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PROSTAGLANDIN E_2 AND I_2 PRODUCTION IN ISOLATED DOG RENAL ARTERIES IN THE ABSENCE OR PRESENCE OF VASCULAR ENDOTHELIAL CELLS

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The spontaneous prostaglandin I_2 production was significantly reduced by the removal of endothelial cells from the isolated dog renal arteries compared with relative slight reduction of prostaglandin E_2 production. The stimulation of prostaglandin I_2 production induced with angiotensin II was also markedly reduced under the absence of endothelial cells, while its potentiation of prostaglandin E_2 production was not inhibited. The results suggest that the vascular endothelial cells are the major sources of prostaglandin I_2 in the dog renal arteries, while prostaglandin E_2 is mainly produced in other cell types, perhaps vascular smooth muscle cells.

Prostaglandins (PGs) play important roles for the vasodilation in dog renal arteries (1). It has also been suggested that the vascular endothelial cells appear to play roles for the relaxation of vasculartures and PGI_2 production (2 - 6). However, the precise sources of PGs in the dog renal arteries still remain to be elucidated. In the present study, we have investigated about the sources of PGE_2 and PGI_2 in the isolated dog renal arteries under the absence or presence of vascular endothelial cells. The results suggest that the vascular endothelial cells are the major sources of PGI_2 in the isolated dog renal arteries, while PGE_2 production is mainly induced in other cells or tissues, perhaps vascular smooth muscle cells.

MATERIALS AND METHODS

Incubation of the isolated dog renal arteries: 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 arteries were quickly stored in ice-cold oxygenated Krebs-Henseleit buffer solution. Then, the arteries were carefully removed free from surrounding tissues and cut into ring segments (about 3 mm in length). The strips were incubated with Krebs-Henseleit buffer solution at 37°C under 95% O_2 - 5% CO_2 gassed. In the experiment for the time course of PG productions, the strips were incubated for 165 min. The incubation medium was sampled at every 15 min. When PG productions were investigated under the absence of endothelial cells, the intimal surface of the strips was rubbed with a wooden stick. To study the effect of angiotensin II (Ang II), the strips were pre-incubated for 90 min, and the buffer solution was replaced every 30 min. The

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10 min incubation was twice performed after the pre-incubation. During the first 10 min incubation, the strips were incubated without the addition of Ang II and in the following 10 min incubation, the strips were incubated with Ang II. Microscopic examination of vessels clearly showed that only endothelial cells from the isolated dog renal arteries are completely removed using this technique.

Measurements of prostaglandin E_2 and I_2 concentrations released from the dog renal arteries by radioimmunoassay: The contents of PGE_2 and PGI_2 (measured as 6-keto-$PGF_{1\alpha}$) released from the strips were measured by the method of Hayashi et al. (7) with slight modifications. Briefly, the incubation medium was adjusted to pH 3.5 and extracted with ethylacetate. The extract was evaporated to dryness and reconstituted to 0.01 M phosphate buffer solution for measuring the PG contents. Each assay mixtures containing extract, anti-PGE_2 or anti-6-keto-$PGF_{1\alpha}$ serum, and [^{3}H]PGE_2 or [^{3}H]6-keto-$PGF_{1\alpha}$ (about 10,000 cpm) were incubated at 37°C for 1 hr. Free and bound [^{3}H]PGE_2 (or [^{3}H]6-keto-$PGF_{1\alpha}$) were separated with the addition of dextran-coated charcoal and the radioactivities of the bound [^{3}H]PGE_2 (or [^{3}H]6-keto-$PGF_{1\alpha}$) were measured in a liquid scintillation counter.

Drugs: Angiotensin II was purchased from Osaka Protein Res., Osaka, Japan. [^{3}H]PGE_2 and [^{3}H]6-keto-$PGF_{1\alpha}$ were purchased from Amercham, Japan. Anti-PGE_2 serum was obtained from Institute Pasteur, Paris, France. Anti-6-keto-$PGF_{1\alpha}$ serum was kindly supplied from Ono Pharmaceutical Co., Ltd., Osaka, Japan.

RESULTS AND DISCUSSION

In the present study, the sources of PGE_2 and PGI_2 in the isolated dog renal arteries under the absence or presence of the vascular endothelial cells were investigated. As shown in Fig. 1, under the presence of vascular endothelial cells (open columns), PGE_2 concentration increased to a maximum level at 90 - 105 min and persisted to 150 - 165 min, while 6-keto-$PGF_{1\alpha}$ concentration enhanced during 0 - 15 min, but then subsided to lower level at 30 - 45 min and persisted to 150 - 165 min. On the other hand, 6-keto-$PGF_{1\alpha}$ production was significantly reduced by the removal of the vascular endothelial cells from renal arteries compared with relative slight reduction of PGE_2 production (dotted columns). Furthermore, the effect of Ang II on PGE_2 and 6-keto-$PGF_{1\alpha}$ productions under the absence or presence of the vascular endothelial cells were also investigated. As shown in Fig. 2, Ang II at the concentration of 10^{-7} M significantly stimulated the both PGE_2 and 6-keto-$PGF_{1\alpha}$ productions in the presence of endothelial cells. However, the stimulation of 6-keto-$PGF_{1\alpha}$ production induced with Ang II was markedly reduced by the removal of the vascular endothelial cells from the renal arteries, while its potentiation of PGE_2 production was not inhibited. The enhancement of 6-keto-$PGF_{1\alpha}$ production induced with bradykinin was also reduced by the removal of the vascular endothelial cells (data not shown). The results suggest that the vascular endothelial cells are the major sources of PGI_2 in the

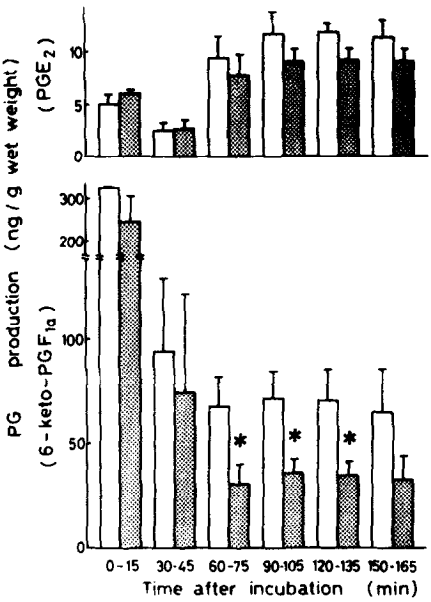

Fig. 1. Time course of PGE_2 and PGI_2 concentrations released from the isolated dog renal arteries under the absence or presence of vascular endothelial cells. The strips were incubated at 37°C for 165 min. The incubation buffer was sampled at every 15 min and measured the concentrations of both PGs. The concentrations of PGE_2 and PGI_2 were measured under the presence (open columns) or absence (dotted columns) of vascular endothelial cells. Significant difference between the presence and absence of the vascular endothelial cells was calucu-lated using Student's t-test. * $p<0.05$. (n=6)

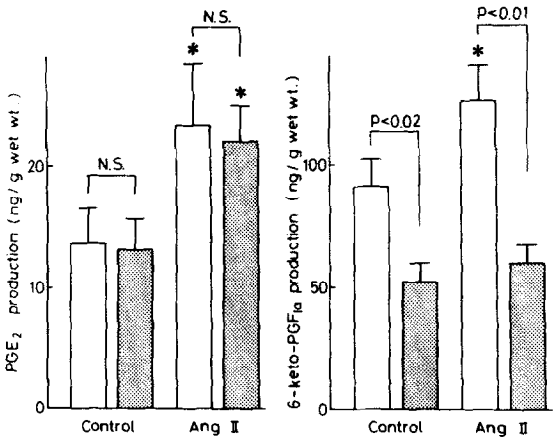

Fig. 2. Effect of angiotensin II (Ang II) on PGE_2 and PGI_2 productions in the isolated dog renal arteries under the absence or presence of vascular endothelial cells. The strips were pre-incubated for 90 min. During the first 10 min incu-bation after the pre-incubation, the strips were incubated without the addition of Ang II and in the following 10 min incubation, the strips were incubated with Ang II (10^{-7} M). The concentrations of PGE_2 and PGI_2 were measured under the presence (open columns) or absence (dotted columns) of the vascular endothelial cells. Significant difference between control and Ang II- treated group ; * $p<0.01$. N.S. indicates not significant. (n=10)

dog renal arteries, although PGI_2 production still occured under the absence of endothelial cells (Fig. 1). It is also extensivly accepted that the vascular endothelial cells are the major sources of PGI_2 in porcine aortic and bovine plumonaly arterial endothelial cells (4 - 6). On the other hand, since the absence of endothelial cells did not influence PGE_2 production (Fig. 1) and the stimulation of PGE_2 production induced with Ang II was not reduced by the removal of endothelial cells (Fig. 2), the other cell types or tissues of vasculatures, perhaps vascular smooth muscle cells may contribute to synthesis of PGE_2.

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REFERENCES

1. Bolger, P.M., Eisner, G.M., Ramwell, P.W., and Slotkoff, L.M. (1978) Nature (London) 271, 467 - 469.
2. Furchgott, R.F., and Zawadzki, J.V. (1980) Nature (London) 288, 373 - 376.
3. Aitura, B.M., and Chand, N. (1981) Br. J. Pharmac. 74, 10 - 11.
4. Cherry, P.D., Furchgott, R.F., Zawadzki, J.V., and Jothianandan, D. (1982) Proc. Natl. Acad. Sci. USA 79, 2106 - 2110.
5. Whorton, A.R., Young, S.L., Data, J.L., Barchowsky, A., and Kent, R.S. (1982) Biochim. Biophys. Acta. 712, 79 - 87.
6. Crutchley, D.J., Ryan, J.W., Ryan, U.S., and Fisher, G.H. (1983) Biochim. Biophys. Acta. 751, 99 - 107.
7. Hayashi, M., Shima, M., Satoh, H., and Satoh, S. (1982) Biochem. Biophys. Res. Commun. 108, 1683 - 1689.