Endothelin Production in Cultured Vascular Smooth Muscle Cells—Modulation by the Atrial, Brain, and C-Type Natriuretic Peptide System

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We examined the regulatory mechanisms of endothelin-1 (ET-1) production in cultured rat vascular smooth muscle cells (VSMC) with a special focus on the roles of protein kinase C (PKC)- and cyclic guanosine-3′,5′-monophosphate (GMP)-mediated signaling systems. Effects of atrial, brain, and C-type natriuretic peptides (ANP, BNP, and CNP) on angiotensin II (Ang II)-, and arginine vasopressin (AVP)-induced production of ET-1 were examined in cultured rat aortic VSMC. Ang II and AVP stimulated ET-1 production in a concentration-dependent manner through angiotensin subtype 1 (AT_1) and vasopressin subtype 1 (V_1) receptors, respectively. The stimulatory effects of Ang II and AVP were markedly abolished in PKC-depleted cells. Rat ANP (1-28), rat BNP-45, and rat CNP-22 potently inhibited Ang II- and AVP-stimulated ET-1 production in a concentration-dependent manner, respectively. The inhibitory effect by CNP on ET-1 production was paralleled by an increase in the cellular level of cyclic GMP. 8-Bromo cyclic GMP reduced the stimulated ET-1 production by Ang II and AVP. These results indicate that Ang II and AVP stimulate ET-1 production in cultured rat VSMC through AT_1 and V_1 receptors by a mechanism probably involving activation of PKC, and that ANP, BNP, and CNP inhibit this stimulated production through a cyclic GMP–dependent process.

ENDOTHELIN-1 (ET-1) is a vasoconstrictive and growth-promoting peptide of 21 amino acids that was first isolated from porcine vascular endothelial cells and proposed to function in vivo as a paracrine regulator of adjacent vascular smooth muscle cells (VSMC).[1] It is now evident that some nonendothelial cells including VSMC[2-4] and glomerular mesangial cells[5-7] can also synthesize and secrete ET-1. Expression of prepro ET-1 transcripts and peptide synthesis in endothelial and nonendothelial cells are increased in response to some growth factors and/or vasoactive hormones.[2,3,6-9]

Atrial natriuretic peptide (ANP) was originally isolated from mammalian hearts.[10,11] A second type of natriuretic peptide was then identified in the porcine brain, designated brain natriuretic peptide (BNP),[12] it has subsequently been isolated from mammalian hearts.[13] These cardiac natriuretic peptides inhibit basal or angiotensin II (Ang II)- and arginine vasopressin (AVP)-induced ET-1 production in cultured vascular endothelial cells[14,15] and glomerular mesangial cells.[7,16]

In the current study, we confirmed previous reports that Ang II and AVP stimulate ET-1 production in cultured rat VSMC[2,3] and examined which receptor subtypes of angiotensin and vasopressin mediate ET-1 production in these cells. Next, we examined whether ANP and BNP inhibit basal or Ang II- and AVP-stimulated production of ET-1 in cultured rat VSMC. In addition, the effect of a third type of natriuretic peptide, designated C-type natriuretic peptide (CNP),[17] on ET-1 production was also examined in these cells.

MATERIALS AND METHODS

Materials

Ang II, saralasin (Sar^1-Val^5-Ala^8), AVP, [1-β-mercapto-β, β-cyclopentamethylene propion acid, 2-(*O*-methyl) tyrosine] arginine vasopressin (PMP), phorbol myristate acetate (PMA), 3-isobutyl-1-methylxanthine (IBMX), and 8-bromo-guanosine-3′,5′-cyclic monophosphate (8-bromo cyclic GMP) were purchased from Sigma (St Louis, MO). Dulbecco's modified Eagle's medium (DMEM), trypsin, Versene, fetal calf serum (FCS), and a specific protein kinase C (PKC) inhibitor, PKC inhibitor peptide, PKC-(19-36), were purchased from Calbiochem (La Jolla, CA). The PKC inhibitor 1-(5-isoquinolinylsulfonyl)-2-methylpiperazine (H7) was purchased from Seikagaku Kogyo (Tokyo, Japan). Flasks were purchased from Becton Dickinson (Oxnard, CA). ET-1, ET-2, ET-3, and big ET-1 (porcine, 1-39) were purchased from the Peptide Institute (Osaka, Japan). ^{125}I-labeled ET-1 was purchased from Amersham Japan (Tokyo, Japan). The cyclic GMP assay kit was purchased from Yamasa Shoyu (Chiba, Japan). The selective angiotensin subtype 1 (AT_1) receptor antagonist, Iosartan, was donated by Du Pont Merck Pharmaceutical (Wilmington, DE). The selective angiotensin subtype 2 (AT_2) receptor antagonist, PD 123319, was donated by Parke-Davis (Ann Arbor, MI).

Cell Culture

Rat VSMC were grown from the explants of Sprague-Dawley rat aorta and cultured in DMEM containing 10% FCS, as previously described.[18,19] Cells were identified as VSMC by morphological and growth characteristics. Briefly, these cells showed a hill-and-valley appearance and had positive fluorescence with antibodies against α-smooth muscle actin, but negative factor VIII antigen.

The cultures were maintained at 37°C with atmospheric air and 5% CO_2, and subculture was performed after treatment with Versene followed by trypsin. Cells after three to seven passages were used for the experiment. Cultures were normally maintained in DMEM containing 10% FCS, but before some experiments, VSMC were rendered quiescent by 48 hours of serum deprivation.

Pharmacological Treatment

The culture medium was removed, and the cell monolayers were washed twice with serum-free DMEM. Cells were exposed to different concentrations (10^{-7}, 10^{-8}, and 10^{-9} mol/L) of Ang II and AVP for 4, 8, and 12 hours. In separate experiments, saralasin, losartan, and PD 123319 were added to the well 5 minutes before addition of AVP and the cells were incubated for 8 hours.

In separate experiments, the effects of PKC inhibitors from three chemical classes, H7 (10^{-4} mol/L), staurosporine (10^{-6} mol/L), and a

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specific PKC inhibitor, PKC-(19-36) (10^{-5} mol/L), on Ang II (10^{-7} mol/L)- or AVP (10^{-7} mol/L)-stimulated ET-1 production were assessed. These PKC inhibitors were added to the well 5 minutes before addition of Ang II or AVP, and the cells were incubated for 8 hours. In addition, to confirm the importance of the PKC-dependent mechanism in stimulating ET-1 production, the effect of PKC depletion was examined. PKC depletion was performed by preincubation with a high dose of PMA (10^{-7} mol/L) for 24 hours in the cells.

Next, effects of various concentrations (10^{-9}, 10^{-8}, and 10^{-7} mol/L) of rat ANP (1-28), rat BNP-45, and rat CNP-22 on ET-1 production in cells treated with 10^{-7} mol/L Ang II or 10^{-7} mol/L AVP were examined. All experiments were performed with 2 mL serum-free DMEM. After the incubation, the medium was aspirated and centrifuged at 3,000 × *g* for 10 minutes, and the supernatant was collected and stored at −80°C until radioimmunoassay. Serum-free DMEM was used to avoid the presence of hormones and enzymes that could have masked the effects of the pharmacological agent added.

Extraction of ET-1

ET-1 was extracted as previously described.[15] Briefly, 1.5 mL from each sample was diluted with 4 mL 4% acetic acid. After centrifugation, the solution was pumped at the rate of 1 mL/min through a Sep-Pak C_{18} cartridge (Millipore, Milford, MA). After the cartridge was washed with 5 mL distilled water, the adsorbed peptides were eluted with 86% ethanol in 4% acetic acid. After evaporation of the eluate by a centrifugal evaporator (model RD-31; Yamato Scientific, Tokyo, Japan), the dry residue was dissolved in the assay buffer (description follows). The recovery rate was found by addition of three different quantities of cold ET-1 (10, 50, and 100 pg/mL) to serum-free DMEM; the recovery was 71% ± 2%.

Radioimmunoassay of ET-1

ET-1 was assayed using ET-1 antiserum and ^{125}I-ET-1 as a tracer. This antibody reacts 100% with ET-1 and cross-reacts 7% with ET-2, 7% with ET-3, and 35% with big ET-1 (porcine, 1-39). The antiserum did not cross-react with rat ANP (1-28), rat BNP-45, rat CNP-22, somatostatin, β-endorphin, human secretin, Ang II, or AVP.

The radioimmunoassay was performed in an assay buffer of 0.01 mol/L sodium phosphate, pH 7.4, containing 0.05 mol/L NaCl, 0.1% bovine serum albumin, 0.1% Nonidet P-40, and 0.01% NaN_3, as described previously.[15] In brief, rehydrated antiserum (100 μL) was added to 100 μL of the sample or 100 μL standard ET-1 dissolved in the assay buffer, and the mixture was incubated for 24 hours at 4°C. Approximately 15,000 cpm ^{125}I-ET-1 was added to each reaction and incubated for an additional 24 hours. After this incubation, the precipitate was collected by centrifugation at 1,700 × *g* for 30 minutes. The supernatant was removed by aspiration, and the pellet was counted for ^{125}I with a gamma counter. The interassay variation was 13% and intraassay variation 7%.

ANP, BNP, CNP, AVP, and Ang II did not interfere with the radioimmunoassay.

Cyclic GMP Measurement

After preincubation, the cell monolayers were washed twice with serum-free DMEM and then stimulated for 30 minutes with various concentrations (10^{-9}, 10^{-8}, and 10^{-7} mol/L) of rat ANP (1-28), rat BNP-45, or rat CNP-22 dissolved in DMEM that contained 0.5 mmol/L IBMX. One milliliter of cell culture medium was collected and stored at −80°C until radioimmunoassay. Cyclic GMP levels were found by radioimmunoassay with the cyclic GMP assay kit as described previously.[7,15]

Calculations and Statistical Analysis

The statistical significance of differences in the results was evaluated by one-way ANOVA, and *P* values were calculated by Scheffe's method.[20] Values are expressed as the mean ± SD.

RESULTS

Effect of Ang II on ET-1 Production

In confluent, quiescent cultured VSMC, Ang II potently stimulated ET-1 production in a time-dependent manner (Table 1). This stimulation by Ang II was concentration-dependent. Nonstimulated quiescent VSMC also produced a small but significant amount of ET-1 (Table 1).

The effects of saralasin, losartan, and PD 123319 on Ang II-stimulated production of ET-1 are shown in Fig 1A. Preincubation of the cells with 10^{-6} mol/L saralasin or 10^{-6} mol/L losartan 5 minutes before addition of 10^{-7} mol/L Ang II abolished the Ang II-mediated increase of ET-1 production. However, preincubation of the cells with 10^{-6} mol/L PD 123319 before addition of 10^{-7} mol/L Ang II had no effect on ET-1 production. This suggests that the AT_1 receptor is coupled to Ang II-mediated ET-1 production in cultured VSMC.

Effect of AVP on ET-1 Production

AVP potently stimulated ET-1 production in a concentration-dependent manner between 10^{-7} and 10^{-9} mol/L (Table 2).

The effects of the selective V_1 receptor antagonist, PMP, on AVP-stimulated production of ET-1 are shown in Fig 1B. Preincubation of the cells with 10^{-6} mol/L PMP 5 minutes before addition of 10^{-7} mol/L AVP abolished the AVP-mediated increase of ET-1 production. This suggests that the V_1 receptor is coupled to AVP-mediated ET-1 production in cultured VSMC.

Effect of PKC Inhibition on Ang II- and AVP-Induced ET-1 Production

The effects of the PKC inhibitors H7 and staurosporine and a specific PKC inhibitor peptide, PKC-(19-36), on Ang II- and AVP-induced ET-1 production are shown in Fig 2A and B. Ang II- and AVP-induced ET-1 production were significantly attenuated in the presence of the PKC inhibitors H7, staurosporine, and PKC (19-36).

To confirm the importance of PKC in the stimulation of ET-1 production, the effect of PKC depletion was examined. ET-1 production by PKC-depleted cells was not significantly in-

Table 1. Effect of Ang II on ET-1 Production in Cultured Rat VSMC

	ET-1 (pg/5 × 10^5 cells) With Incubation of		
	4 h	8 h	12 h
Baseline	9.0 ± 0.7	11.0 ± 0.5	13.8 ± 0.3
Ang II 10^{-9} mol/L	10.4 ± 0.5	13.6 ± 0.7	16.9 ± 1.1*
Ang II 10^{-8} mol/L	13.3 ± 1.1*†	19.3 ± 0.6*†	24.6 ± 1.6*†
Ang II 10^{-7} mol/L	17.8 ± 2.0*†‡	30.0 ± 1.5*†‡	39.7 ± 2.1*†‡

NOTE. Each value is the mean ± SD of assays from 4 cell cultures.
*$P < .05$ *v* baseline.
†$P < .05$ *v* Ang II 10^{-9} mol/L.
‡$P < .05$ *v* Ang II 10^{-8} mol/L.

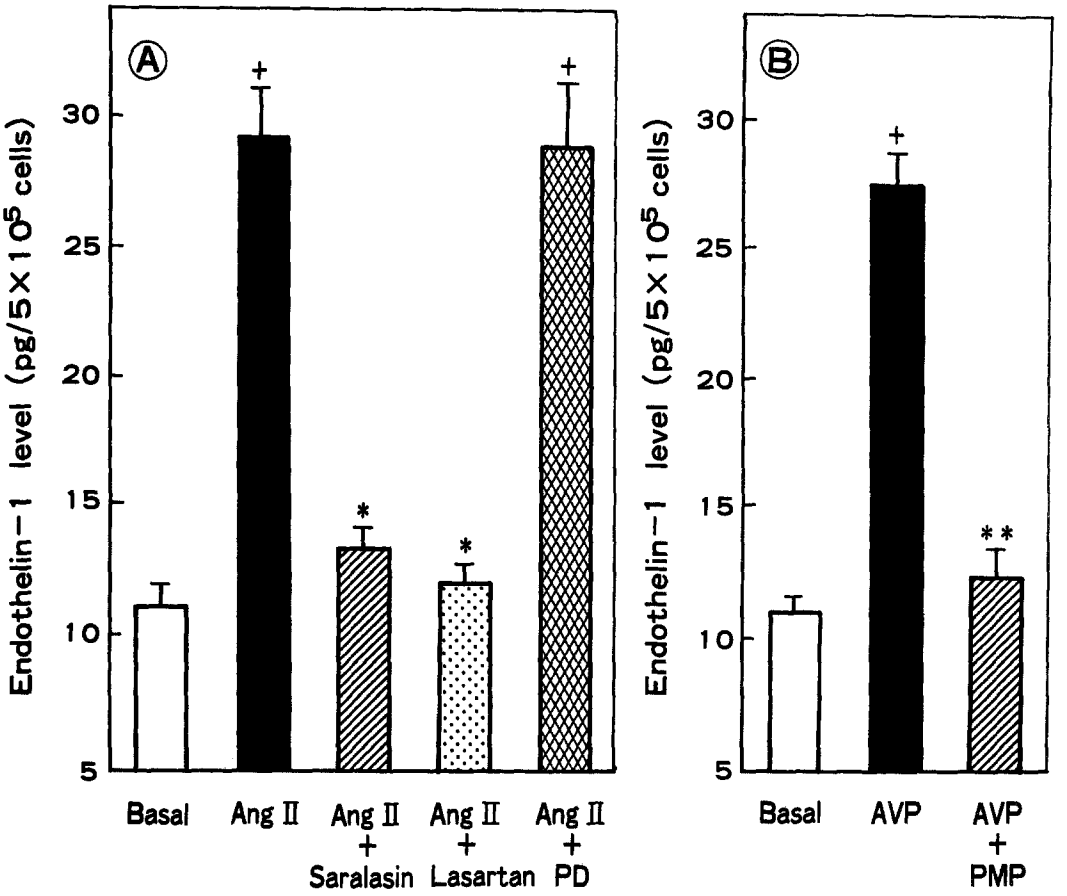

Fig 1. (A) Effects of saralasin, losartan, and PD 123319 (PD) on Ang II-stimulated production of ET-1 in cultured VSMC. Cells were preincubated with 10^{-6} mol/L saralasin, 10^{-6} mol/L losartan, or 10^{-6} mol/L PD 5 minutes before addition of 10^{-7} mol/L Ang II, and the cells were incubated for 8 hours. Each value is the mean ± SD for assays of 4 cell cultures. †$P < .05$ *v* basal production; *$P < .05$ *v* Ang II alone. (B) Effects of the selective V_1 receptor antagonist, PMP, on AVP-stimulated production in cultured VSMC. Cells were preincubated with 10^{-6} mol/L PMP 5 minutes before addition of 10^{-7} mol/L AVP, and the cells were incubated for 8 hours. Each value is the mean ± SD for assays of 4 cell cultures. †$P < .05$ *v* basal production; **$P < .05$ *v* AVP alone.

creased by addition of 10^{-7} mol/L Ang II or 10^{-7} mol/L AVP (Tables 3 and 4).

Effect of ANP, BNP, and CNP on ET-1 Production

Rat ANP (1-28), rat BNP-45, and rat CNP-22 in the absence of Ang II or AVP had no significant effects on ET-1 production compared with baseline levels (Table 5).

Rat ANP (1-28), rat BNP-45, and rat CNP-22 attenuated the effect of Ang II and AVP on ET-1 production in a concentration-dependent manner (Figs 3A and 4A). The inhibitory effect of CNP-22 on ET-1 production was significantly greater than that of ANP (1-28) or BNP-45 (CNP ≫ ANP > BNP). In cells treated with Ang II and AVP, in parallel with the inhibition of ET-1 production, cellular cyclic GMP increased after treatment with ANP (1-28), BNP-45, or CNP-22 (Figs 3B and 4B).

Table 2. Effect of AVP on ET-1 Production in Cultured Rat VSMC

	ET-1 (pg/5 × 10^5 cells) With Incubation of		
	4 h	8 h	12 h
Baseline	9.1 ± 0.4	10.9 ± 0.5	13.6 ± 0.4
AVP 10^{-9} mol/L	10.4 ± 0.6	12.9 ± 0.6	16.2 ± 1.0
AVP 10^{-8} mol/L	11.9 ± 1.1*†	17.8 ± 0.8*†	23.7 ± 1.3*†
AVP 10^{-7} mol/L	17.1 ± 0.4*†‡	26.7 ± 2.3*†‡	37.5 ± 1.6*†‡

NOTE. Each value is the mean ± SD of assays from 4 cell cultures.
*$P < .05$ *v* baseline.
†$P < .05$ *v* AVP 10^{-9} mol/L.
‡$P < .05$ *v* AVP 10^{-8} mol/L.

To determine whether the inhibitory effects of ANP, BNP, and CNP on ET-1 production after stimulation with Ang II or AVP are causally linked to the increase in cellular cyclic GMP, we examined the effect of a cyclic GMP analog on ET-1 production after stimulation with Ang II and AVP (Table 6). 8-Bromo cyclic GMP at a concentration of 10^{-5} and 10^{-4} mol/L significantly reduced stimulation by Ang II or AVP.

DISCUSSION

First, we have confirmed previous reports that cultured VSMC produce ET-1 in a time-dependent manner and that Ang II and AVP stimulate ET-1 production in a concentration-dependent manner in these cells.[2,3] The amount of Ang II- and AVP-stimulated ET-1 production was lower in cultured VSMC compared with cultured vascular endothelial cells.[14,15] Neverthe-

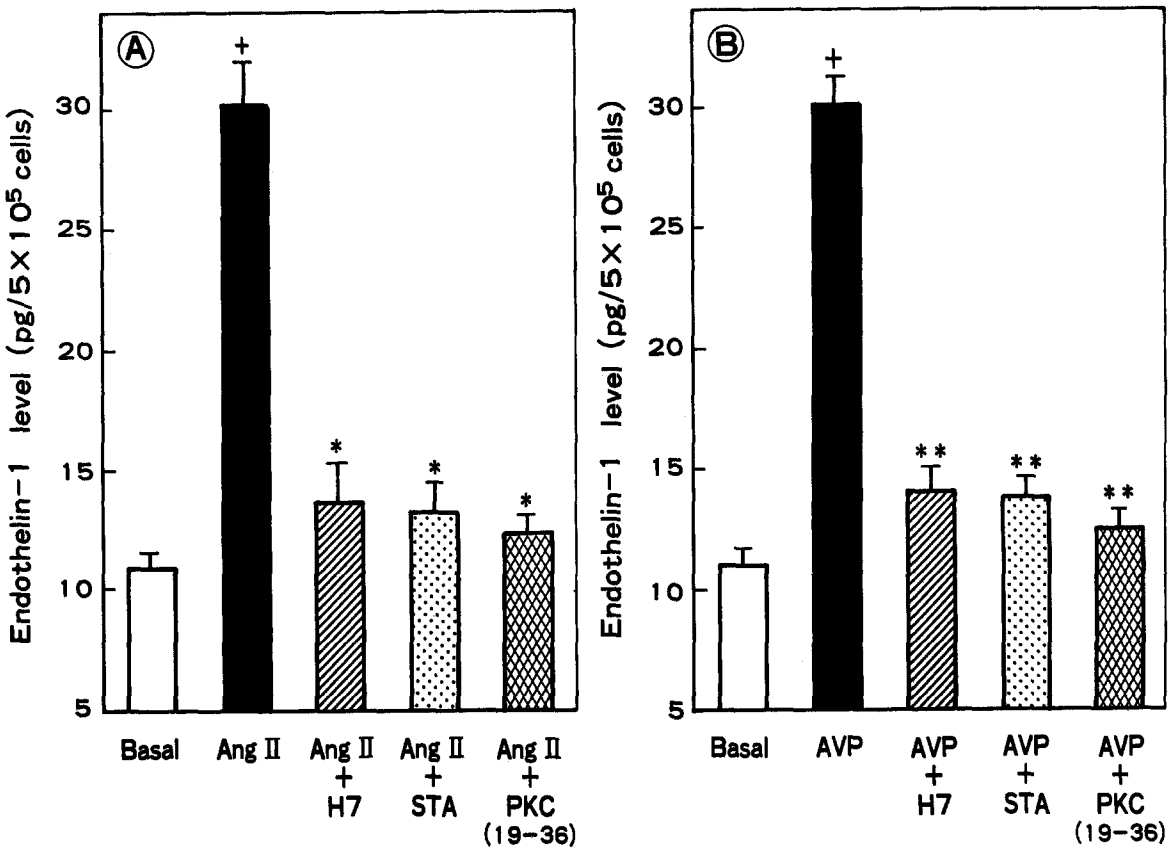

Fig 2. Effect of the PKC inhibitors H7 (10^{-4} mol/L), staurosporine ([STA] 10^{-6} mol/L), and specific PKC inhibitor peptide PKC-(19-36) (10^{-5} mol/L) on ET-1 production in cultured VSMC treated with Ang II (A) or AVP (B). Cells were exposed to PKC inhibitors in addition to 10^{-7} mol/L Ang II or AVP for 8 hours. Each point is the mean of 4 measurements. †$P < .05$ *v* basal production; *$P < .05$ *v* Ang II alone; **$P < .05$ *v* AVP alone.

less, ET-1 in the culture media of Ang II- or AVP-stimulated VSMC appears to attain levels that are within the biologically effective range for this peptide.[1] Furthermore, it is important to note that a low concentration of exogenous ET-1 potentiates the vasoconstrictive or mitogenic action of other vasoconstrictors or growth factors such as norepinephrine,[21] serotonin,[22] or platelet-derived growth factor.[23] Therefore, ET-1 produced by Ang II and AVP may act in concert with Ang II, AVP, or other endogenous substances to stimulate vasoconstriction or proliferation even at low concentrations.

Second, we showed in the current experiment that Ang II-induced ET-1 production was abolished by the Ang II receptor antagonist, saralasin, and the selective AT_1 receptor antagonist, losartan. On the other hand, this stimulation by Ang II was not affected by the selective AT_2 receptor antagonist, PD 123319. This suggests that the AT_1 receptor is coupled to Ang II-mediated ET-1 production in cultured rat VSMC. Further, we showed that AVP-induced ET-1 production was abolished by the selective V_1 receptor antagonist, PMP, and therefore AVP stimulates ET-1 production via the V_1 receptor in VSMC.

Next, we showed that PKC inhibitors from three chemical classes, H7, staurosporine, and PKC-(19-36), inhibited Ang II- or AVP-induced ET-1 production. In addition, stimulation of ET-1 production by Ang II or AVP was abolished in PKC-

Table 3. Effect of Ang II on ET-1 Production With or Without PKC Depletion in Cultured Rat VSMC

	ET-1 (pg/8 h per 5 × 10^5 cells)
Baseline	10.9 ± 0.3
Ang II 10^{-7} mol/L	31.0 ± 1.0*
Ang II 10^{-7} mol/L + PKC depletion	11.3 ± 0.9†

NOTE. Each point represents the mean ± SD of 4 determinations in duplicate. To deplete PKC, cells were preincubated with 10^{-7} mol/L PMA for 24 hours.

*$P < .05$ *v* baseline.

†$P < .05$ *v* Ang II 10^{-7} mol/L.

Table 4. Effect of AVP on ET-1 Production With or Without PKC Depletion in Cultured Rat VSMC

	ET-1 (pg/8 h per 5 × 10^5 cells)
Baseline	10.9 ± 0.4
AVP 10^{-7} mol/L	28.2 ± 1.6*
AVP 10^{-7} mol/L + PKC depletion	11.7 ± 1.4†

NOTE. Each point represents the mean ± SD of 4 determinations in duplicate. To deplete PKC, cells were preincubated with 10^{-7} mol/L PMA for 24 hours.

*$P < .05$ *v* baseline.

†$P < .05$ *v* AVP 10^{-7} mol/L.

Table 5. Effect of the Highest Concentration (10^{-7} mol/L) of Rat ANP (1-28), Rat BNP-45, and Rat CNP-22 on Spontaneous Production of ET-1 in Cultured Rat VSMC

	Baseline	Rat ANP (1-28)	Rat BNP-45	Rat CNP-22
ET-1 (pg/8 h per 5×10^5 cells)	10.8 ± 0.6	9.8 ± 0.3	10.5 ± 0.7	9.7 ± 0.7

NOTE. Cells were exposed to 10^{-7} mol/L rat ANP (1-28), rat BNP-45, and rat CNP-22 for 8 hours. Each point represents the mean ± SD of 4 determinations in duplicate.

depleted cells. These results suggest that Ang II and AVP stimulate ET-1 production in cultured rat VSMC by a mechanism probably involving activation of PKC.

ANP was originally isolated from the mammalian heart.[10,11] A second type of natriuretic peptide, BNP, has subsequently been isolated from mammalian hearts.[13,24,25] ANP and BNP are both secreted through the coronary sinus from the heart, but ANP is secreted mainly from the atria and BNP mainly from the ventricles.[26,27] In addition, ANP is found to counteract the contractile response of aortic rings to ET-1.[28] Subsequently, Zimmerman et al[29] have shown that ANP blocks the pressor action of ET-3 by decreasing cardiac output and that ET-3 blocks the natriuretic action of ANP. Previously, we have shown that ANP and BNP can inhibit ET-1 production in cultured vascular endothelial cells.[15] These observations suggest that natriuretic peptides and ET-1 may have important interactions at the vascular level. In this study, we found that both rat ANP (1-28) and rat BNP-45, which are the major forms of ANP and BNP,[30,31] respectively, in rats, significantly inhibited ET-1 production stimulated by Ang II and AVP in cultured rat VSMC. Under the current experimental conditions, concentrations of ANP and BNP higher than the plasma concentrations were required to inhibit Ang II and AVP effects on ET-1 production. However, plasma ANP and BNP are found at high levels in spontaneously hypertensive rats[30-33] and deoxycorticosterone acetate salt–hypertensive rats.[34] In the malignant or severe phase of rat and human hypertension, both plasma ANP and BNP are found to be markedly increased.[29-31] These observations raise the hypothesis that ANP and BNP may regulate the production of ET-1 induced by Ang II and AVP in VSMC in certain pathological states.

Recently, a third type of natriuretic peptide, designated CNP, was identified in the NH_2-terminal five amino acids, and it ends at the second cysteine with no COOH-terminal tail.[17] In this study, we showed that rat CNP-22 potently inhibited Ang II- and AVP-stimulated ET-1 production in a concentration-dependent manner. Furthermore, the inhibitory effect of CNP-22 on ET-1 production was significantly greater than that of ANP (1-28) or BNP-45 (CNP ≫ ANP > BNP). Recently, CNP-22

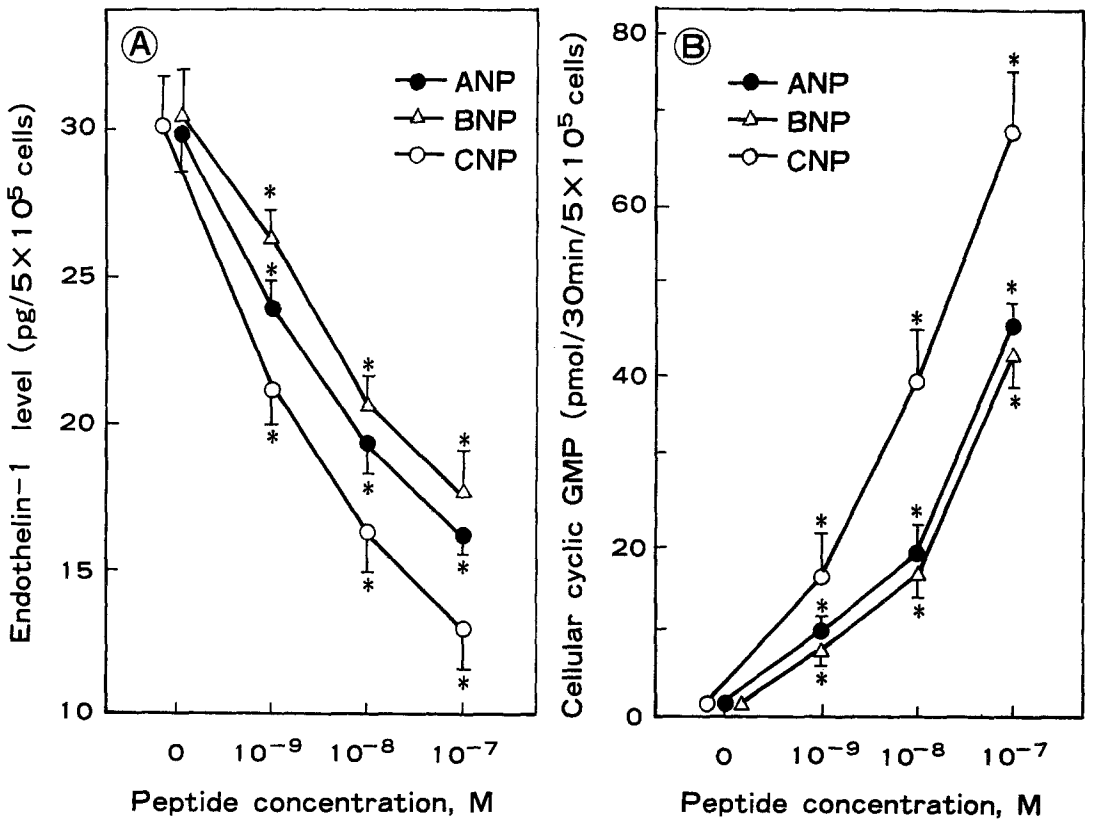

Fig 3. (A) Effects of ANP, BNP, and CNP on ET-1 production in cells treated with Ang II. Cells were exposed to different concentrations of rat ANP (1-28), rat BNP-45, and rat CNP-22 in addition to 10^{-7} mol/L Ang II for 8 hours. Each point is the mean of 6 measurements. (B) Effects of ANP, BNP, and CNP on cellular cyclic GMP levels in cells treated with 10^{-7} mol/L Ang II. Cells were exposed to different concentrations of rat ANP (1-28), rat BNP-45, and rat CNP-22 for 30 minutes in the presence of 0.5 mmol/L IBMX. Each point is the mean of 6 measurements. *$P < .05$ *v* Ang II alone.

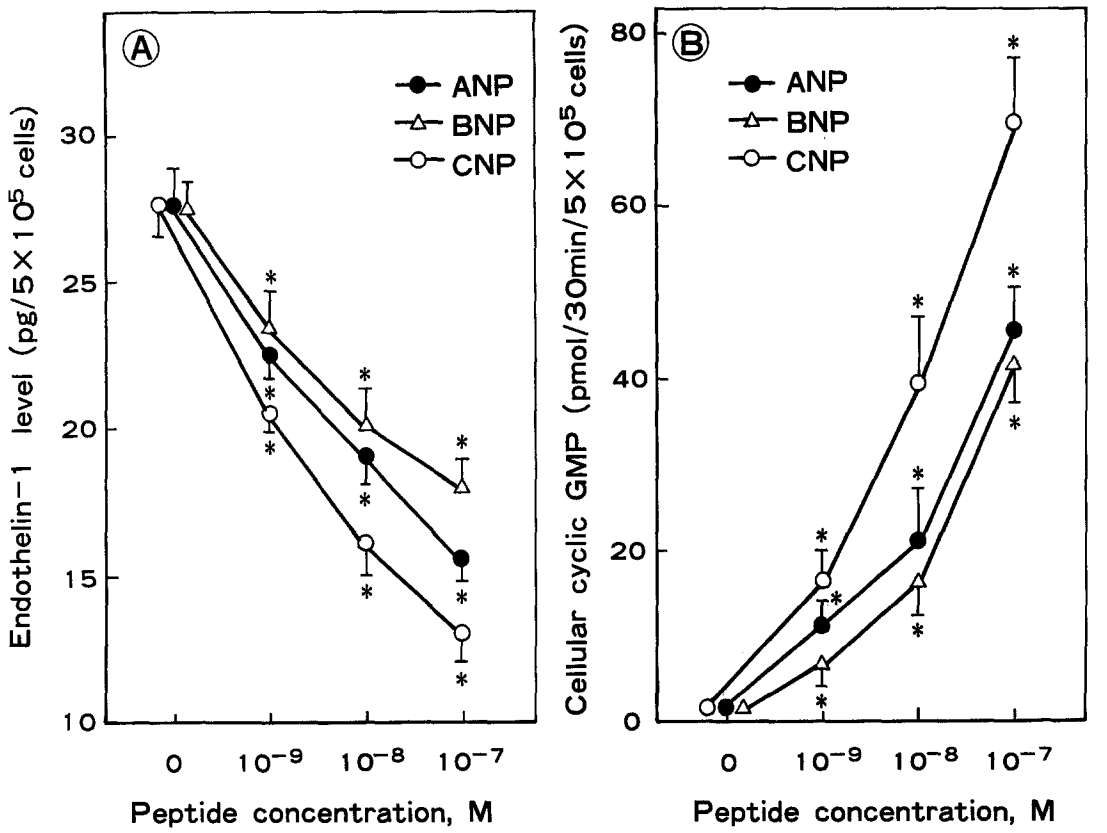

Fig 4. (A) Effects of ANP, BNP, and CNP on ET-1 production in cells treated with AVP. Cells were exposed to different concentrations of rat ANP (1-28), rat BNP-45, and rat CNP-22 in addition to 10^{-7} mol/L AVP for 8 hours. Each point is the mean of 6 measurements. (B) Effects of ANP, BNP, and CNP on cellular cyclic GMP levels in cells treated with AVP. Cells were exposed to different concentrations of rat ANP (1-28), rat BNP-45, and rat CNP-22 for 30 minutes in the presence of 0.5 mmol/L IBMX. Each point is the mean of 6 measurements. *$P < .05$ v AVP alone.

was found to be produced by cultured vascular endothelial cells.[35] Therefore, ET-1 production in VSMC may be, in part, regulated by CNP-22 from adjacent vascular endothelial cells, as well as ANP (1-28) and BNP-45 from the heart.

We have obtained three pieces of evidence for a causal link between cyclic GMP production and inhibition of ET-1 production by natriuretic peptides in cultured rat VSMC. First, rat ANP (1-28) and BNP-45 increased cyclic GMP levels, and these effects paralleled the inhibition of ET-1 production. Second, rat CNP-22 had significantly greater effects than rat ANP (1-28) or rat BNP-45 with respect to inhibiting ET-1 production and increasing cyclic GMP levels in cells stimulated with Ang II or AVP. Third, a cyclic GMP analog reduced Ang II- or AVP-stimulated ET-1 production. These results suggest that natriuretic peptides inhibit Ang II- and AVP-stimulated ET-1 production from cultured rat VSMC, probably through a cyclic GMP–dependent process. However, further studies are necessary to elucidate the exact role of cyclic GMP in the inhibition of ET-1 production by natriuretic peptides in VSMC.

Table 6. Effect of 8-Bromo Cyclic GMP on ET-1 Production in Cultured Rat VSMC Treated With Ang II or AVP

	ET-1 (pg/8 h per 5 × 10^5 cells)
Baseline	10.9 ± 0.5
Ang II 10^{-7} mol/L	30.9 ± 1.1*
Ang II 10^{-7} mol/L + 8 Br 10^{-5} mol/L	21.8 ± 2.0*†
Ang II 10^{-7} mol/L + 8 Br 10^{-4} mol/L	16.0 ± 1.1*†
AVP 10^{-7} mol/L	28.2 ± 1.1*
AVP 10^{-7} mol/L + 8 Br 10^{-5} mol/L	24.1 ± 1.3*‡
AVP 10^{-7} mol/L + 8 Br 10^{-4} mol/L	17.9 ± 0.6*‡

NOTE. Cells were exposed to 10^{-5} or 10^{-4} mol/L 8-bromo cyclic GMP (8 Br) for 8 hours. Each value is the mean ± SD of assays from 4 cell cultures.

*$P < .05$ v baseline.

†$P < .05$ v Ang II 10^{-7} mol/L.

‡$P < .05$ v AVP 10^{-7} mol/L.

Basal production of ET-1 was not significantly altered by ANP, BNP, and CNP, so spontaneous production by cultured VSMC appears to be insensitive to modulation by these natriuretic peptides. This finding seems consistent with our previous observation that basal ET-1 production in vascular endothelial cells is insensitive to modulation by ANP and BNP.[15]

Overall, the present study suggests that ANP, BNP, and CNP reduce the excess production of ET-1 caused by Ang II or AVP, probably through a cyclic GMP–dependent process. Taken together with the profound effects of ET-1 on the contraction and proliferation of VSMC, the ANP-BNP-CNP system may modulate vascular tone, in part, through inhibition of Ang II- or AVP-induced ET-1 production in VSMC. If so, this action in VSMC may be added to natriuresis, vasodilation, and antiproliferation as yet another effect of natriuretic peptides beneficial in certain pathological states.

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