EFFECT OF CYCLIC GMP AND SULFHYDRL ON PROSTACYCLIN PRODUCTION BY HUMAN VASCULAR ENDOTHELIAL CELLS

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The relationship between sulphydryls and cGMP has been observed in several biological processes. Captopril is a sulphydryl-containing angiotensin converting enzyme (ACE) inhibitor, that decreases PG\textsubscript{I}\textsubscript{2} production in cultured human vascular endothelial cells. Enalapril does not appear to have this property. The role of cyclic GMP (cGMP) and sulphydryls in the regulatory mechanisms in captopril-induced PG\textsubscript{I}\textsubscript{2} production and Ca\textsuperscript{++} kinetics was investigated. Bradykinin and Ca ionophore A23187 enhanced PG\textsubscript{I}\textsubscript{2} production, and increased the cytosolic free Ca\textsuperscript{++} concentration ([Ca\textsuperscript{++}]). It was observed that 8-bromo cGMP increased intracellular cGMP concentration ([cGMP]), and decreased PG\textsubscript{I}\textsubscript{2} production without changing [Ca\textsuperscript{++}]. Sulphydryl containing compounds such as captopril. N-acetylcysteine and glutathione decreased PG\textsubscript{I}\textsubscript{2} production via increased [cGMP]. Enalapril, an ACE inhibitor without sulphydryls, has no effect on PG\textsubscript{I}\textsubscript{2} production, [Ca\textsuperscript{++}], and [cGMP]. These results suggested that the presence of sulphydryl groups is an important factor in the ability of vasoactive substances to induce PG\textsubscript{I}\textsubscript{2} production.

Captopril and enalapril are angiotensin I (AI) converting enzyme (ACE) inhibitors that have been used widely as anti-hypertensive drugs, but their precise mechanisms of action are not fully understood. They are thought to reduce blood pressure by lowering plasma angiotensin II (AII) concentration via the inhibition of conversion of AI to AII! It has been additionally postulated that their vasodepressor effect might be due to enhanced prostacyclin (PG\textsubscript{I}\textsubscript{2}) production? It is known that PG\textsubscript{I}\textsubscript{2} production is regulated by many vasoactive substances along with intracellular second messengers such as Ca\textsuperscript{++}, Na\textsuperscript{+}, cyclic nucleotides, diacylglycerol and inositol trisphosphate. It has been reported previously that captopril inhibited PG\textsubscript{I}\textsubscript{2} production although the precise mechanism is unclear? It was also shown that cyclic GMP (cGMP) inhibited PG\textsubscript{I}\textsubscript{2} production by decreasing Ca uptake? Recently Tomera et al suggested that sulphydryl groups played a role in the relaxation of vascular smooth muscle through the accumulation of intracellular cGMP, and captopril is a sulphydryl-containing ACE inhibitor. The possibility is therefore raised that sulphydryl groups affect PG\textsubscript{I}\textsubscript{2} production, and captopril may decrease PG\textsubscript{I}\textsubscript{2} production through an increase in cGMP con-

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TABLE I  EFFECT OF A23187, BRADYKININ(BK), 8-BROMO cGMP AND ANGIOTENSIN II(AII)ON PGI2 PRODUCTION AND 45Ca KINETICS IN CULTURED HUMAN VASCULAR ENDOTHELIAL CELLS

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>A23187 (10^{-8}M)</th>
<th>BK (10^{-5}M)</th>
<th>8-bromo cGMP (10^{-4}M)</th>
<th>AII (10^{-5}M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-keto PGFi2α</td>
<td>16.06±0.63</td>
<td>75.02±2.03*</td>
<td>70.33±0.91*</td>
<td>0.20±0.09*</td>
<td>16.15±0.90</td>
</tr>
<tr>
<td>45Ca uptake</td>
<td>100</td>
<td>144.8±7.2*</td>
<td>147.1±5.7*</td>
<td>75.0±3.1*</td>
<td>97.1±3.7</td>
</tr>
<tr>
<td>45Ca release</td>
<td>100</td>
<td>109.4±2.1*</td>
<td>107.6±1.3*</td>
<td>76.4±3.6*</td>
<td>95.8±4.2</td>
</tr>
</tbody>
</table>

(6-keto PGFi2α:ng/ml/2×10⁵ cells, % change of 45Ca uptake and release, Mean±S.E., n=6, *: p<0.01)

Ca++ kinetics in cultured human vascular endothelial cells was investigated.

METHODS

Endothelial cells were obtained from human umbilical cord veins according to the modified method of Jaffe et al. The primary cultured cells which formed confluent monolayers were used in the experiments. The cells were identified as vascular endothelial cells by detecting Weibel-Palade bodies on electronmicroscopy.

1. Measurement of 45Ca uptake and release
   45Ca uptake and release through the plasma membrane were assayed by the previously reported methods.

2. Measurement of cytosolic free Ca++ concentration
   Cytosolic free Ca++ concentration was measured by the modified method of Gryniewicz.

Fig.1. Effect of A23187 (A), bradykinin (BK) (B), 8-bromo cGMP and angiotensin II (AII) and sulfhydryl (SH)-containing compounds (C) on cytosolic free Ca++ concentration ([Ca++]) in cultured human vascular endothelial cells.
Role of cGMP & Sulphhydrals in PG12 Generation 645

Fig.2. Effect of sulphhydryl (SH)-containing compounds on PG12 generation in cultured human vascular endothelial cells. **: p<0.001, *: p<0.01

TABLE II EFFECT OF SULFHYDRYL(SH)-CONTAINING COMPOUNDS ON THE LEVEL OF INTRACELLULAR CYCLIC NUCLEOTIDES IN CULTURED HUMAN VASCULAR ENDOTHELIAL CELLS

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>8-bromo cGMP (10^-4M)</th>
<th>Glutathione (10^-5M)</th>
<th>N-acetyl cysteine (10^-5M)</th>
<th>Captopril (10^-5M)</th>
<th>Enalapril (10^-5M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>level of cGMP</td>
<td>74.4±9.2</td>
<td>16000&lt; *</td>
<td>97.9±8.5</td>
<td>99.4±9.5</td>
<td>101.9±10.2</td>
<td>69.3±7.9</td>
</tr>
<tr>
<td>(fmol/ml/2×10^6 cells)</td>
<td></td>
<td>97.9±8.5</td>
<td></td>
<td>99.4±9.5</td>
<td>101.9±10.2</td>
<td>69.3±7.9</td>
</tr>
<tr>
<td>level of cAMP</td>
<td>36.9±6.1</td>
<td>33.1±4.5</td>
<td>35.7±5.4</td>
<td>34.3±6.5</td>
<td>37.7±7.3</td>
<td>35.2±6.0</td>
</tr>
<tr>
<td>(fmol/ml/2×10^6 cells)</td>
<td></td>
<td>33.1±4.5</td>
<td></td>
<td>35.7±5.4</td>
<td>34.3±6.5</td>
<td>37.7±7.3</td>
</tr>
</tbody>
</table>

(Mean±SE, n=7, *p<0.01)

CAF-100 (Japan Spectroscopic Co., Ltd.)

3. Assay of prostacyclin concentration
The prostaglandin primarily observed was confirmed as PG_12 by thin layer chromatography with [1-^{14}C]-arachidonic acid. The amount of PG_12 released from endothelial cells was determined by measuring its stable metabolite, 6-keto prostaglandin F_1 alpha, using [3H] 6-keto prostaglandin F_1 alpha assay kit (New England Nuclear).

4. Assay of cyclic nucleotide concentration
After the sampling of the supernatant of the monolayered cells, the cells were rinsed three times with Buffer A (containing 1.8 mM CaCl_2), and 1 ml of 6% trichloroacetic acid added. The cells were scraped from the dishes, collected in test tubes, and sonicated with an ultrasound sonicator (TOMY SEIKO Co., Ltd. UR-200P, 50W, 10 sec). Intracellular cyclic nucleotides were then extracted with water-saturated ether and intracellular cAMP and cGMP concentrations ([cAMP] and [cGMP]) measured using either a [^{125}I] cyclic AMP or cyclic GMP assay kit (YAMASA Co., Ltd.) The cross reactivity of the two assays was less than 1%.

5. Reagents
Bovine serum albumin (Sigma fraction V), fura-2 acetoxy methoxy pentaester (fura-2/AM, Calbiochem.), Ca ionophore A23187 (A23187, Calbiochem.), 8-bromo cGMP (Sigma), bradykinin (BK, Sigma Chemical Co.), angiotensin II (All, Peptide Institute,
Inc.), glutathione (Nacalai Tesque Inc.), N-acetylcysteine (Nacalai Tesque Inc.), captopril (Sankyo Pharm. Co., Ltd.), and enalapril (Merk Bannyu Pharm. Co., Ltd.) were used in this experiment.

6. Statistical analysis

Statistical analysis was performed by Student's t test. The percent change was compared by Wilcoxon parametric test. A p value less than 0.05 was considered to be significant. All data are presented as mean ± SE.

RESULTS

1) The [Ca++]i, 45Ca uptake and PG12 production increased rapidly following incubation, and reached a plateau within 5 min (30.16 ± 3.04 nM in [Ca++]i, and 21.91 ± 3.08 ng/ml/2 x 10^5 cells in PG12 production). It was observed that 45Ca uptake and release reached an equilibrium after 10 min from the start of incubation. Therefore, measurements of PG12 production and 45Ca uptake and release were performed at 15 min after incubation.

2) Both BK and A23187 enhanced PG12 production, 45Ca uptake and release (Table I) and [Ca++]i, (Fig. 1). The [Ca++]i enhancement caused by BK was transient, that by A23187 was sequential. This suggests BK and A23187 increased PG12 production via an increase of [Ca++]i. All appeared to have no effect on these parameters (Table I, Fig. 1).

3) Captopril, a sulphydryl-containing ACE inhibitor, decreased PG12 production (Fig. 2), increased [cGMP]i, (Table II) and decreased 45Ca uptake (Table III), but had no effect on [Ca++]i and [cAMP]i (Fig. 1, Table II). These results suggest that captopril decreases PG12 production with no change in [Ca++]i, and an increase in [cGMP]i might lead to an inhibition of PG12 production.

4) Endothelial cells were incubated in Buffer A with 8-bromo cGMP to investigate whether increases of [cGMP]i inhibited PG12 production. It was observed that 8-bromo cGMP increased [cGMP]i, (Table II) and decreased both PG12 production and 45Ca uptake and release (Table I) without any change in [Ca++]i, or [cAMP]i, (Fig. 1).

5) Glutathione and N-acetylcysteine, which are sulphydryl-containing compounds, without any ACE-inhibiting properties, increased [cGMP]i, (Table II) and decreased PG12 production (Fig. 2) without any change in [Ca++]i, or [cAMP]i, (Fig. 1, Table II). Sulphydryl-containing compounds inhibited PG12 production via increased [cGMP]i.

6) Enalapril, a non sulphydryl-containing ACE-inhibitor, had no effect on any of the parameters (Figs. 1, 2, Table II, III).

DISCUSSION

The role of the sulphydryl group in the PG12 production in cultured human vascular endothelial cells was investigated with specific attention to Ca++ kinetics and cyclic nucleotides. The effect of the sulphydryl-containing ACE inhibitor captopril on PG12 production and Ca++ kinetics was compared with that of the non sulphydryl-containing ACE inhibitor enalapril along with glutathione and N-acetylcysteine which contain sulphydryl, but have no ACE-inhibiting properties. The results suggest that sulphydryl-containing compounds such as captopril, glutathione and N-acetylcysteine decreased PG12 production by increasing [cGMP]i. The non sulphydryl-containing
ACE inhibitor enalapril had no effect on either PGI$_2$ production or [cGMP]. This suggests that sulfhydryl-containing compounds decrease PGI$_2$ production by increasing [cGMP].

Adunyah and Dean$^{10}$ reported that sulfhydryl played a role in Ca$^{++}$ release. It was observed that sulfhydryl played an important role in Ca$^{++}$ release in the human platelet sarcoplasmic reticulum. Tomera$^5$ et al reported that sulfhydryl groups had a regulatory role in the contraction and relaxation of vascular smooth muscle cells through control of $^{45}$Ca$^{++}$ kinetics and cyclic nucleotide metabolism.

This study investigated the function of sulfhydryl groups in the Ca$^{++}$ kinetics and [Ca$^{++}$]. Captopril decreased $^{45}$Ca uptake and release, while glutathione, N-acetylcysteine and enalapril had no effect. These three substances did not affect [Ca$^{++}$], either. A decrease of $^{45}$Ca uptake or an increase of $^{45}$Ca release does not necessarily result in a decrease of [Ca$^{++}$]. It was demonstrated that captopril decreased $^{45}$Ca uptake, but did not affect [Ca$^{++}$], and that Ca ionophore A23187 or bradykinin increased both $^{45}$Ca uptake and release while increasing [Ca$^{++}$]. This suggests that changes in [Ca$^{++}$]$_i$ are of primary importance in the effects of agents on Ca$^{++}$ kinetics. These findings support the hypothesis that the inhibitory effects of SH-containing compounds on PGI$_2$ production in vascular endothelial cells are governed by [cGMP]-dependent mechanisms.

While captopril, glutathione and N-acetylcysteine contain the same amount of sulfhydryl per mole, and they increase [cGMP], almost equally, there were differences in the magnitude of the decrease in PGI$_2$ production observed. It may be that captopril inhibits PGI$_2$ production via not only increased [cGMP], but also by other, unknown mechanisms.

Sulfhydryl-containing compounds such as captopril, glutathione and N-acetylcysteine increased [cGMP], and decreased PGI$_2$ production without changing [Ca$^{++}$]. Enalapril, a non sulfhydryl-containing ACE inhibitor, failed to cause any significant changes in these parameters. The presence of sulfhydryl groups in vasoactive substances appears to have great significance in their ability to inhibit PGI$_2$ production.

REFERENCES