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Regulation of endothelin-1 production in cultured rat mesangial cells

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Regulation of endothelin-1 production in cultured rat mesangial cells. We investigated the regulatory mechanisms of endothelin (ET)-1 production in cultured rat mesangial cells (MC), with a special focus on the roles of protein kinase A (PKA)- and protein kinase C (PKC)-mediated signaling systems. Vasoactive agents and growth promoting factors, including platelet-derived growth factor, vasopressin and thrombin, which act through receptors coupled to the phospholipase C-mediated signaling system, as well as phorbol ester and fetal calf serum stimulated ET-1 production. This effect was attenuated in PKC-depleted or H-7 (a PKC inhibitor) treated MC. On the other hand, an increase in intracellular cyclic AMP by forskolin or β-adrenergic agonist, isoproterenol, which act as anti-mitogenic agents, inhibited serum-stimulated ET-1 production. In addition this effect was mimicked by the addition of 8-bromo-cyclic AMP to the medium. The effect of isoproterenol was abolished by propranolol. H-8, a PKA inhibitor, attenuated the inhibitory effect of forskolin. These findings suggest that ET-1 production in MC is regulated by interaction of both positive and negative signals mediated by PKC- and PKA-dependent mechanisms.

We recently reported the production of endothelin (ET)-1 and the gene expression of prepro-ET-1 mRNA in cultured rat mesangial cells (MC) [1], but the regulation of this production remains to be clarified. In endothelial cells (EC), the production of ET-1 is potentiated by several substances, such as Ca^{2+} ionophore, vasoconstricting hormones, and growth promoting factors [2–5]. Some of these factors act through the receptors coupled to phospholipase C (PLC). Accordingly, both the mobilization of intracellular Ca^{2+} and activation of protein kinase C (PKC) may be involved in the stimulation of ET-1 production. Indeed, phorbol esters, activators of PKC, as well as Ca^{2+} ionophore cause a marked and immediate stimulation of ET-1 production, accompanied by induction of prepro-ET mRNA, in human EC [2–4]. Two phorbol ester-responsive elements are known to locate in the 5′-flanking region of the human prepro-ET gene [6]. These findings indicate that early signaling events through the PLC/Ca^{2+}/PKC system are involved in the regulation of ET-1 production.

Another important cellular signaling system is the adenylate cyclase (AC)/cyclic AMP (cAMP)/protein kinase A (PKA) system. Some investigators have shown the inhibition of mitogenesis of MC by cAMP [7–10]. Recently, Simonson and Dunn showed in rat MC that ET activates PLC activity, and that it also potentiates β-adrenergic-stimulated cAMP accumulation [11]. They hypothesized that the AC/cAMP system antagonizes the PLC/Ca^{2+}/PKC system and works as a negative feedback signal for ET action. Thus, it may be expected that if activation of the PLC/Ca^{2+}/PKC system stimulates ET-1 production in MC, as in EC, activation of the AC/cAMP/PKA system will inhibit ET-1 production. The present study was undertaken in cultured rat MC to examine the regulation of ET-1 production by PKA- and PKC-mediated signaling systems.

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Methods

Cell cultures

Cultures of rat mesangial cells were prepared as previously described [1]. For studies of ET-1 production, 25 cm^2 flasks (PRIMARIA, Falcon, Lincoln Park, New Jersey, USA) were used. For studies of double-stranded DNA measurement, cells were plated on 6-well dishes (Falcon, Lincoln Park, New Jersey, USA). Subcultured mesangial cells (5 to 15th passage) were used in the present experiments.

RIA of ET-1

Confluent mesangial cells washed with phosphate buffered saline (PBS) were preincubated for 48 hours with RPMI 1640 in the absence of FCS. Subsequently the cells were incubated for 8 or 24 hours with RPMI 1640 that contained FCS as indicated. Test substances were added in this incubation medium. After the incubation, media were collected and centrifuged. Then supernatant was collected and stored at −80°C until measurements were performed. RIA for ET-1 was essentially the same as previously described [12].

Measurement of double-stranded DNA

The concentration of double stranded DNA was measured by fluorescence of the complex of mithramycin and double-stranded DNA (Nuclesan 100, Sanbio, Netherlands) [13]. After a 48 hour incubation with RPMI 1640, which contained a test substance or vehicle, the cells were harvested by 0.25% trypsin and 1.0 mM EDTA. The cells were washed twice in PBS and resuspended in 965 μl of distilled water. After the addition of pronase the cells were agitated briefly. Then the cells were incubated for one hour in a gently shaking water bath at 37°C,

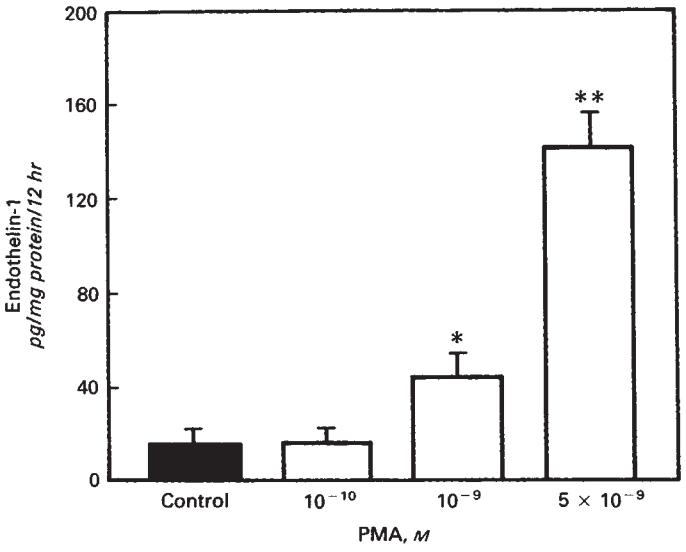

Fig. 1. *Effect of PMA on ET-1 production.* Serum-depleted mesangial cells were incubated with PMA (10^{-10} to 5×10^{-9} M) or 0.5% FCS alone (Cont) for 12 hours. *$P < 0.05$; **$P < 0.01$ compared to Cont.

and subsequently they were agitated vigorously for a few seconds. Samples were placed on ice, then mithramycin was added, followed by vigorous agitation for at least 10 seconds. The sample tubes were centrifuged at 5,000 g for 15 minutes to spin down any undissolved materials. The fluorescence of the samples was measured using a spectrofluorophotometer (RF5000, Shimadzu Co., Kyoto, Japan) at an excitation wavelength of 440 nm and an emission wavelength of 540 nm.

Measurement of $[Ca^{2+}]_i$

Mesangial cells were plated on cover slide and deprived of FCS for at least 24 hours before the experiments. The cells were loaded with 2 μM Fura-2 acetoxymethylester (Molecular Probes, Eugene, Oregon, USA) for 30 minutes. Intracellular calcium concentration ($[Ca^{2+}]_i$) in a single cell was measured by a computer-assisted Ca^{2+} measurement system (OSP-3, Olympus, Tokyo, Japan); the cells were excited (10 msec) alternatively at 340 and 380 nm, and emmission fluorescence at 510 nm was measured. At the end of each experiment, the bath was changed to an ionomycin (10 μM) containing solution (2 mM Ca^{2+}) to measure R_{max}, and then to a calcium-free modified solution containing 1 mM EGTA and 20 μM ionomycin to measure R_{min}. $[Ca^{2+}]_i$ was calculated by the following formula:

$$[Ca^{2+}]_i = K_d([R - R_{min}]/[R_{max} - R]) \times (380_{min}/380_{max})$$

where K_d is the dissociation constant of the Fura-2-Ca^{2+} complex (224 nM) [14].

Materials

RPMI 1640 and fetal calf serum (FCS) were obtained from Gibco Laboratories (Grand Islands, New York, USA). Forskolin, phorbol myristate acetate (PMA), isoproterenol, and propranolol were purchased from Sigma Chemical Co. (St. Louis, Missouri, USA). N[2-(methylamino)ethyl]-5-isoquinoline-sulfonamide (H-8) and 1-(5-isoquinolinylsulfonyl)-2-methylpiperazine (H-7) were obtained from Seikagaku Kogyo Co. (Tokyo, Japan). Ionomycin and dioctanoyl glycerol (DiC8) were purchased from Calbiochem-Behring Corp. (La Jolla, California, USA). Cell protein was measured by a dye-binding method (Bio-Rad, Richmond, California, USA) using bovine serum albumin as a standard.

Table 1. Effect of protein kinase C activator, DiC8, on endothelin-1 production

	Endothelin-1 *pg/mg protein/12 hr*
Basal	30.9 ± 5.0
DiC8 10^{-5} M	35.3 ± 4.5
10^{-4} M	38.3 ± 6.2[a]
3×10^{-4} M	50.0 ± 4.1[a]
10^{-3} M	100.3 ± 10.2[b]

DiC8 is dioctanoyl glycerol.
[a] $P < 0.05$ as compared to basal value
[b] $P < 0.01$ as compared to basal value

Statistical analysis

All results were expressed as mean ± SE. The statistical significance of differences was calculated by the Student's *t*-test or variance analysis, and was considered significant when $P < 0.05$.

Results

Effect of PKC activation on ET-1 production

To examine the role of PKC on ET-1 production, the effect of PMA, a direct activator of PKC, was examined. After 48 hours of serum deprivation, the cells were cultured with 0.5% FCS medium with or without PMA for 12 hours. ET-1 production was corrected for cell protein content. As shown in Figure 1, PMA stimulated the production of ET-1. PMA at 10^{-10} M did not cause any effect, but 10^{-9} or 5×10^{-9} M PMA significantly stimulated ET-1 production. Another stimulator of PKC, dioctanoyl glycerol (DiC8) was also examined. Addition of DiC8 (10^{-4} to 10^{-3} M) to the medium significantly stimulated the production of ET-1 (Table 1).

We further examined the effect on ET-1 production of several substances that are known to act through specific responses coupled to the PLC-mediated signaling system in mesangial cells. The tested substances were PDGF (5 U/ml), thrombin (10 U/ml), arginine vasopressin (AVP, 10^{-7} M), and angiotensin II (Ang II, 10^{-7} M). As shown in Figure 2, each agent except for Ang II significantly potentiated the production of ET-1 compared to the control, which was treated with 0.5% FCS alone. Effects of PMA (10^{-8} M) and 5% FCS are also included in Figure 2 for comparison. Among these substances, 5% FCS showed the most potent stimulation of ET-1 production. Since we could not find the stimulatory effect of Ang II on ET production, we checked the response of Ang II by measuring intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). Ang II (10^{-7} M) induced a spike-shaped Ca^{2+} increase. $[Ca^{2+}]_i$ increased from 171 ± 15 nM to 342 ± 45 nM (peak value, $N = 5$, $P < 0.01$). These results are similar to the values reported by others [15, 16], suggesting that our MC responded to Ang II.

To confirm the importance of PKC-dependent mechanisms in the stimulation of ET-1 production, the effect of PKC-depletion was examined. PKC-depletion was made by preincubation with

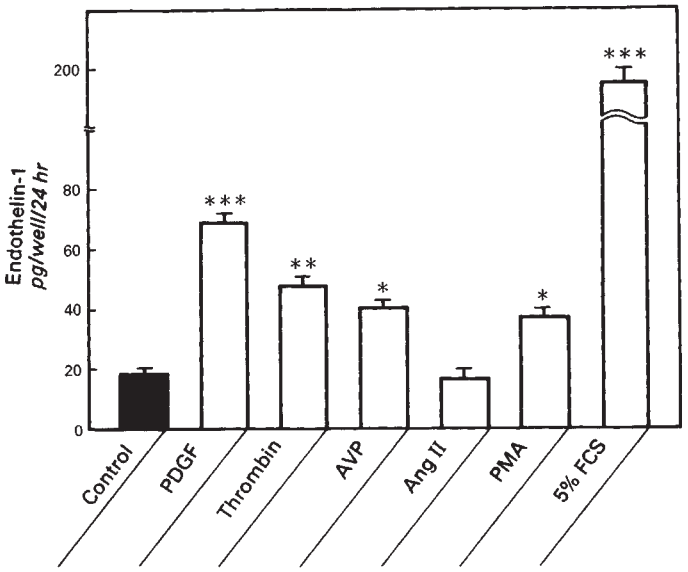

Fig. 2. *Effect of various agents on ET-1 production.* The effect of individual agents on ET-1 production was compared with that of 0.5% FCS alone (Cont). The concentration of each agent was as follows PDGF 5 U/ml, thrombin 10 U/ml, AVP 10^{-7} M, PMA 10^{-8} M and FCS 5%. Abbreviations are in the text. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

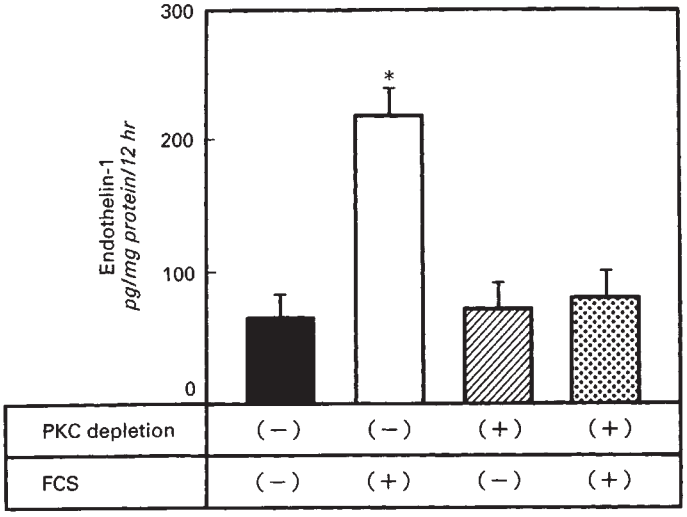

Fig. 3. *Effect of FCS on ET-1 production in the mesangial cells with or without PKC depletion.* To deplete PKC, mesangial cells were preincubated with 10^{-7} M PMA for 24 hours. $^{*}P < 0.01$ compared to FCS- and PKC depletion-free.

a high dose of PMA (10^{-7} M) for 24 hours. Then, the medium was removed, and a new culture medium which contained 5% FCS was added. Figure 3 shows that the ET-1 production by PKC-depleted cells was not increased by the addition of 5% FCS, which significantly stimulate ET-1 production under the normal condition. Contribution of PKC-dependent mechanisms was also examined by using PKC inhibitor, H-7. H-7 (10^{-5} to 10^{-4} M) significantly inhibited the PMA-stimulated ET-1 production (Table 2). These findings suggest that the stimulation of ET-1 production is dependent on PKC.

Table 2. Effect of protein kinase C inhibitor, H-7, on endothelin-1 production induced by PMA

	Endothelin-1 *pg/mg protein/12 hr*
PMA 10^{-8} M	165.5 ± 14.0
+ H-7 10^{-5} M	93.4 ± 11.2[a]
+ H-7 10^{-4} M	55.9 ± 10.1[a]

PMA is phorbol myristate acetate H-7, 1-(5-isoquinolinylsulfonyl)-2-methylpiperazine

[a] $P < 0.01$ as compared to PMA (10^{-8} M) alone

Effect of cAMP on ET-1 production and mitogenesis

First, the effect of forskolin, a substance that stimulates AC by bypassing physiological receptors [17–19], on ET-1 production was examined. Forskolin dose-dependently inhibited the serum-stimulated (5% FCS) production of ET-1 in an eight hour culture. Forskolin 20 μM inhibited ET-1 production by 67% compared to FCS alone (Fig. 4A). The ET-1 production corrected for cell protein content was significantly inhibited, and therefore these findings indicate the inhibition of ET-1 production in each cell. Mitogenesis (as assessed by the content of double-stranded DNA) of MC, incubated in a 5% FCS-containing medium for 48 hours, was also attenuated by forskolin. In the presence of 20 μM forskolin, FCS-stimulated increase in double-strand DNA was inhibited by 60% (Fig. 4B). 8Br-cAMP (10^{-3} M) added to the culture medium mimicked the forskolin effect in terms of ET-1 production and mitogenesis (data not shown), implying that the inhibition of ET-1 production by forskolin is mediated by cAMP.

Next we examined the effect of the receptor-mediated intracellular accumulation of cAMP on ET-1 production. We used a β-adrenergic agonist, isoproterenol, which is known to stimulate AC in mesangial cells [7, 8, 20]. As shown in Figure 5A, isoproterenol (10^{-5} to 10^{-7} M) dose-dependently inhibited the serum-stimulated ET-1 production. This inhibitory effect was antagonized by propranolol, an antagonist of β-adrenergic receptors (Fig. 5B). Propranolol (10^{-5} M) was added five minutes prior to the exposure to isoproterenol (10^{-5} M). Accordingly, these findings suggest that isoproterenol affects ET-1 production via β-adrenergic receptor.

To determine whether the effect of cAMP is mediated by PKA, H-8, a relatively specific inhibitor of PKA [21], was used. As shown in Figure 6, 50 μM H-8 itself had little effect on the FCS (5%)-stimulated ET-1 production, whereas H-8 significantly prevented the inhibition of ET-1 production by 20 μM forskolin (115 ± 14 vs. 42 ± 14 pg/mg protein/8 hr). This finding suggests that cAMP inhibits serum-stimulated ET-1 production at least partly by PKA-dependent mechanism(s).

Discussion

In the present study, we suggest that the production of ET-1 is potentiated by activation of PKC, whereas it was attenuated by activation of PKA in rat MC. To our knowledge, this is the first report of the inhibition of ET-1 production in ET-1-producing cells.

In cultured rat MC, our data showed that receptor-mediated PLC-activating agents, including thrombin, AVP, and PDGF, but not Ang II, significantly potentiate ET-1 production (Fig. 3).

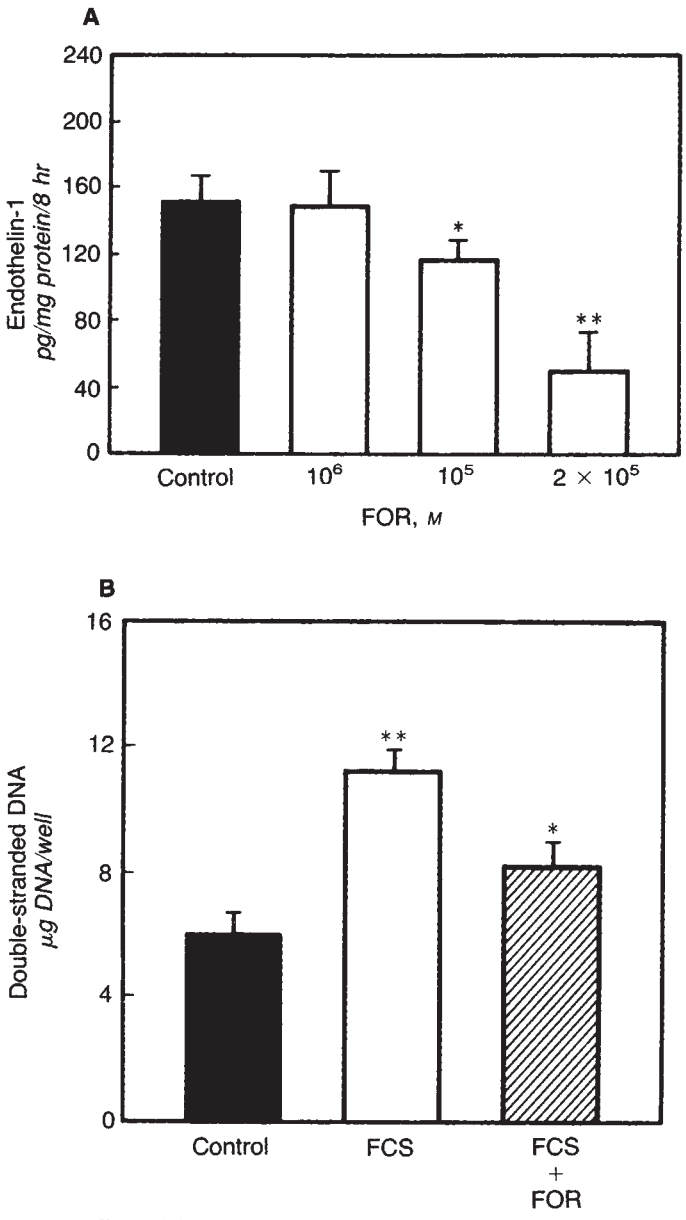

Fig. 4. *Effect of forskolin on serum-stimulated ET-1 production and mitogenesis.* **A.** Dose-response of forskolin (FOR) to serum-stimulated ET-1 production. The ET-1 concentration of conditioned medium was measured 8 hours after addition of the agent in the presence of 5% FCS. **B.** Effect of forskolin (20 μM) on mitogenesis stimulated by 5% FCS for 48 hours. Mitogenesis was assessed as the content of double stranded DNA. Control (Cont) indicates the content of double-stranded DNA in the cells cultured in the absence of FCS. $*P < 0.05$; $**P < 0.01$ compared to Controls.

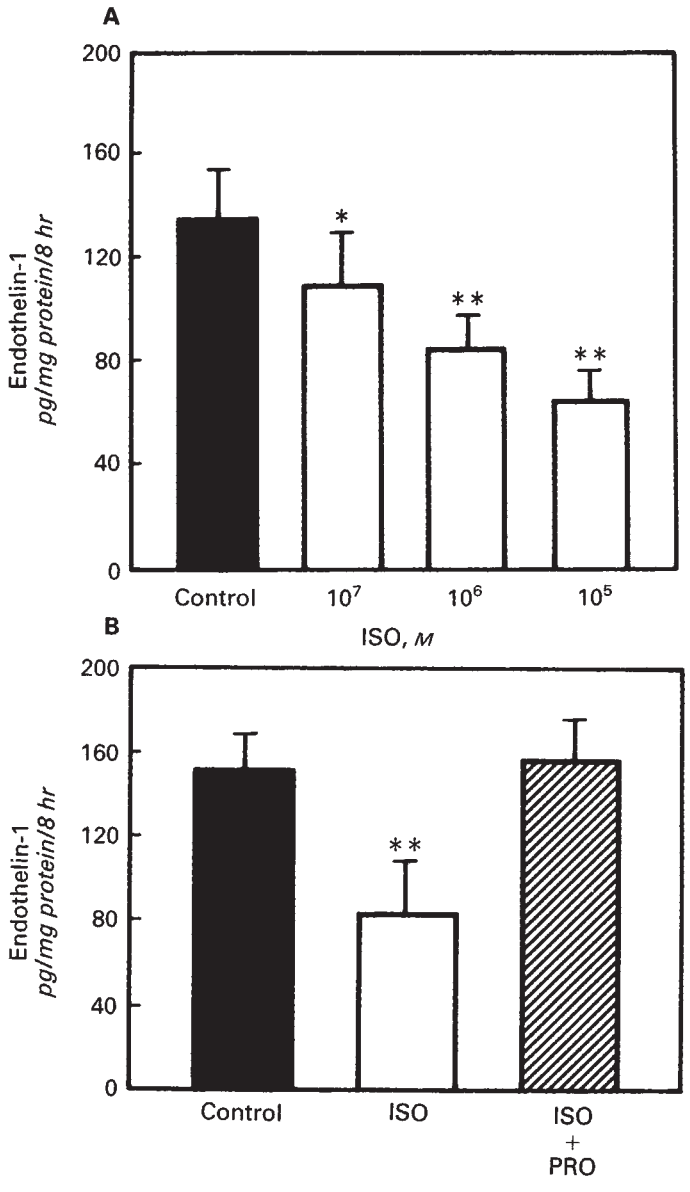

Fig. 5. *Effect of isoproterenol (ISO) on serum-stimulated ET-1 production.* **A.** Dose-response of isoproterenol to serum (5% FCS)-stimulated ET-1 production. **B.** Effect of propranolol (PRO; 10^{-5} M) on the inhibition of ET-1 production by isoproterenol (10^{-5} M). Propranolol was added 5 minutes prior to the exposure to isoproterenol. Control (Cont) indicates the ET-1 production in the cells cultured with 5% FCS alone. $*P < 0.05$; $**P < 0.01$ compared to Cont.

Previous studies have also demonstrated that these agents stimulate ET-1 production in endothelial [2, 4] and non-endothelial cells, such as vascular smooth muscle cells [22] and renal tubular cells [23]. Since the common feature of these agents is their action on the receptors coupled to PLC, both the mobilization of intracellular Ca^{2+} and activation of PKC may be closely involved in the stimulation of ET-1 production. This view is supported by the findings that phorbol esters and Ca^{2+} ionophore cause an induction of prepro-ET mRNA and a marked and immediate stimulation of ET production in human endothelial cells [2, 3, 6]. Our results in rat MC that PMA and DiC8 stimulate ET-1 production, and that the stimulation of ET-1 production is abolished in the PKC-depleted or H-7 treated cells also support this thesis. In the presence of low dose serum, the effects of PMA and DiC8 were evident, but they were not seen in the total absence of serum, suggesting that cofactor(s) such as competence factor in serum is necessary for PKC-induced endothelin production.

Ang II, however, did not stimulate the ET-1 production in

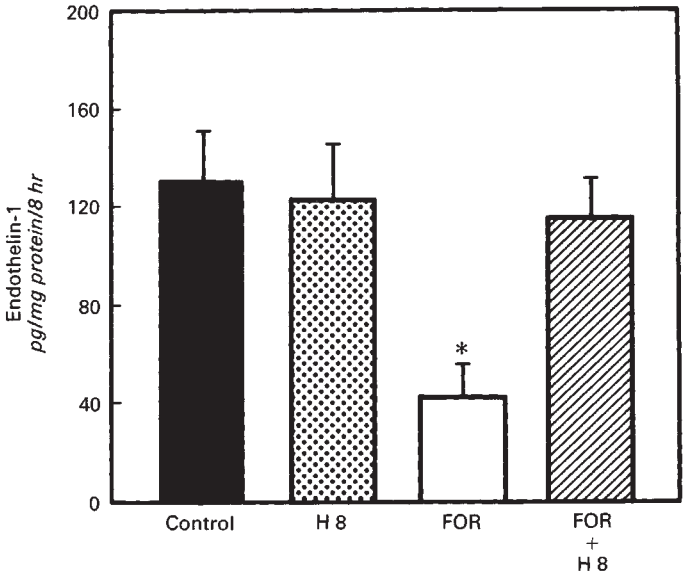

Fig. 6. *Effect of protein kinase A inhibitor, H-8, on inhibition of ET-1 production by forskolin (FOR).* Cells were exposed to H8 for 15 minutes before and during incubation without or with forskolin (20 μM) in the presence of 5% FCS (Cont). *$P < 0.01$ compared to Cont.

cultured rat MC, despite that this substance is known to act through a receptor coupled to PLC [24]. Interestingly, Ganz, Perfetto and Boron recently showed that Ang II can produce a typical transient increase in cell Ca^{2+}, but it does not cause cell proliferation in rat cultured MC [25]. In their study, other agonists, such as AVP and PDGF can stimulate MC proliferation with concomitant increase in cell Ca^{2+} and stimulation of the phosphoinositide cascade. Therefore, these results taken together may imply that another signaling pathway(s) activated by Ang II inhibits ET production.

Contrary to the stimulatory effect of the PLC/Ca^{2+}/PKC system on ET-1 production, we showed that either an increase in intracellular cAMP or the administration of exogenous cAMP inhibited serum-stimulated ET-1 production in cultured rat MC (Fig. 4). This is the first demonstration of an inhibitory factor on ET-1 production. Since the effect of cAMP on ET-1 production was abolished by H-8, cAMP may act at least partly through a PKA-dependent mechanism(s). However, interpretation of the data may need some caution since H-8 is not a pure specific inhibitor of PKA, and the dose we used (50 μM) was high, therefore it may cause non-specific effects. The cellular mechanisms of PKA on ET-1 production are not clear at present. Among the possibilities, the effect of PKA on transcription of the ET-1 gene is interesting, and this should be evaluated in future studies.

A β-adrenergic agonist, isoproterenol, inhibited serum-stimulated ET-1 production, and this effect was abolished by β-adrenergic antagonist, propranolol (Fig. 5). These effects may be due to an accumulation of cAMP caused by the stimulation of AC by isoproterenol [7, 8, 20]. The physiologic implication of this finding is that ET-1 production in MC is neurologically regulated by the signals through β-adrenergic receptor. Recently, Simmonson and Dunn demonstrated that exogenously administered ET-1 potentiates β-adrenergic-stimulated intracellular cAMP accumulation in addition to increasing the intracellular Ca^{2+} concentration [11] and hydrolyzing of inositol phosphates [26]. Since an increase in intracellular cAMP concentration could potentially dampen cell contraction caused by activation of the Ca^{2+}/PKC signaling system [26], they speculated that the cAMP-dependent signaling pathway may act as a negative feedback signal in the regulation of MC functions. Our present study may further expand this thesis regarding ET-1 production. Exogenous application of ET-1 to MC activates PLC-mediated signaling systems [26], and activation of the systems stimulates ET-1 production. Thus, the net effect would be an autonomous increase in ET-1 production. Therefore, negative feedback signals are needed to regulate ET-1 production. The cAMP/PKA system may play this role, since exogenous ET-1 addition also amplifies cAMP production if β-adrenergic stimulus is present [11]. Thus, ET-1 production in MC may be adequately regulated by the interaction of both positive and negative signals mediated by PKC- and PKA-dependent mechanisms.

The physiological role(s) of ET-1 in MC is not clear at present, but two possibilities are suspected: First, ET-1 may work as an autocrine growth factor for MC. MC are known to produce several growth factors, such as interleukin-1 [27, 28], PDGF [29], and insulin-like growth factor [30, 31], and these substances stimulate mitogenesis of their own cells in an autocrine fashion. ET-1 has also been shown to stimulate mitogenesis in MC [26, 32]. Accordingly, ET-1 may play some role in the proliferation of MC, especially in glomerulonephritis. Second, ET-1 may work as a constrictor of MC and contribute to the regulation of glomerular filtration rate. Therefore, precise regulation of ET-1 production in MC may be important in normal and pathophysiological states.

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References

1. Sakamoto H, Sasaki S, Hirata Y, Imai T, Ando K, Ida T, Sakurai T, Yanagisawa M, Masaki T, Marumo F: Production of endothelin-1 by rat cultured mesangial cells. *Biochem Biophys Res Commun* 169:462–468, 1990
2. Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, Yazaki Y, Goto K, Masaki T: A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature (Lond)* 332:411–415, 1988
3. Yanagisawa M, Inoue A, Takuwa Y, Mitsui Y, Kobayashi M, Masaki T: The human preproendothelin-1 gene: Possible regulation by endothelial phosphoinositide turnover signaling. *J Cardiovasc Pharmacol* 13 (Suppl 5):S13–S17, 1989
4. Emori T, Hirata Y, Ohta K, Shichiri M, Marumo F: Secretory mechanism of immunoreactive endothelin in cultured bovine endothelial cells. *Biochem Biophys Res Commun* 160:93–100, 1989
5. Kurihara H, Yoshizumi M, Sugiyama T, Takaku F, Yanagisawa M, Masaki T, Hamaoki M, Kato H, Yazaki Y: Transforming growth factor-β stimulates the expression of endothelin

mRNA by vascular endothelial cells. *Biochem Biophys Res Commun* 159:1435–1440, 1989
6. INOUE A, YANAGISAWA M, TAKUWA Y, MITSUI Y, KOBAYASHI M, MASAKI T: The human preproendothelin-1 gene; complete nucleotide sequence and regulation of expression. *J Biol Chem* 264:14954–14959, 1989
7. SCHLONDORFF D: The glomerular mesangial cell: An expanding role for a specialized pericyte. *FASEB J* 1:272–281, 1987
8. KREISBERG J: Cell biology and biochemistry of the glomerular mesangium. *Miner Electrol Metab* 14:167–175, 1988
9. ABBOUD HE, THROCKMORTON D, WEINSHELL E, JAFFER F: Differential modulation of mesangial cell proliferation by peptide growth factors and cyclic adenosine monophosphate (cAMP). (abstract) *FASEB J* 2:A627, 1988
10. HOMMA T, ICHIKAWA I, HOOVER RL: Prostaglandins of mesangium origin inhibit mesangial cells (MC) proliferation and matrix synthesis. (abstract) *Kidney Int* 33:268, 1988
11. SIMONSON MS, DUNN MJ: Endothelin-1 stimulates contraction of rat glomerular mesangial cells and potentiates β-adrenergic-mediated cyclic adenosine monophosphate accumulation. *J Clin Invest* 85:790–797, 1990
12. ANDO K, HIRATA Y, SHICHIRI M, EMORI T, MARUMO F: Presence of immunoreactive endothelin in human plasma. *FEBS Lett* 245: 164–166, 1989
13. HUKKELHOVEN MWAC, VROMANS E, MARKSLAG AMG, VERMORKEN AJM: A simple fluorimetric microassay for DNA in hair follicles or fractions of hair follicles. *Anticancer Res* 1:341–344, 1981
14. GRYNKIEWICZ G, PECNIE M, TSIEN RY: A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. *J Biol Chem* 260:3440–3450, 1985
15. TAKEDA K, MEYER-LEHNERT H, KIM JK, SCHRIER RW: Effect of angiotensin II on Ca^{2+} kinetics and contraction in cultured rat glomerular mesangial cells. *Am J Physiol* 254:F254–F266, 1988
16. HASSID A, PIDIKITI N, GAMERO D: Effects of vasoactive peptides on cytosolic calcium in cultured mesangial cells. *Am J Physiol* 251:F1018–F1028, 1986
17. METZGER H, LINDER E: Forskolin, a novel adenylate cyclase activator. *IRCS Med Sci* 9:99, 1981
18. SEAMON K, PADGETT W, DALY JW: Forskolin: A unique diterpene activator of adenylate cyclase in membranes and in intact cells. *Proc Natl Acad Sci USA* 78:3363–3367, 1981
19. SEAMON K, PADGETT W, DALY JW: Activation of adenylate cyclase by the diterpene forskolin does not require the guanine nucleotide regulatory protein. *J Biol Chem* 256:9799–9801, 1981
20. TROYER DA, GONZALEZ OF, VENKATACHELAM MA, KREISBERG JI: Elevation of cAMP in cultured mesangial cells diminished vasopressin-stimulated increase of phosphate uptake and ^{32}P-specific activity in ATP but has no effect on phosphoinositide metabolism. *J Biol Chem* 262:1614–1617, 1987
21. HIDAKA H, INAGAKI M, KAWAMOTO S, SASAKI Y: Isoquinolinesulfonamides, novel and potent inhibitors of cyclic nucleotide dependent protein kinase and protein kinase C. *Biochemistry* 23:5036–5041, 1984
22. RESINK TJ, HAHN AWA, SCOTT-BURDEN T, POWELL J, WEBER E, BUHLER FR: Inducible endothelin mRNA expression and peptide secretion in cultured human vascular smooth muscle cells. *Biochem Biophys Res Commun* 168:1303–1310, 1990
23. OHTA K, HIRATA Y, IMAI T, KANNO K, EMORI T, SHICHIRI M, MARUMO F: Cytokine-induced release of endothelin-1 from porcine renal epithelial cell line. *Biochem Biophys Res Commun* 169:578–584, 1990
24. PFEILSCHIFTER J: Tumor promoter 12-O-tetradecanoylpholbol 13-acetate inhibits angiotensin II-induced inositol phosphate production and cytosolic Ca^{2+} rise in rat renal mesangial cells. *FEBS Lett* 203:262–266, 1986
25. GANZ MB, PERFETTO MC, BORON WF: Effects of mitogens and other agents on rat mesangial cell proliferation, pH and Ca^{2+}. *Am J Physiol* 259:F269–F278, 1990
26. SIMONSON MS, MENE SWP, DUBYAK GR, KESTER M, NAKAZATO Y, SEDOR JR, DUNN MJ: Endothelin stimulates phospholipase C, Na/H^+ exchange, c-*fos* expression, and mitogenesis in rat mesangial cells. *J Clin Invest* 83:708–712, 1989
27. LOVETT DH, SZAMEL M, RYAN JL, STERZEL RB, GEMSA D, RESCH K: Interleukin 1 and the glomerular mesangium. I. Purification and characterization of a mesangial cell-derived autogrowth factor. *J Immunol* 136:3700–3705, 1986
28. WERBER HI, EMANCIPATOR SN, TYKOCINSKI ML, SEDOR JR: The interleukin 1 gene is expressed by rat glomerular mesangial cells and is augmented in immune complex glomerulonephritis. *J Immunol* 138:3207–3212, 1987
29. ABBOUD HE, POPTIC E, DICORLETO P: Production of platelet-derived growth factor like protein by rat mesangial cells in culture. *J Clin Invest* 80:675–683, 1987
30. ARON DC, ROSENSWERG JL, ABBOUD HE: Synthesis and binding of insulin-like growth factor I by human glomerular mesangial cells. *J Clin Endocrinol Metab* 68:585–591, 1989
31. CONTI FG, STRIKER LJ, ELLIOT SJ, ANDREANI D, STRIKER GE: Synthesis and release of insulin-like growth factor I by mesangial cells in culture. *Am J Physiol* 255:F1214–F1219, 1988
32. JAFFER FE, KNAUSS TC, POPTIC E, ABBOUD HE: Endothelin stimulates PDGF secretion in cultured human mesangial cells. *Kidney Int* 38:1193–1198, 1990