Cellular Mechanism of Endothelin-1 Release by Angiotensin and Vasopressin

Toshiaki Emori, Yukio Hirata, Kazuki Ohta, Kazuo Kanno, Satoru Eguchi, Taihei Imai, Masayoshi Shichiri, and Fumiaki Marumo

Release of endothelin-1, a novel potent vasoconstrictor peptide originally isolated from endothelial cells, from cultured bovine endothelial cells has been shown to be stimulated by arginine vasopressin and angiotensin II. To elucidate the cellular mechanism by which endothelin-1 is released by these vasoconstrictors, we tested the effects of several compounds on the agonist-induced endothelin-1 release and studied the changes of cytosolic free Ca\(^{2+}\) concentrations and phosphoinositide breakdown by these agonists in cultured bovine endothelial cells. Protein kinase C inhibitors (H-7, staurosporine), an intracellular Ca\(^{2+}\) chelator, and an inhibitor of phospholipase C (neomycin), all abolished the agonist-induced endothelin-1 release, whereas the Ca\(^{2+}\) channel blocker nicardipine was ineffective. Although synthetic 1,2-diacylglycerol (diolein) dose dependently stimulated endothelin-1 release, downregulation of protein kinase C after pretreatment with phorbol ester resulted in decreased effects to increase endothelin-1 release by the agonists. Both arginine vasopressin and angiotensin II induced immediate and transient increases in intracellular Ca\(^{2+}\) levels of fura-2-loaded endothelial cells as well as formation of inositol trisphosphate; the agonist-induced intracellular Ca\(^{2+}\) increases were not affected either by nicardipine or by chelating extracellular Ca\(^{2+}\). The arginine vasopressin- and angiotensin II–induced intracellular Ca\(^{2+}\) increases, inositol trisphosphate formation, and endothelin-1 release were completely abolished by V\(_{1}\)-receptor antagonist and saralasin, respectively. It is concluded that arginine vasopressin and angiotensin II stimulate the release of endothelin-1 by a common mechanism, involving receptor-mediated mobilization of intracellular Ca\(^{2+}\) and activation of protein kinase C in endothelial cells. (Hypertension 1991;18:165–170)

Endothelin (ET)-1, a novel vasoconstrictor peptide with 21-amino acid residues, was originally isolated and sequenced from the supernatant of cultured porcine endothelial cells (ECs).\(^1\) ET-1 has not only strong and sustained vasoconstrictive action on a variety of blood vessels from various species\(^1\)\(^,\)\(^2\) but also a wide spectrum of pharmacological effects such as release of eicosanoids and endothelium-derived relaxing factor from perfused vascular beds,\(^3\) aldosterone from cultured calf adrenal zona glomerulosa cells,\(^4\) atrial natriuretic peptide from cultured rat atrial myocytes,\(^5\) and mitogenic action in cultured vascular smooth muscle cells.\(^6\) These effects are dependent on extracellular Ca\(^{2+}\) and inhibited by Ca\(^{2+}\) channel antagonists, suggesting the importance of Ca\(^{2+}\) influx in the mechanism of its actions.\(^7\)

Supported in part by Grants-in-Aid from the Ministry of Education, Science and Culture (03454512, 02304055), by the Ministry of Health and Welfare, Japan, and by a fund from Uehara Memorial Foundation, Tokyo.

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Received July 17, 1990; accepted in revised form April 19, 1991.

The induction of preproET-1 messenger RNA (mRNA) in porcine EC is augmented by several agents, such as epinephrine, thrombin, Ca\(^{2+}\) ionophore,\(^1\) phorbol ester,\(^8\) transforming growth factor (TGF)-\(\beta\),\(^9\) cytokines,\(^10\) and shear stress.\(^11\) Our recent studies have shown that two vasoconstrictive hor- mones, arginine vasopressin (AVP) and angiotensin (Ang) II, also stimulate the release of immunoreactive ET-1 from cultured bovine ECs, and these effects are mimicked by phorbol ester and Ca\(^{2+}\) ionophore.\(^12\) These findings raise the possibility that the AVP- and Ang II–induced ET-1 release from EC may involve a common mechanism (i.e., phosphoinositide breakdown with the resultant mobilization of intracellular Ca\(^{2+}\) and activation of protein kinase C [PKC]). To address this question, the present study was designed to elucidate the cellular mechanism of AVP and Ang II responsible for ET-1 release in bovine EC.

Methods

Cell Culture and Incubation

ECs from bovine carotid artery were prepared by digestion with collagenase and elastase,\(^13\) and cul-
tured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum and antibiotics (penicillin, 100 units/ml; streptomycin, 100 μg/ml) at 37°C in a humidified atmosphere of 95% air–5% CO₂. The cells were identified as EC by the contact-inhibited monolayer growth with a "cobblestone" appearance and the presence of Factor VIII antigen by immunocytochemical study.13 After serial subculture by 0.05% trypsin–0.02% ethylenediaminetetra-acetic acid (EDTA), ECs harvested between the 15th and 24th passage were used in the experiments. To study the release of ET-1, confluent cells (≈5×10⁶) were replaced with 1 ml fresh serum-free DMEM and incubated at 37°C for 4 hours with or without the following agents: AVP, Ang II, [Sar¹, Ala⁶]Ang II (saralasin, Peptide Institute, Osaka, Japan), [1-(β-mercapto-β-cyclopentamethylene propionic acid), 2-O-methyltyrosine] (POMT)-AVP (Peninsula Laboratories, Belmont, Calif.), 1-5-isooquinolinolsulfonyl]-2-methylpiperezine (H-7), 1,2-bis(2-aminophenoxy)ethane N,N',N'-tetra-acetic acid (BAPTA), tetraacetoxymethyl ester (Dojin Chemicals, Kumamoto, Japan), staurosporine (Kyowa Medex Co., Ltd., Tokyo), neomycin, diolein, 12-O-tetradecanoylphorbol 13-acetate (TPA) (Sigma Chemical Co., St. Louis, Mo.). Dioline and TPA were stocked in dimethyl sulfoxide and diluted with DMEM before use.

Radioimmunoassay

ET-1-like immunoreactivity in medium was measured by a specific radioimmunoassay for ET-1 using rabbit anti-ET-1 serum as previously described.12 The antibody has full cross-reactivity with ET-1 (100%), ET-2 (200%), and ET-3 (100%) but none with big ET-1 (1–39), AVP, Ang II, or other unrelated peptide hormones. In brief, 0.2 ml standard or sample and 0.1 ml antibody (final dilution, 1:12,000) were preincubated at 4°C for 24 hours, followed by the addition of 0.1 ml [¹²⁵I]ET-1 (specific activity: ~74 TBq/mmol, Amersham Japan, Tokyo) and further incubation for 24 hours. The bound ligands were separated from the free ones by the double antibody method. The sensitivity of ET-1 RIA was 1 fmol/tube, and the 50% intercept was 14 fmol/tube. The coefficients of intraassay and interassay variations were 3.2% (n=6) and 8.6% (n=5), respectively.

Determination of Cytosolic Free Ca²⁺ Concentration

Measurement of cytosolic free Ca²⁺ concentration ([Ca²⁺]) was performed by Ca²⁺ fura-2 fluorescence method.2 After incubation in serum-free DMEM for 24 hours, ECs were trypsinized and incubated with 4 μM fura-2 acetoxymethyl ester (Dojin Chemicals) at 37°C for 20 minutes in N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid] (HEPES)–buffered physiological salt solution essentially as previously reported.7 The Ca²⁺ fura-2 fluorescence of the suspended cells (∼5×10⁶ cells/ml) was measured by a spectrofluorimeter (CAF-100, JASCO Co., Ltd., Tokyo) using excitation of 340 and 380 nm and emission of 500 nm. Values of [Ca²⁺] were determined according to the method of Grynkiewicz et al.14 The equation for calculating [Ca²⁺], is

\[
[\text{Ca}^{2+}] (\text{nM}) = K_d \times \left(\frac{R - R_{\text{min}}}{R_{\text{max}} - R}\right) \times \beta
\]

where \(K_d\) is the dissociation constant of fura-2 for Ca²⁺ at 37°C (224 nM), \(R\) represents the ratio of fluorescence of the sample at 340 and 380 nm, \(R_{\text{max}}\) and \(R_{\text{min}}\) are the ratios for fura-2 free acid at the same wavelengths in the presence of saturating Ca²⁺ and nominally zero Ca²⁺, obtained by adding Triton-X and EGTA, respectively, and \(\beta\) is the ratio of fluorescence of fura-2 at 380 nm in zero and saturating Ca²⁺. The effects of the Ca²⁺ channel blocker nicardipine (Yamanouchi Pharmaceutical Co., Ltd., Tokyo) and ethylene glycol-bis(β-aminoethyl ether) N,N',N'-tetra-acetic acid (EGTA) (Dojin Chemicals) on the agonist-induced [Ca²⁺] changes were studied.

**Determination of Inositol 1,4,5-Trisphosphate**

Confluent ECs were incubated at 37°C in Hanks' medium containing 10 mM LiCl for indicated times. Incubation was terminated by the rapid removal of medium and the addition of ice-cold 15% trichloroacetic acid (TCA), and the cells were placed on ice for 30 minutes. The TCA extract was washed with ethylether and neutralized with 1N sodium acetate. Inositol 1,4,5-trisphosphate (IP₃) levels were then determined by a competitive protein binding assay kit (Amersham Japan).

**Determination of Doses for Half-Maximal Stimulation**

The approximate values of half-maximal stimulation (EC₅₀) for the agonist-stimulated [Ca²⁺], increase, IP₃ formation, and ET-1-like immunoreactivity release were determined for each of the dose-response curves.

**Statistical Analysis**

Data are expressed as mean±SEM. Statistical analysis was accomplished by unpaired Student's t test.

**Results**

As shown in Tables 1 and 2, both AVP and Ang II significantly \(p<0.01\) stimulated ET-1-like immunoreactivity release from bovine cultured EC. The stimulatory effects by both agonists were completely abolished by their receptor antagonists, POMT-AVP and saralasin, respectively (Table 1). The agonist-induced ET-1-like immunoreactivity release was completely inhibited by the PKC inhibitor H-7 but not by the Ca²⁺ channel blocker nicardipine. H-7 dose dependently inhibited ET-1-like immunoreactivity release stimulated by Ang II and AVP with the approximate half-maximal inhibitory dose of 5×10⁻⁸ M (Figure 1). Furthermore, staurosporine, another PKC inhibitor; neomycin, a phospholipase C (PLC) inhibitor; and BAPTA, an intracellular Ca²⁺ chelator, all abolished ET-1-like immunoreactivity release induced by AVP and Ang II (Table 2). H-7
**Table 1. Effects of Arginine Vasopressin and Angiotensin II on Endothelin-1 Release From Cultured Bovine Endothelial Cells**

<table>
<thead>
<tr>
<th>Drugs (concentration)</th>
<th>Endothelin-1 released (fmol/4 hr/10^3 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>19.5±3.7</td>
</tr>
<tr>
<td>H-7 (10^-6 M)</td>
<td>16.1±8.4</td>
</tr>
<tr>
<td>Nicardipine (10^-5 M)</td>
<td>16.2±6.8</td>
</tr>
<tr>
<td>AVP (10^-7 M)</td>
<td>69.0±10.4*</td>
</tr>
<tr>
<td>POMT-AVP (10^-6 M)</td>
<td>21.3±1.5</td>
</tr>
<tr>
<td>AVP (10^-7 M)+POMT-AVP (10^-6 M)</td>
<td>21.5±3.1</td>
</tr>
<tr>
<td>AVP (10^-7 M)+H-7 (10^-4 M)</td>
<td>16.2±4.2</td>
</tr>
<tr>
<td>AVP (10^-7 M)+nicardipine (10^-5 M)</td>
<td>79.4±23.7*</td>
</tr>
<tr>
<td>Ang II (10^-7 M)</td>
<td>84.2±19.6*</td>
</tr>
<tr>
<td>Saralasin (10^-4 M)</td>
<td>13.1±3.2</td>
</tr>
<tr>
<td>Ang II (10^-7 M)+saralasin (10^-4 M)</td>
<td>18.4±2.2</td>
</tr>
<tr>
<td>Ang II (10^-7 M)+H-7 (10^-4 M)</td>
<td>17.7±3.9</td>
</tr>
<tr>
<td>Ang II (10^-7 M)+nicardipine (10^-5 M)</td>
<td>81.4±24.5*</td>
</tr>
</tbody>
</table>

Confluent bovine cultured endothelial cells were incubated without and with angiotensin (Ang II), arginine vasopressin (AVP), saralasin, [1-β-mercapto-β,β-cyclopentamethylene propionic acid], 2-O-methylthymosine-AVP (POMT-AVP), H-7, and nicardipine. Each value is mean±SEM (n=6). H-7, 1-(5-isoquinolinylsulfonyl)-2-methylpiperazine; saralasin, [Sar^1, Ala^8]Ang II.

*Statistically significant difference from control (p<0.01).

**Table 2. Effects of Staurosporine, BAPTA, and Neomycin on Arginine Vasopressin- and Angiotensin II-Induced Endothelin-1 Release From Cultured Bovine Endothelial Cells**

<table>
<thead>
<tr>
<th>Drugs (concentration)</th>
<th>Endothelin-1 released (fmol/4 hr/10^3 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>29.2±2.6</td>
</tr>
<tr>
<td>Staurosporine (2×10^-8 M)</td>
<td>31.3±4.6</td>
</tr>
<tr>
<td>BAPTA-AM (10^-4 M)</td>
<td>26.7±2.8</td>
</tr>
<tr>
<td>Neomycin (10^-4 M)</td>
<td>26.5±1.6</td>
</tr>
<tr>
<td>AVP (10^-7 M)</td>
<td>70.4±6.0*</td>
</tr>
<tr>
<td>AVP (10^-7 M)+staurosporine (2×10^-8 M)</td>
<td>27.3±3.6</td>
</tr>
<tr>
<td>AVP (10^-7 M)+BAPTA-AM (10^-4 M)</td>
<td>28.0±4.6</td>
</tr>
<tr>
<td>AVP (10^-7 M)+neomycin (10^-4 M)</td>
<td>28.3±2.4</td>
</tr>
<tr>
<td>Ang II (10^-7 M)</td>
<td>69.4±7.3*</td>
</tr>
<tr>
<td>Ang II (10^-7 M)+staurosporine (2×10^-8 M)</td>
<td>31.7±4.0</td>
</tr>
<tr>
<td>Ang II (10^-7 M)+BAPTA-AM (10^-4 M)</td>
<td>27.2±3.9</td>
</tr>
<tr>
<td>Ang II (10^-7 M)+neomycin (10^-4 M)</td>
<td>27.4±1.9</td>
</tr>
</tbody>
</table>

Confluent bovine cultured endothelial cells were incubated without and with angiotensin (ANG) II, arginine vasopressin (AVP), staurosporine, 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid-tetraacetoxymethyl ester (BAPTA-AM), and neomycin. Each value is mean±SEM (n=6).

*Statistically significant difference from control (p<0.01).

**Figure 1.** Line graph shows inhibitory effects of H-7 on arginine vasopressin (AVP) and angiotensin (Ang) II-induced endothelin (ET)-1 release in cultured bovine endothelial cells (ECs). Confluent ECs were incubated without (●) and with (●) 10^-7 M AVP and 10^-7 M Ang II (○) in the absence and presence of H-7 in various doses (10^-4 to 10^-3 M) for 4 hours. Each point is mean of six experiments; bars show SEM. *Statistically significant difference from the stimulated cells in the absence of H-7 (p<0.05).

**Figure 2.** Line graph shows dose-responsive effect of diolein on endothelin (ET)-1 release in cultured bovine endothelial cells (ECs). Confluent ECs were incubated with various doses (10–100 μg/ml) of diolein for 4 hours. Each point is mean of six experiments; bars show SEM. *Statistically significant difference from the unstimulated control cells (p<0.05).

and staurosporine prevented TPA- but not ionomycin-induced ET-1-like immunoreactivity release, whereas BAPTA did not block TPA-induced ET-1-like immunoreactivity release (data not shown), suggesting the specificity of the inhibitors used in the experiments.

As shown in Figure 2, diolein, a synthetic 1,2-diglyceride, dose dependently (25–100 μg/ml) stimulated ET-1-like immunoreactivity release in a similar fashion as did TPA. To determine whether PKC activation is required for ET-1-like immunoreactivity release by both agonists, ECs were pretreated with 10^-7 M TPA for 24 hours to downregulate PKC activity. As shown in Figure 3, AVP and Ang II caused far less stimulatory effects on ET-1-like
FIGURE 3. Bar graph shows effect of downregulation of protein kinase C on arginine vasopressin (AVP)– and angiotensin (Ang) II–induced endothelin (ET)-1 release in cultured bovine endothelial cells (ECs). After pretreatment with (striped bars) or without (open bars) 10^{-7} M TPA for 24 hours, ECs were incubated with 10^{-7} M AVP and 10^{-7} M Ang II for 4 hours. Each point is the mean of six experiments; bars show SEM. *Statistically significant difference from the unstimulated control cells (p<0.05). Statistical significances between TPA-treated and nontreated cells are indicated at top: NS, not significant (p>0.05).

immunoreactivity release in TPA-treated cells than those of nontreated cells, whereas basal release of ET-1-like immunoreactivity did not differ between TPA-treated and nontreated cells.

As shown in Figure 4, AVP and Ang II induced immediate increases in [Ca^{2+}], that peaked at 1–1.5 minutes and returned to basal levels after 3 minutes. The increments of [Ca^{2+}], by AVP and Ang II were completely abolished by pretreatment with POMT-AVP and saralasin, respectively. In contrast, pretreatment with either nicardipine or EGTA had no significant effect on the agonist induced [Ca^{2+}] increments.

Both AVP and Ang II induced immediate (within 15 seconds) IP₃ formation with maximal effects by 30 seconds (Figure 5). The effects of AVP and Ang II on IP₃ formation were completely abolished by pretreatment with POMT-AVP and saralasin, respectively (Table 3).

The dose-responsive effects by AVP and Ang II on [Ca^{2+}], increases, IP₃ formations, and ET-1-like

![Figure 4](http://hyper.ahajournals.org/)

**TABLE 3.** Effects of Arginine Vasopressin and Angiotensin II on Formation of Inositol Trisphosphate in Cultured Bovine Endothelial Cells

<table>
<thead>
<tr>
<th>Drugs (concentration)</th>
<th>Inositol 1,4,5-trisphosphate (pmol/30 sec×10³ cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10.8±4.2</td>
</tr>
<tr>
<td>AVP (10^{-6} M)</td>
<td>26.5±6.2*</td>
</tr>
<tr>
<td>POMT-AVP (10^{-3} M)</td>
<td>9.7±5.1</td>
</tr>
<tr>
<td>AVP (10^{-6} M)+POMT-AVP (10^{-3} M)</td>
<td>11.4±8.2</td>
</tr>
<tr>
<td>Ang II (10^{-7} M)</td>
<td>19.2±3.0*</td>
</tr>
<tr>
<td>Saralasin (10^{-6} M)</td>
<td>9.9±2.1</td>
</tr>
<tr>
<td>Ang II (10^{-7} M)+saralasin (10^{-6} M)</td>
<td>11.8±6.5</td>
</tr>
</tbody>
</table>

Confluent bovine cultured endothelial cells were incubated without and with angiotensin (Ang) II, arginine vasopressin (AVP), saralasin, and [1-β-mercapto-β,β-cyclopentamethylene propionic acid], 2-O-methyltyrosine]-AVP (POMT-AVP) in medium containing 10 mM LiCl for 30 seconds. Each value is the mean±SEM (n=3).

*Statistically significant difference from control (p<0.01).
immunoreactivity releases were studied (Figure 6). Both AVP ($10^{-7}$ to $10^{-5}$ M) and Ang II ($10^{-8}$ to $10^{-6}$ M) dose dependently stimulated increases in $[Ca^{2+}]_i$ (Figure 6A), increases in IP$_3$ release (Figure 6B), and release of ET-1-like immunoreactivity (Figure 6C). Except for the less maximal increment of $[Ca^{2+}]_i$ by AVP compared with that by Ang II, the approximate

**Discussion**

ET-1 was originally identified as a 21-amino acid residue peptide from the supernatant of cultured porcine ECs.$^1$ It has been suggested that ET-1 is synthesized as a precursor with 203 amino acid residues that is initially processed to yield an intermediate form (big ET-1) with 38 (human) or 39 (porcine) amino acid residues and finally processed to mature ET-1. It has been shown that the expression of mRNA for ET-1 precursor is induced by several local mediators, such as thrombin, TGF-$\beta$, cytokines, and shear stress.$^{11}$

We have recently shown that endogenous vasoconstrictor hormones (AVP and Ang II) are potent secretagogues for ET-1-like immunoreactivity in bovine EC.$^{12}$ Furthermore, the PKC activator TPA and the Ca$^{2+}$ ionophore ionomycin have been shown to stimulate ET-1-like immunoreactivity release and both compounds had synergistic effects.$^{12}$ Because both AVP and Ang II have been shown to induce PLC-mediated phosphoinositide breakdown in vascular smooth muscle cells,$^{15}$ it is possible that these agonists may also induce PLC-mediated phosphoinositide breakdown via their specific receptors in EC. The present study clearly showed that the effects of AVP and Ang II on ET-1-like immunoreactivity release from cultured bovine ECs are exerted by PLC-mediated phosphoinositide breakdown via their specific receptors because their effects were completely abolished by pretreatment with PLC inhibitor neomycin as well as V$_1$-receptor antagonist and saralasin, respectively.

It is well recognized that the Ca$^{2+}$ messenger system consists of two branches, one mediated by Ca$^{2+}$ influx through receptor-operated or voltage-dependent Ca$^{2+}$ channels and the other by a PLC-mediated phosphoinositide breakdown, thereby generating diacylglycerol (DG), which activates PKC, and IP$_3$, which mobilizes Ca$^{2+}$ from intracellular store sites; both DG-PKC and IP$_3$-Ca$^{2+}$ limbs of the phosphoinositide-PLC signal transduction system interact synergistically to induce full biologic responses.$^{16}$ Our results that neither removal of extracellular Ca$^{2+}$ by EGTA nor inhibition of Ca$^{2+}$ influx by nicardipine affected $[Ca^{2+}]_i$, increases by AVP and Ang II suggest that the Ca$^{2+}$ messenger system by both agonists does not involve the former branch (i.e., Ca$^{2+}$ influx through Ca$^{2+}$ channels) in EC.

In the present study, both PKC inhibitors (H-7, staurosporine) and the PLC inhibitor neomycin showed potent inhibitory effect on the AVP- and Ang II-induced ET-1-like immunoreactivity release, and synthetic 1,2-diglyceride (diolein) had a dose-responsive effect on ET-1-like immunoreactivity release. Although we have not directly determined DG con-
tents in the present study, both AVP and Ang II induced immediate and dose-responsive production of IP₃, a hydrolysis product of phosphoinositol 4,5-bisphosphate (PIP₂), suggesting that another hydrolysis product DG from PIP₂ should be formed simultaneously. Taken together, our data strongly suggest that activation of PKC by endogenous and exogenous DG is involved in the mechanism of ET-1 release. Furthermore, downregulation of PKC by pretreatment with TPA attenuated the stimulatory effects by AVP and Ang II, suggesting that PKC activation resulting from the agonist-induced endogenous DG formation is responsible for ET-1-like immunoreactivity release. Since TPA-responsive elements are located in the S' noncoding region of the structural gene of preproET-1, it is possible to postulate that the activation of PKC resulting from phosphoinositide breakdown may be functionally linked to the activation of these transcription factors, thereby affecting the induction of the preproET-1 gene.

In the present study, both the PLC inhibitor neomycin and the intracellular Ca²⁺ chelator BAPTA showed inhibitory effects on the AVP- and Ang II-induced ET-1-like immunoreactivity release, whereas the Ca²⁺ channel blocker nicardipine failed to affect ET-1-like immunoreactivity release. Our data suggest that the IP₃-Ca²⁺ limb, in addition to the DG-PKC limb, of the signal transduction system also plays an important role in the mechanism of ET-1-like immunoreactivity release by AVP and Ang II. The present study further revealed that the dose-response curves for AVP- and Ang II-stimulated ET-1-like immunoreactivity release were almost comparable with those for intracellular Ca²⁺ release and IP₃ formation, although Ang II was apparently one order of magnitude greater than AVP. Thus, our data lend further support to the contention that receptor-mediated release of ET-1-like immunoreactivity is closely linked to increases in [Ca²⁺]ᵢ, as well as IP₃ (and presumably DG) formation.

There has been a growing awareness that vascular endothelial monolayer is capable of intrinsic modulation of the vascular tone by elaborating vasoactive substances, including vasodilators (endothelium-derived relaxing factor and prostacyclin) and vasoconstrictors (ET-1 and thromboxane A₂). From the present study, it appears that there exists a close interrelation between endogenous vasoconstrictor hormones and endothelium-derived local vasoconstrictor ET-1 to regulate vascular tonus and regional blood flow. The pathophysiological significance of endogenous ET-1 in the setting of elevated circulating AVP and Ang II, such as in excessive AVP secretion and renovascular hypertension, should be studied.

In conclusion, the present data show that endogenous vasoconstrictor hormones (AVP and Ang II) are involved in the mechanism of ET-1-like immunoreactivity release through activation of PKC and mobilization of intracellular Ca²⁺ resulting from the common receptor-mediated phosphoinositide breakdown in EC.

References


Keywords • endothelium • endothelin • arginine vasopressin • angiotensin II • protein kinase C • calcium
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Hypertension. 1991;18:165-170
doi: 10.1161/01.HYP.18.2.165

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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