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EFFECTS OF CALCIUM ANTAGONISTS AND CALMODULIN INHIBITORS ON ANGIOTENSIN II- INDUCED PROSTAGLANDIN PRODUCTIONS IN THE ISOLATED DOG RENAL ARTERIES

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SUMMARY: Angiotensin II markedly potentiated both PGE_2 and PGI_2 productions in the isolated dog renal arteries. This angiotensin II- induced response was significantly reduced by the treatments of EGTA and calcium antagonists such as verapamil, nifedipine and 8-(N,N'-diethylamino)-octyl-3,4,5,-trimethoxybenzoate (TMB-8). Calmodulin inhibitors, trifluoperazine and W-7 also inhibited the angiotensin II- induced PG productions while an inactive analogue of W-7, W-5 did not have any effect. The results suggest that angiotensin II may enhance the intracellular Ca^{2+} level through the influx of extracellular Ca^{2+} and then, calmodulin activated with Ca^{2+} will stimulate both PGE_2 and PGI_2 productions via its activation of phospholipase A_2 in the dog renal arteries.

Angiotensin II (Ang II) markedly potentiated both prostaglandin (PG) E_2 and I_2 productions in the isolated dog renal arteries (1,2). This stimulatory action of Ang II is related to the activation of phospholipase A_2 through its specific receptor (2). Furthermore, PG generating system has been reported to require calcium ion (Ca^{2+}) in many tissues (3-5) and to further include the calmodulin-dependent pathway in platelets (6). However, the roles of Ca^{2+} and calmodulin in the Ang II- induced PG productions in the dog renal arteries remain to be elucidated. For investigating the roles of Ca^{2+} and calmodulin in the Ang II- induced response, we have used a variety of drugs; 1) chelating agent, EGTA, 2) calcium antagonists, verapamil and nifedipine, 3) intracellular calcium antagonist, TMB-8, 4) calmodulin inhibitors, trifluoperazine and W-7 and 5) an inactive analogue of W-7, W-5. All results obtained suggest that the Ang II- induced PGE_2 and PGI_2 productions in the isolated dog renal arteries may be stimulated by its activation of phospholipase A_2 through the calcium-calmodulin dependent pathway.

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METHODS AND MATERIALS

Mongrel dogs of either sex weighing 7 to 12 kg were anesthetized with sodium pentobarbital (30 mg/kg, i.v.) and the renal arteries were dissected. The isolated dog renal arteries were quickly stored in the ice-cold oxygenated Krebs-Henseleit buffer solution (KHBS). The arteries were carefully removed free from surrounding tissues and cut into ring segments (approximately 3 mm in length). The strips were incubated with KHBS at 37°C under 95% O_2 - 5% CO_2 stream.

To study the effects of calcium antagonists and calmodulin inhibitors on PG productions, we had used a variety of drugs; verapamil, nifedipine, 8-(N,N'-diethylamino)-octyl-3,4,5,-trimethoxybenzoate (TMB-8), trifluoperazine (TFP), W-7 and W-5. After the pre-incubation with KHBS for 80 min, the strips were pre-treated for 10 min with each inhibitor or not. And then, the incubation was performed further twice under the same condition. During the last 10 min, the strips were incubated with Ang II (0.1 μM) containing each inhibitor or not.

To test the role of extracellular Ca^{2+} on PG productions, after the pre-incubation for 60 min with KHBS, the strips were treated with EGTA (etyleneglycol bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid) in the Ca^{2+} depleted KHBS for 30 min. After the pre-treatment of EGTA (4 mM), the strips were incubated twice for 10 min with Ca^{2+}-free KHBS containing EGTA (0.25 mM). During the last 10 min incubation, the strips were incubated with Ang II (0.1 μM).

The incubation medium was sampled at the end of each 10 min incubation in the last 20 min. The PGE_2 and PGI_2 (determined as 6-keto-$PGF_{1\alpha}$) concentrations released from the strips were measured by using a radioimmunoassay method after extracted with ethylacetate (2).

Significant difference was calculated using Student's t-test.

DRUGS: Angiotensin II (Osaka Protein Res., Osaka, Japan), EGTA (Wako Pure Chemical,Tokyo, Japan), verapamil (Eisai Co., Ltd., Tokyo, Japan), nifedipine (Bayer Yakuhin, Ltd., Osaka, Japan), TMB-8 (Sigma Chemical Co., St.Louis, MO, U.S.A.), trifluoperazine (Sumitomo Chemical, Osaka, Japan), W-7 and W-5 (Rikaken Co., Ltd., Tokyo, Japan), [^{3}H]PGE_2 and [^{3}H]6-keto-$PGF_{1\alpha}$ (Amersham, Tokyo, Japan), anti-PGE_2 serum (Institute Pasteur, Paris, France) and anti-6-keto-$PGF_{1\alpha}$ serum (Ono Pharmaceutical Co., Ltd., Osaka, Japan) were used.

RESULTS AND DISCUSSION

Ang II (0.1 μM) markedly potentiated both PGE_2 and 6-keto-$PGF_{1\alpha}$ productions in the isolated dog renal arteries (Table 1). This Ang II- induced response was significantly reduced by the treatment of EGTA under the absence of Ca^{2+}. Furthermore, calcium antagonist such as verapamil which blocks Ca^{2+} channel inhibited the Ang II- induced response (Fig. 1). Nifedipine also suppressed the stimulation of both PG productions induced with Ang II (data not shown). The results suggest that the Ang II- induced PG productions may be mediated through the influx of Ca^{2+}. This result is also supported by the reports that Ca^{2+} is a primary intracellular mediator in the induction of PG production in the vascular endothelium of human umbilical vein (7), porcine aorta (8) and bovine pulmonary artery (9). Although Ang II stimulated the

Table 1. Effect of EGTA on Ang II- induced PGE_2 and 6-keto-$PGF_{1\alpha}$ productions in the isolated dog renal arteries

	PGE_2 (ng/g wet wt.)		6-keto-$PGF_{1\alpha}$ (ng/g wet wt.)	
	control	Ang II	control	Ang II
Ca^{++} (2.5 mM)	10.19 ± 1.62	20.08 ± 4.45 *	51.75 ± 8.34	69.08 ± 9.61 *
Ca^{++} (0 mM) +EGTA (0.25 mM)	4.44 ± 1.38 +	3.69 ± 1.18	17.75 ± 4.28 ++	18.49 ± 5.50

The strips were incubated with EGTA under the absence of Ca^{2+} or without of EGTA under the presence of Ca^{2+}. Under the absence of Ca^{2+} in the incubation medium, the strips were incubated with 0.25 mM of EGTA after the pre-treated with 4 mM of EGTA for 30 min. Values represent mean±S.E., n=7. Significant difference between the control and the Ang II- treated groups; * $p<0.05$, and difference between the absence and the presence of Ca^{2+} in the control groups; + $p<0.05$ and ++ $p<0.01$.

intracellular Ca^{2+} efflux via its activation of the translocation of Ca^{2+} from the intracellular Ca^{2+} stores (10), in the present study Ang II did not potentiate PG productions under the treatment of EGTA and calcium antagonists (Table 1 and Fig. 1). Therefore, Ang II may not potentiate both PGE_2 and PGI_2 productions in the dog renal arteries through the direct stimulation of the

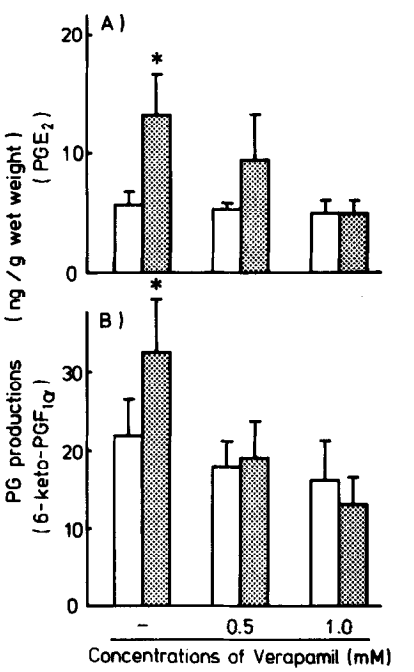

Fig. 1. Effect of verapamil on Ang II- induced PGE_2 (A) and 6-keto-$PGF_{1\alpha}$ (B) productions. Values represent mean±S.E. in control (open columns) and Ang II-treated groups (dotted columns). n=5-8. Significant difference between control and Ang II- treated groups; * $p<0.05$.

release of Ca^{2+} from its stores like mitochondria, salcoplasmic reticulum and membran bound pools. We have further investigated about the role of intracellular Ca^{2+} on Ang II- induced response by using TMB-8, an inhibitor of the mobility of intracellular Ca^{2+}. TMB-8 in the concentrations of 50 and 100 μM failed to inhibit the stimulations of both PGE_2 and 6-keto-$PGF_{1\alpha}$ productions (Fig. 2). But, TMB-8 at 500 μM completely reduced the Ang II- induced response. At almost about same concentration, TMB-8 has also been reported to inhibit the release of arachidonic acid from phosphatidylcholin induced with thrombin in human platelets (11). Therefore, this inhibitory effect of TMB-8 may result from its suppression of the mobility of the intracellular Ca^{2+} from the intracellular stores. This result will lead to that Ca^{2+} accumulated from the extracellular space may probably stimulate further release of Ca^{2+}

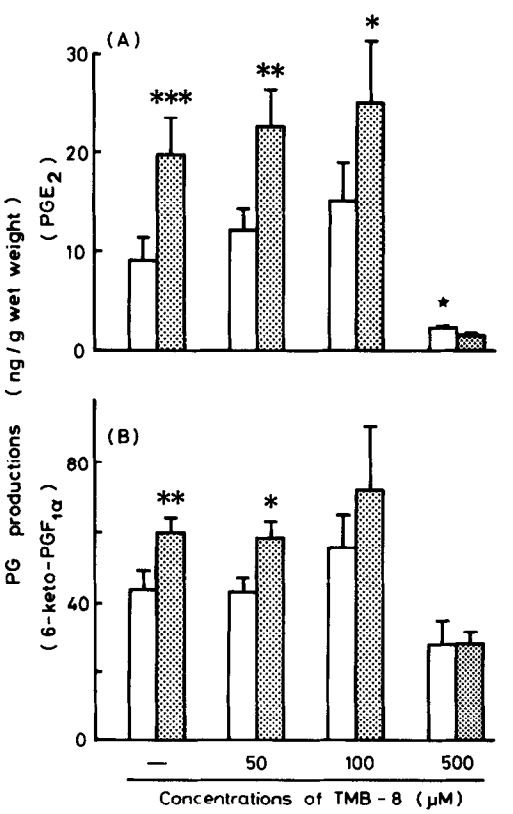

Fig. 2. Effect of TMB-8 on Ang II- induced PGE_2 (A) and 6-keto-$PGF_{1\alpha}$ (B) productions. Values represent mean±S.E. in control (open columns) and Ang II-treated groups (dotted columns). n=6. Significant difference between control and Ang II- treated groups; * $p<0.05$, ** $p<0.01$ and *** $p<0.001$, and difference between the absence and the presence of TMB-8 in the control groups; ★ $p<0.05$.

from the intracellular Ca^{2+} stores. This Ca^{2+}-induced Ca^{2+}-release mechanism has also been reported in norepinephrine-induced vascular contractile response in the rabbit mesenteric arterial smooth muscle (12). However, since TMB-8 at 500 μM also reduced both basal PG productions, same mechanism appears to be also present in the spontenous PG generating system.

Calmodulin activated with Ca^{2+} stimulates PG productions through the activation of phospholipase A_2 in platelets (6). To investigate the role of calmodulin in the Ang II- induced PG productions mediated through the Ca^{2+}-dependent pathway in the dog renal arteries, we have used calmodulin inhibitors such as TFP, W-7 and an inactive analogue of W-7, W-5. Calmodulin inhibitors and W-5 alone did not have any effect of basal PG productions. However, the Ang II- induced PGE_2 and 6-keto-$PGF_{1\alpha}$ productions were markedly inhibited by the treatments of TFP and W-7 while W-5 failed to inhibit (Figs. 3 and 4). The results suggest that calmodulin activated through the Ca^{2+}-dependent pathway may also play an important role in both PGE_2 and PGI_2 productions induced

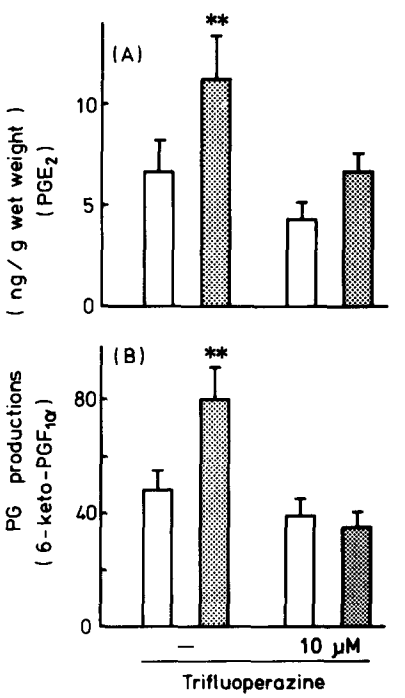

Fig. 3. Effect of trifluoperazine on Ang II- induced PGE_2 (A) and 6-keto-$PGF_{1\alpha}$ (B) productions. Values represent mean±S.E. in control (open columns) and Ang II- treated groups (dotted columns). n=8. Significant difference between control and Ang II- treated groups; * $p<0.05$.

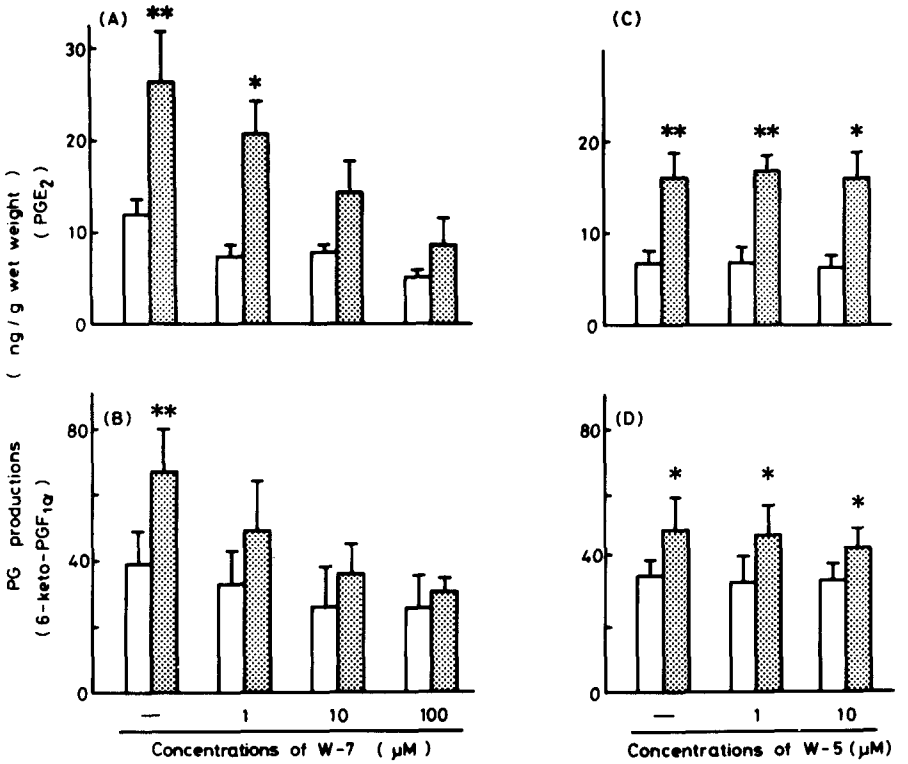

Fig. 4. Effects of W-7 and W-5 on Ang II- induced PGE_2 (A and C) and 6-keto-$PGF_{1\alpha}$ (B and D) productions. Values represent mean±S.E. in control (open columns) and Ang II- treated groups (dotted columns). n=6. Significant difference between control and Ang II- treated groups; * $p<0.05$ and ** $p<0.01$.

with Ang II in the isolated dog renal arteries. This suggestion for PG synthesis is also supported by the reports that bradykinin stimulates the activity of phospholipase A_2 via the activation of calmodulin in vascular endothelial cells from porcine aorta (8) and bovine plumonaly artery (9). Therefore, the following mechanism may be proposed; Ang II may enhance the intracellular Ca^{2+} level through the stimulation of the influx of extracellular Ca^{2+} and then, calmodulin activated with intracellular Ca^{2+} will potentiate both PGE_2 and PGI_2 productions via the activation of phospholipase A_2.

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