Mechanisms of Vasoconstriction Induced by 9,11-Epithio-11,12-methano-thromboxane A$_2$ in the Rabbit Coronary Artery

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The vasoconstrictor effects of 9,11-epithio-11,12-methano-thromboxane A$_2$ (STA$_2$) on smooth muscle strips of the rabbit coronary artery have been investigated in vitro. Right coronary artery (RCA) was more responsive to STA$_2$ than either the left anterior descending or the circumflex coronary artery. On endothelium-denuded RCA strips, the sensitivity and responsiveness to STA$_2$ were greater than observed on intact muscle strips. A thromboxane(Tx)-antagonist, (9,11)-(11,12)-dideoxa-9α, 11α-dimethylmethano-11,12-methano-13,14-dihydro-13-aza-14-oxo-15-cyclopentyl-16,17,18,19,20-pentanol-15-epi-TxA$_2$ (ONO-3708), inhibited the STA$_2$-induced contraction, whereas atropine or prazosin had no effect. Nifedipine partly inhibited the STA$_2$-induced contraction, one half of which was still evoked in Ca$^{2+}$-free solution. When acetylcholine was applied prior to the application of STA$_2$ in Ca$^{2+}$-free solution, the STA$_2$-vasoconstriction disappeared. In saponin-treated chemically skinned muscle strips, STA$_2$ itself had no effect on either the pCa-tension relation or on the release of Ca$^{2+}$ from intracellular stores. However, inositol 1,4,5-trisphosphate released Ca$^{2+}$ from such stores, and 12-o-tetradecanoyl phorbol-13-acetate (TPA) and 1,2-diolein, activators of protein kinase C, enhanced the contraction induced by 0.3 μM Ca$^{2+}$. It is concluded that STA$_2$ acts on the TxA$_2$ receptor and produces contraction due to an increase in both voltage- and agonist(receptor)-dependent Ca$^{2+}$ influx. STA$_2$ also releases Ca$^{2+}$ from ACh- and caffeine-sensitive storage sites. (Circulation Research 1987;60:402-409)

Thromboxane A$_2$ (TxA$_2$), a product of arachidonic acid metabolism in blood platelets, is a powerful constrictor of vascular smooth muscle and a potent inducer of platelet aggregation. Together with prostacyclin released from the endothelium of vascular tissues, these prostanoids may play physiological and pathological roles in the regulation of vascular tone.

Since the degradation of TxA$_2$ is rapid, investigation of its mechanism of action on vascular tissues has been hampered, and more stable derivatives, such as carbocyclic TxA$_2$ (cTxA$_2$) or (15s)-hydroxy-11α, 9α-(epoxymethano)prosta-5Z, 13E-dienoic acid (U46619), have been developed. More recently, another stable TxA$_2$ analog, 9,11-epithio-11,12-methano-TxA$_2$ (STA$_2$), has been synthesized. It has been shown to act on the same receptor as U46619 and to be a more potent agonist on human saphenous vein. Recently, Siess et al reported that endoperoxides and TxA$_2$ might interact with a specific receptor on the human platelet membrane to release Ca$^{2+}$ from intracellular stores, resulting in the hydrolysis of phosphoinositides.

The formation of inositol 1,4,5-trisphosphate (InsP$_3$) and 1,2-diacylglycerol (DG), products of hydrolysis of phosphatidylinositol 4,5-bisphosphate (PI-P$_2$) following agonist-receptor interaction, plays an essential role in the responses of various cells. In rabbit mesenteric artery, Hashimoto et al have shown that activation of the α$_1$-adrenergic receptor by norepinephrine (NE) produces InsP$_3$ and phosphatidic acid by rapid breakdown of PI-P$_2$. Subsequently, the InsP$_3$ causes Ca$^{2+}$ release from nonmitochondrial intracellular storage sites (possibly the sarcoplasmic reticulum). A derivative of the other product, 1-oleoyl-2-acetyl glycerol, enhances Ca$^{2+}$-induced contractions in saponin-skinned muscle strips. However, whether TxA$_2$ directly releases Ca$^{2+}$ from cellular storage sites or indirectly through the actions of a second messenger is still controversial.

The present study was undertaken to characterize the effects of TxA$_2$ receptor stimulation on the mechanical responses of smooth muscle cells of intact and skinned muscle strips of the rabbit coronary artery. For this purpose, STA$_2$ was used instead of TxA$_2$ as the agonist. Furthermore, to clarify the underlying mechanism of action of STA$_2$, the effects of InsP$_3$ and DG were also investigated and compared with those of STA$_2$.

Materials and Methods

Preparation

Male albino rabbits (2.0–2.2 kg) were given sodium pentobarbital (40 mg/kg i.v.) and exsanguinated. The heart was removed rapidly and placed in a dissecting chamber filled with Krebs solution. Branches of the coronary artery (right coronary artery, RCA; left anterior descending coronary artery, LAD; and left circumflex coronary artery, LCX) were carefully excised, including aortic wall and coronary ostia. They were
cleaned for stripping at a distance of 8–10 mm from the
ostium (0.7–0.8 mm diameter), and thin circular strips
(0.3–0.5 mm long, 0.05–0.08 mm wide, and 0.02–
0.03 mm thick) were prepared under a binocular mi-
croscope. The length, width, and thickness of the
strips were then measured under an inverted micro-
scope to allow calculation of the cross-sectional area.
In most experiments, the endothelium was removed
carefully with small knives made from razor blades.
To study the effects of the endothelium on the contrac-
tions evoked by high K+, acetylcholine chloride
(ACh), or STA2, the endothelium in some muscle
strips was preserved. In the present experiments, re-
moval of the endothelium was not confirmed histologi-
ically. However, prior to the experiments, the follow-
ing pharmacologic profiles were observed: In
endothelium intact strips, 10 nM A23187 relaxed and 1
μM methylene blue enhanced the tonic evoked by 0.3 μM ACh or 39 mM K+, but neither agent modi-
fied the K+- or ACh-induced contractions in
denuded strips. These results suggest that adequate
functional denudation of the endothelium had oc-

Recording of Mechanical Activity

Mechanical activity of intact and skinned muscles
was measured by attaching a circular strip to a strain
gauge (UL-2 type, Shinko Co., Tokyo, Japan) in a
chamber of 0.6-ml capacity. The solution was changed
gently and aspirating off simultaneously with a water pump from the other
end.13 To suppress sympathetic nerve activity, 0.3 μM
tetrodotoxin (TTX) and 3 μM guanethidine were
present in the Krebs solution throughout the experi-
ments. All experiments were performed at 25° C.

Skinned Muscles

Skinned muscle preparations were obtained by us-
ing saponin (25 μg/ml) for 20 minutes in relaxing
solution at 25° C.13,14 The tension-pCa relation was
obtained by cumulative application of increasing Ca2+
concentrations in a stepwise manner. Drugs with or
without phosphatidylserine (PS) were applied during the
Ca2+-induced contraction after tension had reached a
steady level.
To estimate the amount of Ca2+ stored within the
skinned muscle, the amplitude of contraction induced
by 25 mM caffeine was measured in the presence of
0.5 mM EGTA after a 2-minute application of 0.3 μM
Ca2+ buffered with 4 mM EGTA, as previously
indicated.13,14

Solutions

The ionic composition of the Krebs solution was as
follows (mM): Na+ 137.4, K+ 5.9, Mg2+ 1.2, Ca2+
2.6, HCO3− 15.5, H2PO4− 1.2, Cl− 134.4, and glu-
cose 11.4. The high K+ solution was prepared by
replacing sodium chloride with potassium chloride isos-
motically. The solution was bubbled with 97% O2 and
3% CO2, and the pH of the solution was adjusted with

Tris to 7.4. In Ca2+-free solutions, CaCl2 was replaced
with MgCl2, and 2 mM EGTA was added.

In skinned muscles, the following relaxing solution
was used (mM): K+ methanesulphonate (KMs) 114,
Tris maleate 20, MgCl2 5.1, adenosine 3′-triphos-
phosphate (ATP) 5.2, creatine phosphate 5, and ethylene-
glycol-bis-(β-aminohexylether)-N,N,N′,N′′-tetraeaetic
acid (EGTA) 4. Various Ca2+ concentrations were pre-
pared by adding appropriate amounts of Ca(Ms)2 to 4
mM EGTA. The binding constants used in this experi-
ment have been previously reported.12,13

Drugs

The chemicals used were 9,11-epithio-11,12-
methano-TxA3 (STA3); 9,11-dimethylmethano-11,12-
methano-16-phenyl-13,14-dihydro-13-aza-15α-
β-ω-tetranor-TxA4 (ONO-11120); and (9,11), (11,
12)-dideoxa-9α,11α-dimethylmethano-11,12-methano-
13,14-dihydro-13-aza-14-oxo-15-cyclopentenyl-16,
17,18,19,20-pentanol-15-epi-TxA3 (ONO-3708; ONO
Pharmaceutical Co., Osaka, Japan); atropine sulfate
(E. Merck, Darmstadt, FRG); prazosin (Pfizer Phar-
macetical Co., Basel, Switzerland); nifedipine (Bayer
Pharmaceutical Co., Basel, Switzerland); caffeine
and guanethidine HCl (Tokyo Kasei Co., Tokyo,
Japan); ethyleneglycol-bis-(β-aminohexylether)-N,N,
N′,N′′-tetraacetic acid (EGTA; Dozin Laboratory,
Kumamoto, Japan); saponin (ICN Pharmaceutical Inc.,
Cleveland, Ohio); phosphatidylserine (PS, beef brain;
Serdary Research Laboratories, London, Ont., Can-
da); acetylcholine chloride (ACh); 12-α-tetradeca-
noyl phorbo1-13-acetate (TPA); indomethacin, acetyl-
salicylic acid (aspirin); tetrodotoxin (TTX); 1,2-diolein;
adenosine 5′-triphosphate disodium salt (ATP Na3);
and creative phosphate disodium salt (Sigma Chemical
Co., St. Louis, Mo.). Insoluble 1,4,5-triphosphate
(InsP3) was kindly provided by Dr. M. Hirata, Faculty
of Dentistry, Kyushu University.

Statistics

The results are expressed as the mean ± SD and
number of observations. Differences between means
within each experiment were evaluated by analysis of
variance. If significant differences were then demon-
strated, Student’s paired or unpaired t test was used to
determine which pairs of means were significantly dif-
ferent. Values of p < 0.05 were considered significant.

Results

Regional Differences in the Action of STA2 on the
Mechanical Activity of Coronary Arteries

Initially, it was determined which region was most
sensitive to STA2 among the three branches of the
coronary artery (RCA, LAD, and LCX). Figure 1
shows a typical example of contractions evoked by 128
mM K+, 10 μM ACh, and 30 nM STA2 in circularly
cut smooth muscle strips obtained from the above three
branches. The endothelium in all preparations was
gently manipulated to preserve its function (intact
muscle strips). In intact muscle strips prepared from all
three branches, contractions evoked by 128 mM K+

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Consisted of an initial large phasic response followed by a small tonic contraction. Among the three stimulants, ACh (10 \( \mu \)M) produced the largest contraction (RCA: 1.15 \( \pm \) 0.05 \( \times \) the maximum contraction evoked by 128 mM K\(^+\), \( n = 5 \); LCX: 1.25 \( \pm \) 0.08 \( \times \), \( n = 5 \); LAD: 1.24 \( \pm \) 0.06 \( \times \), \( n = 5 \)). When 30 nM STA\(_2\) was applied to intact muscle strips, the evoked contraction consisted of a phasic followed by a large tonic response. The phasic contraction developed after a long latency (5–10 seconds). Its rate of rise was low, and the relaxation of muscle strips after removal of STA\(_2\) was very slow (for example, the time required for the RCA to relax completely after removal of 30 nM STA\(_2\) was 20–30 minutes). The maximum amplitude of contraction evoked by 30 nM STA\(_2\) was the smallest among three stimulants (Figure 1). However, the minimum concentration of STA\(_2\) required to produce a contraction was much lower than that of ACh (for STA\(_2\), 1 nM; for ACh, 0.1 \( \mu \)M).

Figure 2 shows the regional differences in the action of STA\(_2\) on the mechanical activity recorded from intact muscle strips of the three coronary branches. The amplitude of contraction evoked by 30 nM STA\(_2\) was largest in muscle strips prepared from the RCA. An increase in STA\(_2\) concentration did not enhance the amplitude of contraction in any branch. Such regional differences in the response to STA\(_2\) were not observed following application of ACh. In 5 animals, the concentration of STA\(_2\) required to produce a half-maximal contraction was 3.2 \( \pm \) 0.1 nM in the RCA, 13.2 \( \pm \) 1.6 nM in the LAD, and 10.5 \( \pm \) 0.8 nM in the LCX. These results suggest that in the presence of the endothelium (intact muscle strips), the RCA may be the region most sensitive to STA\(_2\) among the three coronary branches. Subsequently, muscle strips prepared from the RCA were used to investigate the actions of STA\(_2\) on mechanical activity.

Effects of the Endothelium on STA\(_2\)-Induced Contractions

Figure 3A shows the effect of the endothelium on the contractions evoked by 128 mM K\(^+\), 10 \( \mu \)M ACh, or STA\(_2\) (10 nM and 30 nM). To investigate the role of endothelium on the phasic contraction evoked by 128 mM K\(^+\), the absolute values of the contraction/unit cross-sectional area (KN/m\(^2\)) were measured in intact and denuded muscle strips. There was no significant difference between the responses observed in the presence (25.1 \( \pm \) 8.3 KN/m\(^2\), \( n = 15 \)) and absence (28.5 \( \pm \) 6.7 KN/m\(^2\), \( n = 18 \)) of the endothelium (\( p > 0.05 \)). However, tonic responses evoked by 128 mM K\(^+\) were larger in denuded strips than those of intact preparations. The magnitude of the contractions evoked by 10 \( \mu \)M ACh or STA\(_2\) (10 nM and 30 nM) relative to those evoked by 128 mM K\(^+\) was also increased in denuded strips (the values of ACh-response were 1.15 \( \pm \) 0.03 times, \( n = 5 \), in intact muscle strips and 1.28 \( \pm \) 0.08 times, \( n = 5 \), in denuded strips, \( p < 0.05 \)). Figure 3B shows the dose-response relation of STA\(_2\) in muscle strips of RCA in the presence or absence of endothelium. To compare the contraction amplitudes evoked by various concentrations of STA\(_2\), the maximum amplitude of the contraction induced by 128 mM K\(^+\) was normalized as 1.0. Contractions induced by STA\(_2\) (>0.3 nM) were markedly increased in denuded strips, and the concentration of STA\(_2\) required to produce the half-maximum amplitude of contraction was significantly smaller (the ED\(_{50}\) values in intact and denuded muscle strips were 3.3 \( \pm \) 0.1 nM, \( n = 5 \), and 1.2 \( \pm \) 0.1 nM, \( n = 5 \), respectively, \( p < 0.05 \)). When indomethacin (3 \( \mu \)M) or aspirin (0.1 mM) was applied over 1 hour to both strips, the amplitude of the phasic and subsequent tonic

**Figure 1.** Schematic illustration of the rabbit coronary arteries used in the study and typical mechanical responses of circularly cut smooth muscle strips prepared from three different regions (arrow indicates RCA, right coronary artery; LAD, left anterior descending coronary artery; and LCX, left circumflex coronary artery) to 128 mM K\(^+\), 10 \( \mu \)M ACh, and 30 nM STA\(_2\). The endothelium of all preparations was preserved.

**Figure 2.** Concentration-response curves to STA\(_2\) in vascular muscle artery strips (endothelium intact) prepared from three different coronary artery regions (RCA, LAD, and LCX). Maximal responses to 128 mM K\(^+\) on strips from each region were normalized as 1.0. Curves were fit by eye. Each point is the mean derived from five observations \( \pm \) SD (vertical lines).

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contractions evoked by 128 mM K⁺ or 10 μM ACh remained the same but that evoked by 30 nM STA₂ was increased only in intact muscle strips (not shown). These results indicate that cyclooxygenase product(s) may be released from the endothelium by application of STA₂, resulting in inhibition of contractions provoked by STA₂ but not by K⁺ or ACh. Therefore, denuded vascular muscle strips were used to investigate the mechanism underlying STA₂-induced contractions.

Effects of Atropine, Prazosin, and ONO-3708 on STA₂-Induced Contractions

To investigate the role of the TxA₂ receptor on STA₂ responses, the effects of atropine, prazosin, ONO-3708, or ONO-11120 (the latter two are TxA₂ receptor blockers) on STA₂-induced contractions were observed. Atropine (1 μM) and prazosin (1 μM) did not modify the amplitude of contraction evoked by 1 nM STA₂ (this concentration is near the ED₅₀ value in denuded muscle strips). Phenotolamine (3 μM) had no effect on the STA₂-induced contraction. ONO-3708 (> 1 nM) inhibited contractions evoked by 1 nM STA₂ (0.83 ± 0.05 × the control, n = 5), and at 1 μM, ONO-3708 produced complete suppression (0.03 ± 0.02, n = 5). However, this agent (up to 3 μM) did not modify the contraction evoked by 30 nM STA₂ (0.95 ± 0.08, n = 5). Similar results were obtained by application of ONO-11120 (not shown). These results suggest that STA₂ acts at least in part on the TxA₂ receptor to produce a contraction.

Effects of Nifedipine or Ca²⁺-Free Solution on STA₂-Induced Contractions

Nifedipine inhibits voltage dependent Ca²⁺ influx in rabbit coronary artery. Therefore, the effects of nifedipine were studied to clarify the role of such Ca²⁺ influx on STA₂-induced contractions (Figure 4). When low concentrations of STA₂ (< 3 nM) were applied to denuded muscle strips, a monophasic contraction developed slowly. Nifedipine (> 1 nM) strongly inhibited this contraction in a dose-dependent manner, but a component of the mechanical response remained, even in the presence of greater concentrations of nifedipine (up to 0.3 μM). The contraction evoked by high concentrations of STA₂ (≥ 10 nM) comprised a phasic response with a relatively fast rate of rise and a tonic response. The former was less sensitive to nifedipine than the latter (Figure 4A).

To further investigate the mechanism of the STA₂-induced contraction, the effects of STA₂ in Ca²⁺-free solution containing 2 mM EGTA were observed. In Ca²⁺-free solution, the K⁺-induced contraction ceased within 15 seconds, and ACh produced a transient phasic contraction. Under these conditions, only high concentrations of STA₂ (≥ 10 nM) produced a contraction. Figure 5 shows the effects of successively applied ACh and STA₂ on mechanical responses in Ca²⁺-free solution containing 2 mM EGTA. When 30 nM STA₂ was initially applied, subsequently applied ACh produced only a small contraction (Figure 5A and B). When 10 μM ACh was initially applied, Ca²⁺-free solution, the contraction evoked by subsequently applied STA₂,
mM K+ with 10 μM ACh, n = 5). The pCa-tension relation was a sigmoidal curve (Figure 6C), and the ED$_{50}$ value for pCa was 6.28 (0.52 ± 0.01 μM, n = 5). These relations were almost the same as those previously observed with the rabbit mesenteric artery.$^{13}$

Figure 7 shows the effects of 30 nM STA$_2$ in the presence or absence of PS on the contraction evoked by 0.3 μM Ca$^{2+}$ in skinned muscle strips. After the Ca$^{2+}$-induced contraction had reached a steady amplitude, STA$_2$ alone (Figure 7A) or with PS (Figure 7B) was added to the Ca$^{2+}$-containing solution. STA$_2$ (30 nM) did not modify the amplitude of contraction evoked by 0.3 μM Ca$^{2+}$ in either condition. STA$_2$ (30 nM) also had no effect on the contraction evoked by 1 μM or 10 μM Ca$^{2+}$ (not shown). However, 1,2-diolein (50 μg/ml) increased the amplitude of the contraction evoked by 0.3 μM Ca$^{2+}$, and this effect was enhanced by addition of PS. TPA increased the Ca$^{2+}$ contraction (0.3 μM) more than 1,2-diolein, and PS enhanced this action of TPA even further. The same effects of TPA and 1,2-diolein with PS were observed on the contraction evoked by 0.3 μM Ca$^{2+}$ buffered with 10 mM EGTA or under pretreatment with 3 μM A23187. Thus, in the smooth muscle cell of RCA, TPA and 1,2-diolein may directly act on the contractile proteins and sensitize them to Ca$^{2+}$. Furthermore, 1 mM dithiothreitol did not modify the effects of either agent on the Ca$^{2+}$ contraction. When STA$_2$ with 1,2-diolein was additionally applied during a contraction evoked by 0.3 μM Ca$^{2+}$, the amplitude of the response ceased (Figure 5C and D). These results indicate that STA$_2$ may release Ca$^{2+}$ from the ACh-sensitive, intracellular Ca$^{2+}$ store.

Effects of STA$_2$ on Chemically Skinned Muscle Strips of RCA

Figure 6 shows the relation between the free Ca$^{2+}$ concentration and contraction obtained from saponintreated chemically skinned muscle strips. Various concentrations of Ca$^{2+}$ were cumulatively applied to the strip as shown in Figure 6B. The minimum concentration of Ca$^{2+}$ required to produce a mechanical response was 0.1 μM, and increased concentrations of Ca$^{2+}$ enhanced the amplitude of contraction. The maximum amplitude of Ca$^{2+}$-induced responses was obtained at 10 μM Ca$^{2+}$, and a further increase in Ca$^{2+}$ concentration no longer enhanced the contraction. As shown in Figure 6A and B, the contraction evoked by 10 μM Ca$^{2+}$ in chemically skinned muscle strips was consistently larger than that evoked by 128 mM K+, 10 μM ACh, or 128 mM K+ + 10 μM ACh in unskinned tissues (2.45 ± 0.32 $\times$ the 128 mM K+ contraction, n = 5; 2.23 ± 0.24 $\times$ the 10 μM ACh contraction, n = 5; and 1.53 ± 0.25 $\times$ the contraction evoked by 128 mM K+).
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Figure 6. Effects of calcium on chemically skinned muscle strips of RCA. To compare the effects of Ca²⁺ in skinned muscle strips (B), 128 mM K⁺, 10 μM ACh, or 128 mM K⁺ + ACh were applied to the strip before application of saponin for skinning (A). C: pCa tension relation. The amplitude of the contraction evoked by 10 μM Ca²⁺ was normalized as 1.0. Each point represents the mean ± SD (vertical bars), n = 5.

Discussion

The present results have shown that the responsiveness to STA₂ was greatest in the RCA and was increased by removal of the endothelium. Since indomethacin or aspirin enhanced the amplitude of STA₂-induced contractions in intact muscle strips but not in the denuded ones, cyclooxygenase-related products released from endothelial cells may partly contribute to the observed effects of STA₂.

It has been reported that the contraction induced by TXA₂ mimetics in vascular smooth muscle does not involve the activation of α-adrenergic, serotoninergic, histaminergic H₁, and angiotensin II receptors. In the present study, the STA₂-induced contractions were unaffected by α-adrenergic blocking agents (prazosin and phentolamine) or a muscarinic antagonist (atropine) but were inhibited by ONO-11120 and ONO-3708, which are reported to be selective TXA₂ antagonists in platelets and in vascular smooth muscle. Such results strongly suggest that the effects of STA₂ are mediated by TXA₂ receptors.

Figure 7. Effects of 10 nM TPA, 50 μg/ml 1,2-diolein, or 30 nM STA₂ in the absence (A) or presence (B) of 50 μg/ml phosphatidylserine (PS) on the contraction evoked by 0.3 μM Ca²⁺ in chemically skinned muscle strips prepared from the RCA. The amplitude of contractions evoked by 0.3 μM Ca²⁺ was normalized as 1.0. Abscissa = time after application of 0.3 μM Ca²⁺. Drugs were applied 10 minutes after application of Ca²⁺ (indicated by the second arrow). Mean values obtained from 3–5 different strips are shown.
In the rabbit coronary artery, ACh contracted the tissue by activating both voltage-dependent and receptor-operated Ca\(^{2+}\) influx and stimulating Ca\(^{2+}\) release from caffeine-sensitive intracellular stores (possibly the sarcoplasmic reticulum).\(^{1-5}\) The present results have shown that the STA\(_2\)-induced contraction in this tissue is due to stimulation of voltage-dependent (nifedipine-sensitive) and receptor-operated Ca\(^{2+}\) influx and also to release of Ca\(^{2+}\) from storage sites. This mechanism is similar to that activated by ACh in this tissue.\(^{15,16}\) The STA\(_2\)-induced contraction in Ca\(^{2+}\)-free solution was diminished by prior application of ACh, and, reciprocally, the ACh response was inhibited by pretreatment with STA\(_2\). These results suggest, therefore, that the STA\(_2\)-sensitive Ca\(^{2+}\) store is the same as or closely related to the ACh-sensitive one.

Although the actions of STA\(_2\) were very similar to those of ACh, the responses induced by STA\(_2\) in smooth muscle strips were not exactly the same as those evoked by ACh. First, the rate of rise of contraction after application of STA\(_2\) (especially in low concentration) and the rate of fall after removal of STA\(_2\) were much lower than with ACh. Since the preparations used in the present experiments were very small and thin (see "Materials and Methods"), the time required for agonist diffusion can be assumed to be minimal, as expected from the results obtained from the Ca\(^{2+}\) contraction in skinned muscle strips. Therefore, such features of the STA\(_2\) contraction do not result from the slow diffusion of STA\(_2\), but may be due to a specific characteristic of the STA\(_2\) (TxA\(_2\)) receptor.

Second, the effect of STA\(_2\) on the release of Ca\(^{2+}\) from the storage sites was concentration-dependent. High concentrations of STA\(_2\) produced effects that were comparable to those of ACh in porcine and rabbit coronary arteries\(^{16,20}\) and of NE in rabbit mesenteric artery.\(^{14}\) Since STA\(_2\) is a stable compound, the loss of response evoked by low concentrations of STA\(_2\) in Ca\(^{2+}\)-free solution may not be due to its degradation but rather to an inability to release Ca\(^{2+}\).

In various cells and tissues, InsP\(_3\) and DG have essential roles as signal transducers following the formation of hormone or neurotransmitter-receptor complexes.\(^{9,10}\) NE and ACh rapidly reduce the amount of PI-P\(_2\), and generated InsP\(_3\) and PA in the rabbit mesenteric artery.\(^{14}\) Since STA\(_2\) is a stable compound, the loss of response evoked by low concentrations of STA\(_2\) in Ca\(^{2+}\)-free solution may not be due to its degradation but rather to an inability to release Ca\(^{2+}\).

In various cells and tissues, InsP\(_3\) and DG have essential roles as signal transducers following the formation of hormone or neurotransmitter-receptor complexes.\(^{9,10}\) NE and ACh rapidly reduce the amount of PI-P\(_2\), and generated InsP\(_3\) and PA in the rabbit mesenteric artery.\(^{14}\) Since STA\(_2\) is a stable compound, the loss of response evoked by low concentrations of STA\(_2\) in Ca\(^{2+}\)-free solution may not be due to its degradation but rather to an inability to release Ca\(^{2+}\).

Furthermore InsP\(_3\) releases Ca\(^{2+}\) in many vascular tissues\(^{22-24}\) and produces a contraction due to Ca\(^{2+}\) release from caffeine-sensitive stores in chemically skinned muscle of rabbit mesenteric and main pulmonary arteries.\(^{11,21}\) The production of PA is an indicator of DG synthesis, and this agent increases the Ca\(^{2+}\)-induced contraction in chemically skinned muscle.\(^{12}\)

Following activation of the receptor by TxA\(_2\) or TxA\(_2\) mimetics in human platelets, phosphatidylinositol metabolism was stimulated, and PA, 1,2-DG, and inositol phosphates were produced.\(^{4,26,27}\) Pollock et al.\(^{16}\) reported that TxA\(_2\) receptor occupancy is closely linked to inositol phospholipid metabolism and to elevation of cytosolic free Ca\(^{2+}\). They suggested that Ca\(^{2+}\) and DG (via activation of protein kinase C) may act synergistically to mediate the cellular response following occupancy of the TxA\(_2\) receptor.

In the present study, STA\(_2\) neither released Ca\(^{2+}\) from intracellular stores nor modified the Ca\(^{2+}\)-induced contraction, even in the presence of phosphatidylserine or 1,2-diolenie in skinned coronary arterial smooth muscles. On the other hand, InsP\(_3\) released Ca\(^{2+}\) from caffeine-sensitive stores, and TPA or 1,2-diolenie enlarged the Ca\(^{2+}\)-induced contraction in the presence of phosphatidylserine, as previously observed in the rabbit mesenteric artery.\(^{11,12}\) Since protein kinase C is activated by Ca\(^{2+}\) and acidic phospholipid (e.g., phosphatidylserine\(^{10}\)), these results may indicate that DG activates protein kinase C and enhances the Ca\(^{2+}\) contraction in skinned muscle strips. In preliminary experiments, STA\(_2\) (10 nM) reduced the amount of PI-P\(_2\) in tissue prepared from RCA, as did ACh (T. Sasaguri, personal communication). STA\(_2\) (10 nM) may bind to the TxA\(_2\) receptor and produce InsP\(_3\) (release of Ca\(^{2+}\) from SR) and DG (enhancement of contraction) through hydrolysis of PI-P\(_2\), thus provoking the mechanical response.

In conclusion, in the rabbit coronary artery, STA\(_2\) binds to the TxA\(_2\) receptor and produces a contraction due to activation of voltage-dependent (nifedipine-sensitive) and receptor-operated Ca\(^{2+}\) influx and also to release of Ca\(^{2+}\) from intracellular stores. The latter mechanism is thought to be closely associated with the actions of InsP\(_3\) and DG synthesized within the cell.

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References


Key Words • rabbit coronary artery • chemically skinned muscle • inositol trisphosphate • protein kinase C • epithio-methano-thromboxane A₂
Mechanisms of vasoconstriction induced by 9,11-epithio-11,12-methano-thromboxane A2 in the rabbit coronary artery.

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