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# EFFECTS OF PROSTAGLANDINS ON THE CYTOSOLIC FREE CALCIUM CONCENTRATION IN VASCULAR SMOOTH MUSCLE CELLS

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The effects of prostaglandin (PG) $F_{2\alpha}$ and 9,11-epithio-11,12-methanothromboxane $A_2$ ($STA_2$), a stable analogue of thromboxane $A_2$, on the cytosolic free calcium concentration ($[Ca^{2+}]i$) in vascular smooth muscle cells were studied with a new fluorescent $Ca^{2+}$ indicator fura 2. $PGF_{2\alpha}$ and $STA_2$, which are strong vasoconstrictors, caused rapid phasic and subsequent tonic increases in $[Ca^{2+}]i$. $PGF_{2\alpha}$ caused dose-dependent elevation of $[Ca^{2+}]i$ not only in control solution but also in the calcium-free solution. A first stimulation with $PGF_{2\alpha}$ caused dose-dependent decrease in the response of $[Ca^{2+}]i$ to a second stimulation with $PGF_{2\alpha}$. Pretreatment with 13-Azaprostanoic acid, a receptor level antagonist of thromboxane $A_2$ inhibited the increase of $[Ca^{2+}]i$ induced by $STA_2$. These results suggest that $PGF_{2\alpha}$ induces calcium mobilization followed by smooth muscle contraction through its specific receptors.


There are many reports that prostaglandin (PG)s, which are synthesized in the vascular wall or platelets, may be important not only in regulation of normal vascular flow but also in the pathogenesis of several pathological conditions such as cerebral or coronary vasospasm (1-4). The primary trigger for smooth muscle contraction is known to be elevation of $[Ca^{2+}]i$ in VSMC (5,6). Recently, Capponi et al. measured $[Ca^{2+}]i$ in VSMC with the fluorescent $Ca^{2+}$ indicator quin 2 and they suggested that $[Ca^{2+}]i$ rise induced by angiotensin II was due to a dual mechanism through its specific receptor: release from intracellular stores and calcium influx across the cell membrane (7). Alexander et al. reported that calcium mobilization from intracellular stores by angiotensin II

Abbreviations used : PG, prostaglandin ; $[Ca^{2+}]i$, cytosolic free calcium concentration ; VSMC, vascular smooth muscle cells ; $STA_2$, 9,11-epithio-11,12-methano-thromboxane $A_2$ ; 13-APA, 13-azaprostanoic acid ; $TXA_2$, thromboxane $A_2$.

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interacting with membrane receptors appeared to be mediated through the breakdown of polyphosphoinositide (8). However, the mechanism of action of PGs on VSMC is not known. Kennedy et al. suggested the existence of distinct receptors for $PGE_2$, $PGF_{2\alpha}$ and thromboxane $A_2$ in vascular smooth muscle preparations (9). In the present study, we measured $[Ca^{2+}]i$ in VSMC with a new fluorescent $Ca^{2+}$ indicator fura 2, which is more sensitive than quin 2 (10). With this indicator, we examined whether $[Ca^{2+}]i$ of VSMC is involved in PG-induced vasoconstriction and whether the changes of $[Ca^{2+}]i$ induced by PGs are mediated through their specific receptors.

## MATERIALS AND METHODS

$PGF_{2\alpha}$, $STA_2$ and 13-APA were donated by Ono Pharmaceutical Co. (Osaka, Japan). Fura 2 and fura 2 acetoxymethyl ester (fura 2 AM) were purchased from Molecular Probes Inc. (Junction City, OR). Other materials used were commercial products of the highest grade available. VSMC were prepared from female Wistar rat aorta by the explant method (11). $[Ca^{2+}]i$ was measured with the fluorescent $Ca^{2+}$ indicator fura 2 (10). Confluent VSMC were preincubated in serum-free Dulbecco's modified Eagle's Medium (DMEM) for 24 h at 37°C under 5% $CO_2$ in air. Then the cells were harvested in 0.06 % trypsin solution containing 0.02 % EDTA, washed twice with DMEM, and incubated at 2 x $10^6$ cell/ml in Earle's balanced salt solution (116 mM NaCl, 5.3 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, 1 mM $NaH_2PO_4$, 5.5 mM glucose, 26 mM $NaHCO_3$, 10 mM HEPES, pH 7.3 at 37°C) containing 4 $\mu$M fura 2 AM for 50 min. The cells were then washed twice with the same buffer to remove extraneous dye. Loading of cells with fura 2 AM did not affect their viability; after the loading period, more than 99 % of the cells excluded trypan blue dye. The cells (1 x $10^6$ /ml) were then placed in a 1 $cm^2$ quartz cuvette for measurement of $[Ca^{2+}]i$ at 37°C with continuous stirring. Fluorescence was recorded in a Hitachi Fluorescence Spectrophotometer 650-60 (Tokyo, Japan), with excitation and emission wavelength of 340 nm and 495 nm respectively. $[Ca^{2+}]i$ was calculated from the fluorescence signals as described Tsien et al. (10,12).

## RESULTS

The addition of $PGF_{2\alpha}$, one of the strongest known vasoconstrictors, evoked an immediate and striking increase of $[Ca^{2+}]i$ in VSMC. Typical increases of $[Ca^{2+}]i$ in response to various concentrations of $PGF_{2\alpha}$ are shown in Fig. 1-A. The basal level of $[Ca^{2+}]i$ was 125 $\pm$ 6.2 nM (mean $\pm$ S.E. ; n=32). After the addition of $PGF_{2\alpha}$, there were rapid phasic and subsequent tonic rises in $[Ca^{2+}]i$ dose-dependently. Moreover,

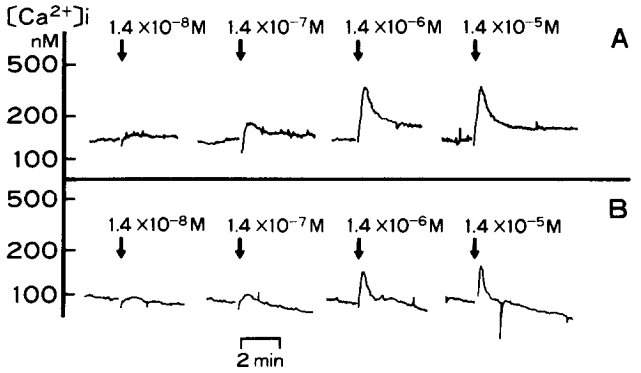


Fig. 1. Effects of various concentrations of $PGF_{2\alpha}$ on $[Ca^{2+}]i$ in VSMC in the presence or absence of extracellular calcium. Arrows indicate the time of addition of $PGF_{2\alpha}$ to the cell suspension in control solution (A) or calcium-free solution (B), which was obtained by omitting $CaCl_2$ from the control solution and adding 2mM EGTA. Traces were obtained with parts of the same cell suspension in the same experiment.

$PGF_{2\alpha}$ still caused dose-dependent increase of $[Ca^{2+}]i$ in calcium-free solution, obtained by omitting $CaCl_2$ from the control solution and adding 2 mM EGTA, although there were no tonic rises in $[Ca^{2+}]i$ after the phasic responses (Fig. 1-B).

Next we investigated the effects of successive additions of $PGF_{2\alpha}$ on $[Ca^{2+}]i$ in calcium-free solution. A first addition of $1.4 \times 10^{-5}$ M $PGF_{2\alpha}$ almost abolished the response of $[Ca^{2+}]i$ to a subsequent addition of $1.4 \times 10^{-5}$ M $PGF_{2\alpha}$ within 5 min, though by this time the $[Ca^{2+}]i$ level had already returned to the basal value (Fig. 2-A). However, addition of $1 \times 10^{-7}$ M angiotensin II elicited increase in $[Ca^{2+}]i$ even after 2 times stimulations with $1.4 \times 10^{-5}$ M $PGF_{2\alpha}$, but the extent of $[Ca^{2+}]i$ increase was less than that of angiotensin II alone (Fig. 2-A,C). When the dose of $PGF_{2\alpha}$ on its first addition was reduced to $1.4 \times 10^{-7}$ M, increase in $[Ca^{2+}]i$ was observed after its second addition at $1.4 \times 10^{-5}$ M, but the extent of $[Ca^{2+}]i$ increase was less than that after a first treatment with the same dose of $PGF_{2\alpha}$ (Fig. 2-B). Fig. 3 shows the effects of $STA_2$, a stable analogue of $TXA_2$ (13), and 13-APA, a specific antagonist of $TXA_2$ (14), on the $[Ca^{2+}]i$ in the control solution. The addition of $1 \times 10^{-5}$ M $STA_2$ evoked a rapid increase of $[Ca^{2+}]i$, and

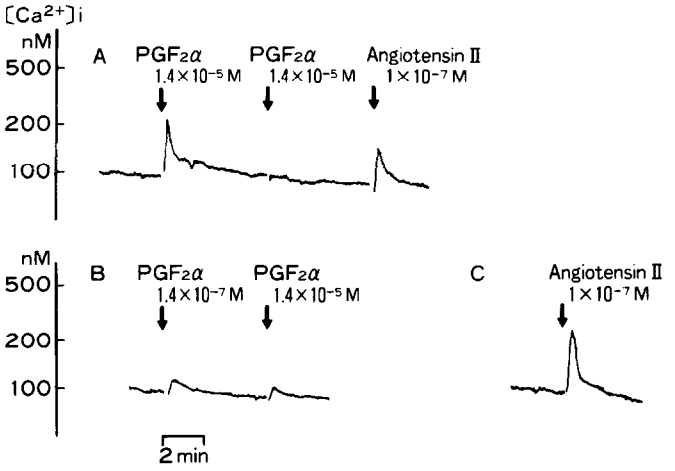


Fig. 2. Effects of successive stimulations with $PGF_{2\alpha}$ on $[Ca^{2+}]i$ in VSMC in the absence of extracellular calcium. Arrows indicate the times of $PGF_{2\alpha}$ addtions. Traces were obtained in the same experiment with portions of a single cell suspension.

the pretreatment with 2.5 x $10^{-6}$ M 13-APA for 5 min inhibited the increase of $[Ca^{2+}]i$ induced by $STA_2$.

## DISCUSSION

$PGF_{2\alpha}$ is one of the strongest known vasoconstrictors of cerebral and coronary arteries (2,3). In the present study we demonstrated that addition of $PGF_{2\alpha}$ evoked an immediate and dose-dependent increase of $[Ca^{2+}]i$ in VSMC (Fig. 1-A).

In calcium-free solution, in which calcium influx across the cell membrane is negligible, $PGF_{2\alpha}$ could still cause clear dose-dependent increase of $[Ca^{2+}]i$, but the tonic rises in $[Ca^{2+}]i$ after the phasic responses induced by $PGF_{2\alpha}$ were not observed (Fig. 1-B). These results

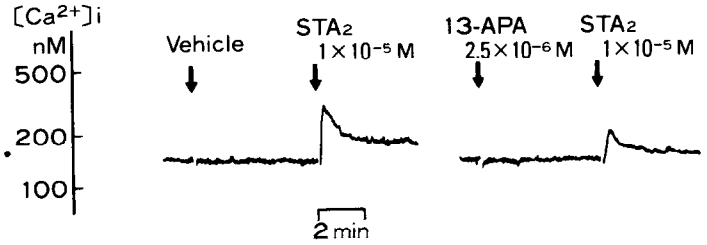


Fig. 3. Effects of $STA_2$ and 13-APA on $[Ca^{2+}]i$ in VSMC. Traces were obtained in the same experiment.

suggest that $PGF_{2\alpha}$ elicited calcium mobilization from intracellular stores, and the tonic rise after the phasic response induced by $PGF_{2\alpha}$ might indicate $Ca^{2+}$ influx into the cells.

As shown in Fig 2-A and B a first stimulation with $PGF_{2\alpha}$ caused dose-dependent decrease in the response of $[Ca^{2+}]i$ to a subsequent stimulation with $1.4 \times 10^{-5}$ M $PGF_{2\alpha}$ in calcium free-solution. Angiotensin II could still cause the increase of $[Ca^{2+}]i$ even after the stimulation with $1.4 \times 10^{-5}$ M $PGF_{2\alpha}$. Moreover, pretreatment with 13-APA, a specific antagonist of $TXA_2$ at a receptor level (14), inhibited the increase of $[Ca^{2+}]i$ induced by $STA_2$, stable analogue of $TXA_2$ (Fig. 3). From these results we suggest that the decreased response in $[Ca^{2+}]i$ upon repeated $PGF_{2\alpha}$ stimulation is due to down regulation of specific receptors and that $PGF_{2\alpha}$ induces calcium mobilization followed by smooth muscle contraction through its specific receptors. However, the depletion of intracellular stores might also play a role in this decreased response, because the extent of $[Ca^{2+}]i$ increase induced by angiotensin II after the stimulation with $PGF_{2\alpha}$ was less than that of angiotensin II alone in calcium-free solution (Fig. 2-C). Further investigations are necessary to clarify this point.

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