PROSTACYCLIN GENERATION BY CULTURED HUMAN VASCULAR ENDOTHELIAL CELLS WITH REFERENCE TO ANGIOTENSIN I-CONVERTING ENZYME

SHOHEI SAWADA, M.D., TAKEO TOYODA, M.D., HAJIME TAKAMATSU, M.D.
ISAMU NIWA, M.D., NORIHICO MAEBO, M.D., HAJIME TSUJI, M.D.
MASAO NAKAGAWA, M.D., AND HAMAO IITI, M.D.

Prostacyclin (PGI₂) generation has been known to be regulated by several endogenous vasoactive substances, and in this study the relationship between angiotensin I-converting enzyme (ACE) related substances and PGI₂ generation was investigated using cultured human vascular endothelial cells. Addition of angiotensin I (AI) or bradykinin (BK) enhanced PGI₂ generation and increased the level of ACE activity in the culture medium, while the addition of ACE inhibitor (captopril) caused a dose dependent suppression of PGI₂ generation and ACE activity. The enhancement of PGI₂ generation induced by AI or BK was not affected by pretreatment with captopril, and angiotensin II (AII) did not show any effect on either PGI₂ generation or ACE activity. Through these experimental results, the conversion of AI to AII by ACE was considered not to cause the enhancement of PGI₂ generation. Captopril solely inhibited PGI₂ generation and the reported hypothesis that captopril enhances PGI₂ generation by the accumulation of AI or BK via inhibition of ACE was not confirmed in this experimental system. Rather, it is proposed that AI or BK induced PGI₂ generation may be regulated by the increased breakdown of AI or BK, as an autoregulation mechanism, that is derived from increased ACE activity by AI or BK.

PROSTACYCLIN (PGI₂) generation has been known to be regulated by several endogenous mediators and various agents. It is well known that captopril is an inhibitor of angiotensin I-converting enzyme (ACE), and so is effective in reducing blood pressure in hypertensive patients by inhibiting the conversion of angiotensin I (AI) to angiotensin II (AII).

Key Words:
- Prostacyclin
- Angiotensin I-converting enzyme
- Angiotensin I
- Angiotensin II
- Bradykinin

But at the same time captopril lowers blood pressure even in patients with low renin hypertension. There are several reports suggesting that this might originate in its inhibitory effects on ACE activity which result in an increase in kinin levels and in enhanced prostaglandin generation, probably due to activation of phospholipases by bradykinin (BK). Thus there is a possibility that the kinin-prostaglandin system may contribute to the hypotensive action of captopril in low renin hypertension. In this study the relationship between ACE related substances and PGI₂ generation was investigated using cultured human vascular endothelial cells.

(Received August 7, 1985; accepted December 26, 1985)
Second Department of Medicine, Kyoto Prefectural University of Medicine, Kyoto, Japan
Mailing address: Shohei Sawada, M.D., Second Department of Medicine, Kyoto Prefectural University of Medicine, 465 Kajii-cho, Kawaramachi Hirokoji-agaru, Kamigyoku, Kyoto 602, Japan

242 Japanese Circulation Journal Vol. 50, March 1986
MATERIALS AND METHODS

Endothelial cells were obtained from human umbilical cord vein according to the modified method of Jaffe et al. Briefly, the cells were collected by collagenase treatment, and an aliquot of this cell suspension was poured into several plastic 35 mm Petri dishes and incubated at 37°C under 5% CO₂. After 5–7 days, primary cultured cells formed confluent monolayers. The cells were identified as vascular endothelial cells by the presence of Weibel-Palade body in their cytoplasm. Endothelial cells were washed with conditioned buffer (Buffer A: 150 mM

*Japanese Circulation Journal Vol. 30, March 1986*
Fig.3. Effect of bradykinin (BK) and angiotensin I (AI) on ACE activity (a) and PGI₂ generation (b) in medium of cultured human vascular endothelial cells.

Fig.4. Effect of angiotensin II (AII) on ACE activity and PGI₂ generation in medium of cultured human vascular endothelial cells.

NaCl, 5 mM KCl, 1.8 mM CaCl₂, 5 mM Glucose, 1 mM MgCl₂, 10 mM HEPES, pH 7.4) twice, and were prepared for this experimental study by incubation at 37°C with 500 µl of Buffer A containing several agents, with or without pretreatment of the cells by captopril (10⁻⁵ M) for 30 min. The incubated media were employed to investigate the assay of PGI₂ and activity of ACE. The main prostaglandin generated from these cells was PGI₂ by thin layer chromatography from preliminary experiment using ¹⁴C-arachidonic acid.⁴ PGI₂ level was measured as
Fig. 5. Effect of pretreatment with captopril on enhancement of PGI₂ generation induced by angiotensin I (AI) in medium of cultured human vascular endothelial cells.

Fig. 6. Effect of pretreatment with captopril on enhancement of PGI₂ generation induced by bradykinin (BK) in medium of cultured human vascular endothelial cells.

6-keto PGF₁α by radioimmunoassay with New England Nuclear 6-keto PGF₁α[^3H] RIA kit. ACE activity was assayed by Lieberman's method[^4]. Bradykinin triacetate were purchased from Sigma Chemical Co. AI and AII was from Peptide Institute, Inc. Captopril was donated from Sankyo Pharm. Co. Ltd, Japan.

Statistical analysis was performed by Student’s t test. All data are presented as mean ± SE.

[^3H]: Tritium
[^4]: Ref. 2
RESULTS

As shown in Fig. 1a, ACE activity was detected at 15 min after incubation in the buffer solution, its activity gradually increased, it attained a plateau after 30 min, and the activity at this point was $0.90 \pm 0.16$ n mole/ml/min $2 \times 10^5$ cells. Under the same experimental conditions, 6-keto PGF$_{1\alpha}$ level recovered in the medium increased more rapidly than the ACE activity, and attained a plateau after 15 min (the amount of 6-keto PGF$_{1\alpha}$ at this point was $16.11 \pm 0.64$ ng/ml/$2 \times 10^5$ cells, Fig. 1b). These results indicate that the peak time of ACE activity is rather later than that of PGI$_2$ generation. Based on these results, assays of ACE activity and PGI$_2$ generation were performed at 30 min after incubation in the following experiments. Captopril caused a dose dependent decrease of ACE activity and it was inhibited completely at the higher concentration of more than $10^{-5}$M (Fig. 2a). PGI$_2$ generation was also inhibited in a dose dependent manner (Fig. 2b).

Figure 3a & 3b represent the effect of BK, AI and AII on PGI$_2$ generation and ACE activity in the medium. Both BK and AI increased ACE activity (Fig. 3a), and PGI$_2$ generation was increased in a dose dependent manner (Fig. 3b). AII did not show any effect on either of them (Fig. 4). When the cells were pretreated with captopril, PGI$_2$ generation induced by both of Buffer A and AI was decreased remarkably, but the degree of enhancement of PGI$_2$ generation by AI was unchanged in both the presence and absence of pretreatment with captopril (Fig. 5); AII did not show any effect on either the enhancement of PGI$_2$ generation or ACE activity after pretreatment with captopril.

The degree of enhancement of PGI$_2$ generation by BK was also not affected by pretreatment with captopril (Fig. 6).

After the cells were pretreated with captopril ($10^{-5}$M) for 30 min, ACE activity decreased remarkably in the fresh incubated medium without captopril (data not shown).

So the suppressive effect of the ACE activity induced by captopril in our experimental system may not originate from the direct action of captopril to the assay system of the ACE activity.

The results of these experiments are summarized in Fig. 7; PGI$_2$ generation of endothelial cells was stimulated in response to BK and AI, but not to AII, and the peak time of ACE activity was considered to be later than that of PGI$_2$ generation.

DISCUSSION

ACE has been demonstrated to be localized on the luminal surface of the plasma membrane of vascular endothelium. In this report we detected ACE activity in the incubation medium of cultured vascular endothelial cells obtained

*Japanese Circulation Journal Vol. 50, March 1986*
from human umbilical cord vein, and it was confirmed that the endothelial cells produce and release ACE.

The hypothesis that AII stimulates PGI₂ generation was not confirmed in our experimental system. There have been several reports about the effect of AII or AI on PGI₂ generation of vascular tissues. Kono et al. reported that the action of AI was entirely due to the action of AII converted from AII.

Some investigators reported that AII increased the generation of PGI₂ or PGI₂-like substances\(^2\)\(^-\)\(^8\); on the other hand, Ahnenic-Gelas et al., Whorton et al. and Ody et al. reported that AII had no effect on PGI₂ generation by cultured endothelial cells\(^9\)\(^-\)\(^11\). These previous reports and our results do not answer the question of why AII could not increase PGI₂ generation in cultured vascular endothelial cells, and why AI could enhance it.

Recently, Gunther et al. found specific AII receptors on the surface of cultured smooth muscle cells\(^12\). However, Ody et al. reported that they failed to detect specific AII receptors on the surface of cultured endothelial cells, and speculated that AII induced enhancement of PGI₂ generation in whole vessel (containing both endothelial cells and smooth muscle cells) was due to mechanical stimulation elicited by contraction of the underlying smooth muscle cells\(^11\).

Concerning PGI₂ generation induced by captopril, Mullan and Moncada reported that it increased PGI₂ generation\(^2\) and Vio et al. reported that it could not increase PGI₂ generation in its higher concentration\(^13\). Abe et al. reported that PGI₂ generation was enhanced by oral administration of captopril through the enhanced accumulation of BK via inhibition of ACE activity in human subjects\(^1)\(^-\)\(^4\). On the other hand, our experimental results revealed that captopril solely inhibited PGI₂ generation apart from the inhibition of ACE activity, and the degree of enhancement of PGI₂ generation by AI or BK was not affected by pretreatment with captopril. Through these experimental results captopril was not considered to enhance PGI₂ generation by the suspected accumulation of BK or AI via inhibition of ACE activity by captopril, and was rather considered to inhibit PGI₂ generation of endothelial cells directly, and therefore PGI₂ does not play an important role in the hypotensive effect of captopril.

From the results that BK or AI increased PGI₂ generation, which was followed by the release of ACE activity, it can also be speculated that PGI₂ generation may be modulated by the breakdown of BK or AI via increased ACE activity induced by BK or AI, as an autoregulation mechanism.

REFERENCES
8. SATOH H, SATOH S: Prostaglandin E₂ and I₁ production in isolated dog renal arteries in the absence or presence of vascular endothelial cells. Biochem Biophys Res Commun 188: 873, 1984

Japanese Circulation Journal Vol. 50, March 1986