (homologous desensitization).

In the rat portal vein, endothelin, in concentrations over 0.5 nM, depolarized the smooth muscle membrane mainly due to increase in the Na permeability, and endothelin (0.15 nM) enhanced the spike discharge with or without depolarization of the membrane (37). Furthermore, endothelin increased the opening probability of the voltage dependent L- and T-types of Ca channel, nonselectively, as estimated using the patch clamp procedure (38). In the present experiments, endothelin (10 nM) produced contraction of intact tissues, and the contraction was markedly enhanced after removal of the endothelial cells. The membrane was depolarized by endothelin but by only a few mV at the resting membrane potential of $-60$ mV. However, when a low concentration (0.5 nM) of endothelin was applied during the contraction induced by STA$_2$, a dual action occurred; i.e., an initial relaxation and subsequent contraction and when a high concentration (10 nM) was applied, only contraction occurred. The above results may indicate that endothelin acts directly on smooth muscle cells and also acts on endothelial cells which release EDRF (7) but not EDHF. In the present experiments, we confirmed the previous observations that in vivo and in vitro experiments, actions of endothelin in low concentrations are thought to be a relaxant due to release of EDRF and in high concentrations, it acts as a stimulant on vascular beds (7, 39, 40). Presumably thrombin and endothelin release EDRF from the endothelial cell and produce relaxation of vascular beds together with direct and long lasting contracting actions on vascular smooth muscle cells.

The results obtained in the smooth muscle tissue excised from the left anterior descending branch of the guinea pig coronary artery can be summarized as follows: ACh releases EDRF and EDHF; STA$_2$, thrombin and endothelin release EDRF but not EDHF; and ATP releases neither EDRF nor EDHF. The ability of thrombin to release endothelin was not detected in the present experiments; however, both endothelin and thrombin show much the same action on the mechanical and electrical properties of the guinea pig coronary artery. The hyperpolarization observed by application of ACh is likely to be due to release of EDHF, and that induced by ATP is likely due to a direct action of ATP on the PII receptor distributed on the smooth muscle cell membrane.

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