Protein kinase C-dependent prostaglandin production mediates angiotensin II-induced atrial-natriuretic peptide release

Dennis J. CHURCH,* Silvia BRACONI, Vincent VAN DER BENT, Michel B. VALLOTTON and Ursula LANG

Department of Medicine, Division of Endocrinology, University Hospital, CH-1211 Geneva 4, Switzerland

The respective roles of protein kinase C (PKC) and endogenous prostaglandin formation in angiotensin II (Ang II)-induced myocardial secretion of atrial natriuretic peptide (ANP) was studied in cultured, spontaneously beating, neonatal-rat cardiomyocytes. Incubation of cardiomyocytes with 0.1 μ M Ang II led to a rapid but transient increase in particulate-bound PKC activity, a response accompanied by marked increases in cellular 6-oxo-prostaglandin $F_{1\alpha}$ (6-oxo-PGF $_{1\alpha}$) generation and ANP secretion. A role for PKC in Ang II-induced 6-oxo-PGF $_{1\alpha}$ formation and ANP secretion was apparent, insofar as both responses were suppressed in the presence of the PKC inhibitors staurosporine (1 μ M) and CGP 41251 (1 μ M), as well as in cells in which PKC had been previously down-regulated by pretreatment with phorbol diester. Furthermore, Ang II-induced 6-oxo-

PGF $_{1\alpha}$ production was found to be strongly correlated with Ang II-induced ANP release ($r = 0.87$, $P < 0.001$, $n = 6$), indicating a role for prostacyclin (PGI $_2$) in Ang II-induced ANP secretion in these cells. This hypothesis was confirmed by finding that both Ang II-induced 6-oxo-PGF $_{1\alpha}$ production and ANP release were abolished in the presence of the respective phospholipase A_2 and cyclo-oxygenase inhibitors quinacrine (10 μ M) and indomethacin (10 μ M), whereas exogenously applied PGI $_2$ (1 μ M) and prostaglandin E_2 (0.1 μ M) mimicked Ang II-induced ANP secretion in this system. Taken together, these results suggest that Ang II induces ANP secretion in spontaneously beating rat cardiomyocytes via a PKC-dependent autocrine pathway involving a cyclo-oxygenase product and a yet-to-be-identified myocardial prostanoid receptor.

INTRODUCTION

The mammalian heart synthesizes and secretes atrial natriuretic peptide (ANP), a vasorelaxant factor displaying natriuretic and diuretic properties [1]. Although atrial stretch is generally considered to be the prime physiological stimulus inducing ANP release [2,3], little is known about the intracellular signalling mechanisms governing hormone-induced secretion of this peptide. Perfused heart, isolated atria and/or cultured cardiomyocytes secrete ANP upon stimulation with a number of vasoactive agents [4–8], and it has been suggested that protein kinase C (PKC) activation, Ca^{2+} influx, cyclic AMP formation and/or endogenous prostaglandin production play important roles in this response [9].

Numerous studies have shown the importance of angiotensin II (Ang II) in the regulation of blood pressure and the pathogenesis of hypertension [10]. In this respect, Ang II displays a multitude of cardiovascular effects, regulating intravascular volume and peripheral vascular resistance by increasing aldosterone production, renal Na^+ reabsorption and arterial smooth-muscle cell contraction [11]. Not surprisingly, Ang II also exerts a direct effect on the heart, affecting cardiac contractility and promoting ANP release [6,12,13], processes that are mediated by high-affinity myocardial Ang II receptors whose activation leads to both phospholipase C (PLC)-dependent phosphoinositide formation and Ca^{2+} mobilization in this tissue [14,15].

PKC is likely to play an important role in Ang II-induced cardiac responses, as increases in PLC activity lead to the formation of PKC-activating diacylglycerols [16]. In agreement with this hypothesis, PKC activation is a major requirement for the induction of ANP secretion by the PLC-activating agents [9].

Interestingly, we have previously reported that the activation of PKC leads to increased prostacyclin (PGI $_2$) and prostaglandin E_2 (PGE $_2$) production in spontaneously beating cultured cardiomyocytes [17], whereas others have shown that cyclo-oxygenase inhibition abolishes ANP secretion induced by PLC-activating agonists in isolated atria [18]. Considering that prostaglandins are potent ANP secretagogues in their own right [19], PKC-mediated prostaglandin production may well be at the basis of Ang II-induced myocardial ANP secretion.

We investigated this hypothesis by stimulating spontaneously beating cultured neonatal-rat cardiomyocytes with Ang II and by measuring the effect in terms of PKC activation, 6-oxo-PGF $_{1\alpha}$ formation and ANP release both in the absence and presence of various PKC, phospholipase A_2 (PLA $_2$) and cyclo-oxygenase inhibitors, and in PKC-down-regulated cells. Results indicate that Ang II-induced 6-oxo-PGF $_{1\alpha}$ production and ANP secretion are PKC-mediated events, and that endogenous agonist-induced prostaglandin production is indeed at the basis of ANP release in this system.

MATERIALS AND METHODS

Materials

[γ - ^{32}P]ATP and 6-oxo-[3H]PGF $_{1\alpha}$ were obtained from Amersham International (Amersham, Bucks., U.K.). ^{125}I -ANP was purchased from Novabiochem (Basel, Switzerland). ATP, histone III-S, 1,2-diolein, staurosporine, quinacrine and indomethacin were obtained from Sigma (St. Louis, MO, U.S.A.), as was 4 β -phorbol 12-myristate 13-acetate (PMA), dithiothreitol, γ -globulin, leupeptin, trypsin, DNAase I and Triton X-100. DEAE-cellulose DE52 was purchased from Whatman (Maidstone,

Abbreviations used: PKC, protein kinase C; Ang II, angiotensin II; ANP, atrial natriuretic peptide; 6-oxo-PGF $_{1\alpha}$, 6-oxo-prostaglandin $F_{1\alpha}$; PGI $_2$, prostacyclin; PGE $_2$, prostaglandin E_2 ; PLA $_2$, phospholipase A_2 ; PLC, phospholipase C; PMA, 4 β -phorbol 12-myristate 13-acetate.

* To whom correspondence should be addressed.

Kent, U.K.). McCoy's modified 5A medium, Hanks' $\text{Ca}^{2+}/\text{Mg}^{2+}$ -free balanced salt solution (HBSS) and foetal-calf serum (FCS) were from Gibco (Basel, Switzerland). The PKC inhibitor CGP 41251 was kindly given by Ciba-Geigy (Basel, Switzerland), and anti-6-oxo-PGF_{1 α} antiserum was generously given by Dr. M. J. Dunn (Division of Nephrology, Case Western Reserve University, Cleveland, OH, U.S.A.). Anti-ANP antiserum was obtained from Peninsula Labs (Belmont, CA, U.S.A.).

Cell culture

Neonatal-rat ventricular cardiomyocytes were obtained from 1–2-day-old Wistar rats by a modification of the method of Kem et al. [20]. Briefly, the lower (apical) two-thirds of 10–40 hearts were excised from decapitated rats and placed in 40 ml of ice-cold sterile HBSS containing 100 i.u./ml penicillin and 10 mg/ml streptomycin at 4 °C. The tissue was washed with 40 ml HBSS, cut into small pieces, further washed with 40 ml of HBSS and enzymically digested for 6 min with 10 ml of trypsin/DNAase (2.5 mg/ml and 0.3 mg/ml respectively) at 37 °C in a 50 ml sterile conical tube subjected to constant stirring. The supernatant from the first incubation was discarded, 10 ml of fresh enzyme solution was added, and the incubation procedure was repeated. Subsequent supernatants were collected and centrifuged at 200 *g* for 6 min and the resulting cell pellets were resuspended in HBSS containing 10% FCS at 37 °C. Once the sequential digestions were terminated, the cells were pooled, washed in 40 ml of McCoy's modified 5A medium containing 10% FCS, 1% insulin/transferrin/sodium selenite medium supplement (ITS), 100 i.u./ml penicillin and 10 mg/ml streptomycin, and seeded in 90 mm plastic Petri dishes as described by Blondel et al. [21]. After 3 h of incubation, the Petri dishes were shaken and the supernatants containing the cardiomyocytes were pooled and seeded in 90 mm plastic Petri dishes or six-well culture plates (Costar, Cambridge, MA, U.S.A.). Most of the cultured cells (i.e. > 90%) began to contract spontaneously within 24–48 h of plating (30–60 beats/min) and exhibited positive staining for pro-ANP, the precursor form of rat ANP (Ile¹²- α -hANP; h = human) and a specific cardiomyocyte marker [1]. 6-Oxo-PGF_{1 α} production co-purified with cellular ANP secretion when mesenchymal cells were eliminated from culture in accordance with Blondel et al. [21], indicating that 6-oxo-PGF_{1 α} production originates from myoblasts and not from contaminating cell types. Substantiating this, cultured myocytes did not contain ϵ -type PKC (rat fibroblast marker; see [17] and [22]) or bind acetylated low-density lipoprotein (endothelial-cell marker; see [23]). Confluent spontaneously beating cells were used on day 3 of culture for all experiments described herein.

Determination of PGI₂ production and ANP release

For assessment of PGI₂ formation and ANP release, six-well tissue-culture plates containing confluent spontaneously contracting cardiomyocyte monolayers were washed with 2 ml of Krebs–Ringer buffer containing 0.2% BSA and 0.2% glucose as previously described [24]. After replacing the supernatant with 1 ml of fresh buffer, the cells were incubated at 37 °C for the indicated times in the presence of the various pharmacological agents, and 500 μ l samples of the supernatants were collected and assayed for 6-oxo-PGF_{1 α} and ANP contents by radio-immunological methods previously described [25,26], omitting the semi-purification step required for assaying ANP levels in human plasma. The relative affinity of the anti-ANP antiserum

for rat atriopeptides was as follows: α -hANP and rat ANP (Ile¹²- α -ANP), 100%; rat atriopeptin III, 100%; β -hANP, 50%; γ -hANP, 40%; rat atriopeptin II, 5%; B-type natriuretic peptide (BNP), < 0.001%; and both angiotensin II and atriopeptin I, 0%. Cross-reactivity of the antiserum to 6-oxo-PGF_{1 α} has been previously studied in detail [25].

Subcellular fractionation and determination of PKC activity

Ang II-stimulated cells cultured in 90 mm plastic Petri dishes were subfractionated, and semi-purification of membrane and cytosolic PKC was carried out by DEAE-cellulose chromatography as described by Lang and Vallotton [27]. Eluates were assayed for PKC activity by measuring the incorporation of ³²P from [γ -³²P]ATP into histone III-S in the absence and presence of lipid activators as previously indicated [25].

Statistical analysis

Student–Fisher unpaired bilateral *t* tests and/or ANOVA using the Scheffe F-test criterion for unbalanced groups was used where applicable. A value of *P* < 0.05 was accepted as statistically significant. Results represent the means $\pm$ S.E.M. of at least three experiments performed in duplicate or triplicate.

RESULTS

PKC mediates Ang II-induced 6-oxo-PGF_{1 α} production and ANP release

Incubation of spontaneously beating cultured rat cardiomyocytes with 0.1 μ M Ang II led to a rapid increase in particulate-bound PKC activity (Figure 1). The response was transient, with maximal activation of PKC occurring at 5 min ($+56 \pm 20.1\%$; *n* = 3) and being followed by a rapid return to basal membrane PKC activity at 15 min. Overall, the transient increase in particulate-bound PKC activity was accompanied by a corresponding decrease in cytosolic PKC activity ($-20.8 \pm 4.2\%$, *n* = 4 at 5 min), indicating that Ang II induces the activation of cardiomyocyte PKC in a manner similar to, if not identical with, that previously reported for phorbol diesters in this preparation [17].

Figure 1 Ang II-induced PKC activation in rat cardiomyocytes

Cultured cardiomyocytes were stimulated with 0.1 μ M Ang II for the indicated times, subfractionated and assayed for PKC activity as described in the Materials and methods section. Membrane (●) and cytosolic (○) PKC activity is expressed as a percentage of control (basal) values. Results represent means $\pm$ S.E.M. of 3–4 separate experiments performed in duplicate determinations: **P* < 0.05 versus control.

Figure 2 Ang II-induced 6-oxo-PGF_{1α} production

Cultured cardiomyocytes were incubated with 0.1 μM Ang II for the indicated times (a) or with various concentrations of Ang II for 1 h (b), and 6-oxo-PGF_{1α} was determined as described in the Materials and methods section. (a) Time course of Ang II-induced 6-oxo-PGF_{1α} production: ●, Ang II-stimulated cells; ○, controls. (b) Concentration-dependence of Ang II-induced 6-oxo-PGF_{1α} production (C, control). Results shown represent means ± S.E.M. of 4 separate experiments performed in triplicate determinations: **P* < 0.05 versus control.

As shown in Figures 2 and 3, Ang II-induced PKC activation was accompanied by marked increases in both cellular PGI₂ production and ANP release. The kinetics of both responses were similar, with 0.1 μM Ang II inducing significantly increased 6-oxo-PGF_{1α} production (from 44 ± 10 to 181 ± 31 pg/mg of protein, *n* = 4) and ANP secretion (from 0.74 ± 0.24 to 1.85 ± 0.36 ng/mg of protein, *n* = 4) within 15 min of application to cell monolayers (Figures 2a and 3a respectively). Not surprisingly, Ang II exhibited similar concentration-dependent

Figure 3 Ang II-induced ANP secretion

Cultured cardiomyocytes were incubated with 0.1 μM Ang II for the indicated times (a) or with various concentrations of Ang II for 1 h (b), and ANP release was determined as described in the Materials and methods section. (a) Time course of Ang II-induced ANP secretion: ●, Ang II-stimulated cells; ○, controls. (b) Concentration-dependence of Ang II-induced ANP secretion (C, control). Results shown represent means ± S.E.M. of 4 separate experiments performed in triplicate determinations: **P* < 0.05 versus control.

properties in both instances, the EC₅₀s for Ang II-induced 6-oxo-PGF_{1α} production and ANP release being estimated at 12 ± 7.6 nM and 3 ± 1.1 nM respectively (Figures 2b and 3b, *n* = 4).

As PKC activation is a potent signal for both cardiomyocyte prostaglandin formation and ANP secretion [9,17], we further investigated the role of PKC in Ang II-induced cellular responses. As shown in Table 1, incubation of cells with 0.1 μM Ang II in

Table 1 Effect of PKC inhibition on Ang II-induced 6-oxo-PGF_{1α} production and ANP secretion

Cultured cardiomyocytes were incubated with 0.1 μM Ang II for 1 h in the absence or presence of staurosporine (1 μM) or CGP 41251 (1 μM), and 6-oxo-PGF_{1α} production and ANP secretion were determined as described in the Materials and methods section. Results represent means ± S.E.M. of *n* experiments (in parentheses) performed in triplicate determinations: **P* < 0.05 versus control.

Treatment	6-Oxo-PGF _{1α} (pg/mg of protein)		ANP release (ng/mg of protein)	
	Control	Ang II (0.1 μM)	Control	Ang II (0.1 μM)
None (<i>n</i> = 9)	99.5 ± 14.2	394 ± 58.8*	2.4 ± 0.16	3.6 ± 0.24*
Staurosporine (1 μM) (<i>n</i> = 8)	71.7 ± 19.0	137.3 ± 34.6	2.2 ± 0.28	2.5 ± 0.30
CGP 41251 (1 μM) (<i>n</i> = 4)	81.3 ± 10.5	187.0 ± 41.8	2.8 ± 0.48	3.0 ± 0.40

Figure 4 Effect of PKC down-regulation on Ang II-induced 6-oxo-PGF_{1α} production and ANP secretion

Cultured cardiomyocytes were pretreated with 0.1 μ M PMA, washed twice in Krebs–Ringer buffer and further incubated with 0.1 μ M Ang II for 1 h. 6-Oxo-PGF_{1α} formation (●) and ANP secretion (○), expressed as percentages of Ang II-induced 6-oxo-PGF_{1α} formation and ANP release in untreated (control) cells, were determined as described in the Materials and methods section. Results shown represent means $\pm$ S.E.M. of 5 separate experiments performed in triplicate determinations: * P < 0.05 versus control.

the presence of either staurosporine (1 μ M) or CGP 41251 (1 μ M), a staurosporine derivative displaying highly selective PKC-inhibitory properties [28], decreased Ang II-induced 6-oxo-PGF_{1α} production by 83% and 64% respectively. Similar inhibitory effects of both staurosporine and CGP 41251 were observed for Ang II-induced ANP secretion (75% and 83% inhibition respectively; see Table 1), suggesting that PKC activation accounts for a substantial portion of Ang II-induced PGI₂ production and ANP release in this preparation.

Confirming this hypothesis, Ang II-induced 6-oxo-PGF_{1α} production and ANP release were severely decreased in cells pretreated with PMA (0.1 μ M), a widely used experimental method for the investigation of PKC-dependent processes [16]. In untreated cells, Ang II increased 6-oxo-PGF_{1α} formation and ANP release from 123 ± 13 to 343 ± 38 pg/mg of protein and from 2.98 ± 0.27 to 3.64 ± 0.28 ng/mg of protein respectively. In contrast, after 3 h of exposure to 0.1 μ M PMA, Ang II did not significantly change 6-oxo-PGF_{1α} and ANP release, which only increased from 208 ± 28 to 270 ± 41 pg/mg of protein and from 3.25 ± 0.45 to 3.32 ± 0.50 ng/mg of protein respectively. Thus, as shown in Figure 4, pretreatment of cells with 0.1 μ M PMA for 3 h led to the inhibition of both Ang II-induced 6-oxo-PGF_{1α}

Table 3 Effect of endogenously produced prostaglandins on ANP secretion

Cultured cardiomyocytes were incubated at 37 °C for 1 h with either 1 μ M rat ANP (Ile¹²-α-ANP) or 0.5 mM 8-bromo cyclic GMP (8Br-cGMP), and 6-oxo-PGF_{1α} production (panel a) was determined as described in the Materials and methods section. Alternatively, cultured cells were incubated at 37 °C for 1 h with either 1 μ M PGI₂ or 0.1 μ M PGE₂, and ANP secretion was determined as described in the Materials and methods section (panel b). Results shown represent means $\pm$ S.E.M. of 3 separate experiments performed in triplicate determinations: * P < 0.05 versus control.

(a)	
Treatment	6-Oxo-PGF _{1α} (pg/h per mg of protein)
Control	125.6 $\pm$ 16.5
ANP	169.3 $\pm$ 20.6
8-Br-cGMP	161.3 $\pm$ 44.0
(b)	
Treatment	ANP release (ng/h per mg of protein)
Control	2.7 $\pm$ 0.08
PGI ₂	3.6 $\pm$ 0.04*
PGE ₂	3.9 $\pm$ 0.13*

production and ANP release (69% and 88% respectively), an inhibitory response nearly identical with that obtained with staurosporine and CGP 41251.

Endogenous prostaglandin production is at the basis of Ang II-induced ANP secretion

The profile of Ang II-induced 6-oxo-PGF_{1α} production is similar to that obtained for Ang II-induced ANP release, suggesting that endogenous prostaglandin production is at the basis of ANP secretion in this system. Indeed, Ang II-induced 6-oxo-PGF_{1α} production was strongly correlated with cellular ANP release in experiments in which both variables were simultaneously tested ($r = 0.87$, P < 0.001, $n = 6$ at 15 min), whereas inhibition of both PLA₂ and cyclo-oxygenase severely decreased Ang II-induced ANP secretion in this preparation. As shown in Table 2, the purported PLA₂ inhibitor quinacrine (10 μ M) decreased Ang II-induced 6-oxo-PGF_{1α} production and ANP release by 61% and 76% respectively, an effect mimicked by the cyclo-oxygenase inhibitor indomethacin (10 μ M; 67% and 83% inhibition respectively).

Table 2 Inhibition of Ang II-induced 6-oxo-PGF_{1α} production and ANP secretion by quinacrine and indomethacin

Cultured cardiomyocytes were incubated with 0.1 μ M Ang II for 1 h in the absence or presence of quinacrine (10 μ M) or indomethacin (10 μ M), and 6-oxo-PGF_{1α} production and ANP secretion were determined as described in the Materials and methods section. Results represent means $\pm$ S.E.M. of n experiments (in parentheses) performed in triplicate determinations: * P < 0.05 versus control.

Treatment	6-Oxo-PGF _{1α} (pg/mg of protein)		ANP release (ng/mg of protein)	
	Control	Ang II (0.1 μ M)	Control	Ang II (0.1 μ M)
None ($n = 4$)	109.6 $\pm$ 26.8	380.7 $\pm$ 40.4*	2.6 $\pm$ 0.16	3.7 $\pm$ 0.17*
Quinacrine (10 μ M) ($n = 3$)	58.7 $\pm$ 8.0	158.1 $\pm$ 35.2	2.8 $\pm$ 0.52	3.1 $\pm$ 0.36
Indomethacin (10 μ M) ($n = 4$)	70.3 $\pm$ 22.9	132.0 $\pm$ 24.4	3.0 $\pm$ 0.22	3.2 $\pm$ 0.26

Spontaneously beating cultured cardiomyocytes simultaneously produce both PGI₂ and PGE₂ after PKC activation [17]. In this light, we finally incubated cells with both PGI₂ and PGE₂ and tested for enhanced ANP release. As shown in Table 3(a), neither 1 μ M ANP nor 0.5 mM 8-bromo cyclic GMP (a non-hydrolysable analogue of the intracellular effector of ANP-receptor activation; see [1]) had an effect on cardiomyocyte 6-oxo-PGF_{1 α} formation. In contrast, incubation of cells with either 1 μ M PGI₂ or 0.1 μ M PGE₂ led to significant increases in ANP release (Table 3b), responses confirming the hypothesis that endogenous prostaglandin production is at the basis of Ang II-induced ANP release in this system.

DISCUSSION

Taken together, the present results strongly suggest that Ang II-induced ANP secretion in spontaneously beating rat cardiomyocytes occurs via a PKC-dependent autocrine pathway involving a cyclo-oxygenase product and a yet-to-be-identified myocardial prostanoid receptor. This is supported by evidence that incubation of cardiomyocytes with Ang II simultaneously augments cellular 6-oxo-PGF_{1 α} formation, ANP release and membrane PKC activity, and that Ang II-induced ANP secretion is severely decreased in the presence of PKC, PLA₂ and cyclo-oxygenase inhibitors, as well as in PKC-down-regulated cells. Indeed, Ang II-induced 6-oxo-PGF_{1 α} production and ANP release exhibited strikingly similar kinetic profiles, and the EC₅₀s for these responses are indistinguishable from the K_d for Ang II-binding in rat myocardial tissue [14]. Finally, PGI₂ and/or PGE₂ appear to be the prostaglandin mediators underlying Ang II-induced ANP release in this system, as both compounds induced an increase in ANP secretion in this preparation.

These results are consistent with the observation that PKC activation leads to rapid PGI₂, PGE₂ and ANP release in cultured rat cardiomyocytes [9,17] and are also in agreement with the finding that cardiomyocytes contain angiotensin II receptors of the AT₁ subtype [14], the activation of which leads to PLC-mediated phosphoinositide formation and PKC activation in this and/or in other systems [15,27]. Indeed, PKC activation appears to be the principal signalling component underlying Ang II-induced responses in these cells, as phorbol diester pretreatment severely inhibited both Ang II-induced 6-oxo-PGF_{1 α} production and ANP release. In this context, it can be surmised that a certain amount of cytosolic PKC must be readily available in the basal (i.e. non-stimulated) state in order for Ang II to induce PKC translocation and subsequent prostaglandin-mediated ANP secretion, a conclusion in agreement with previous reports indicating that PKC activation is at the heart of ANP secretion induced by most PLC-activating agonists [9].

Nevertheless, the exact nature of the cyclo-oxygenase metabolite mediating Ang II-induced ANP release remains to be fully elucidated. The concentration of exogenous PGI₂ required for inducing ANP secretion was approx. 1000-fold higher than the amount of 6-oxo-PGF_{1 α} released by cardiomyocytes after stimulation with Ang II, suggesting that PGE₂ is a more likely candidate for prostaglandin-mediated Ang II-induced ANP release. In agreement with this, nanomolar concentrations of PGE₂ suffice in promoting ANP secretion in cultured cardiomyocytes (EC₅₀ = 32 $\pm$ 5 nM, *n* = 4; D. J. Church, unpublished work) and it has previously been shown that PKC activation leads to substantial PGE₂ synthesis in these same cultures [17]. Alternatively, the discrepancy between 6-oxo-PGF_{1 α} and PGI₂ levels may be explained in terms of PGI₂ stability, 6-oxo-PGF_{1 α}

hydrophobicity, and/or by the fact that 6-oxo-PGF_{1 α} may not be the only stable PGI₂ metabolite present in cardiomyocyte cultures. If this is the case, 6-oxo-PGF_{1 α} levels may only represent a fraction of the PGI₂ which is actually synthesized by these cells. Clearly, future studies may require the identification of the PGI₂ metabolites present in cardiomyocyte cultures, as well as prostaglandin-receptor antagonist studies in order to determine which endogenously produced prostaglandin is at the basis of Ang II-induced ANP secretion in this system.

Ang II-induced increases in ANP secretion have previously been demonstrated both *in vivo* and in isolated atrial preparations [6,13]. Although the latter studies suggest that Ang II-induced ANP release is unrelated to secondary pressor effects that occur upon intravascular injection of Ang II *in vivo* [11], the innervation of the atrium is a rich source of catecholamines, agents which are potent ANP secretagogues *in vitro* [29]. In this respect, our results constitute the first report indicating that Ang II-induced ANP secretion occurs via the direct activation of spontaneously beating cardiomyocytes, a conclusion reinforcing the hypothesis that elevations of plasma renin, and subsequently Ang II, are directly responsible for the elevation of plasma ANP observed in models of heart failure [30]. Interestingly, ANP has been shown to inhibit both aldosterone secretion and renin release [31,32], illustrating a subtle retro-control of the mechanism of Ang II-induced ANP secretion at the physiological level.

Finally, ANP has been shown to inhibit PKC-mediated responses in a variety of systems, a property carried out via the activation of cellular ANP receptors possessing guanylate cyclase properties [1]. In this light, the lack of effect of both ANP and 8-bromo cyclic GMP on cellular 6-oxo-PGF_{1 α} formation is consistent with the view that ANP exerts a negative feedback inhibition on cardiac tissue [33] and thus does not contribute to Ang II-induced cardiomyocyte stimulation. Conversely, both PGI₂ and PGE₂ are noted for their myocardium-stimulating effects, a property carried out through the receptor-mediated activation of adenylate cyclase [34], an event leading to increases in both cellular contraction frequency and Ca²⁺ influx in this tissue [35,36]. Interestingly, it has been shown that Ang II induces similar chronotropic and Ca²⁺-influx-activating effects in cultured neonatal cardiomyocytes [12], suggesting that PKC-dependent prostaglandin formation is also at the basis of Ang II-induced changes in cardiomyocyte contractility, a hypothesis indirectly confirmed by the observation that phorbol diesters mimic Ang II-induced chronotropic responses in cultured myocytes [37], whereas PMA induces a cyclo-oxygenase-product dependent increase in cyclic AMP formation in similar preparations [38].

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REFERENCES

- 1 Inagami, T. (1989) *J. Biol. Chem.* **264**, 3043–3046
- 2 Dietz, J. R. (1984) *Am. J. Physiol.* **237**, R1093–R1096
- 3 Lang, R. E., Tholken, H., Ganten, D., Luft, F. C., Ruskoaho, H. and Unger, T. (1985) *Nature (London)* **314**, 264–266
- 4 Mäntymä, P., Leppaluoto, J. and Ruskoaho, H. (1990) *Endocrinology (Baltimore)* **126**, 587–595
- 5 Gibbs, D. M. (1987) *Regul. Pept.* **19**, 73–78
- 6 Veress, A. T., Milojevic, S., Yip, C., Flynn, T. G. and Sonnenberg, H. (1988) *Am. J. Physiol.* **254**, R809–R814

- 7 Schiebinger, R. J. and Santora, A. C. (1989) *Endocrinology* (Baltimore) **124**, 2473–2479
- 8 Schiebinger, R. J. (1988) *Am. J. Physiol.* **255**, H1429–H1433
- 9 Ruskoaho, H., Kinnunen, P. and Mäntymaa, P. (1991) *Can. J. Physiol.* **69**, 1514–1524
- 10 Swales, J. (1993) in *The Renin–Angiotensin System* (Iän, J., Robertson, S. and Nicholls, M. G., eds.), vol. 2, pp. 62.1–62.12. Gower Medical Publishing, London
- 11 Vallotton, M. B. (1987) *Trends Pharmacol. Sci.* **8**, 69–74
- 12 Allen, I. S., Cohen, N. M., Dhallan, R. S., Gaa, S. T., Lederer, W. J. and Rogers, T. B. (1988) *Circ. Res.* **62**, 524–534
- 13 Sonnenberg, H., Milojevic, S., Yip, C. and Veress, A. T. (1989) *Can. J. Physiol. Pharmacol.* **67**, 1365–1368
- 14 Rogg, H., Schmid, A. and de Gasparo, M. (1990) *Biochem. Biophys. Res. Commun.* **173**, 416–422
- 15 Baker, K. M. and Singer, H. A. (1988) *Circ. Res.* **62**, 896–904
- 16 Nishizuka, Y. (1992) *Science* **258**, 607–614
- 17 Church, D. J., Braconi, S., Vallotton, M. B. and Lang, U. (1993) *Biochem. J.* **290**, 477–482
- 18 Zongazo, M. A., Carayon, A., Masson, F., Maistre, G., Noe, E., Eurin, J., Barthelemy, C., Komajda, M. and Legrand, J. C. (1991) *Eur. J. Pharmacol.* **209**, 45–55
- 19 Kovacic-Milivojevic, B., Schultz, H. D. and Gardner, D. G. (1990) *Can. J. Physiol.* **69**, 1493–1499
- 20 Kem, D. C., Johnson, E. I. M., Capponi, A. M., Chardonnens, D., Lang, U., Blondel, B., Koshida, H. and Vallotton, M. B. (1991) *Am. J. Physiol.* **261**, C77–C85
- 21 Blondel, B., Roijen, I. and Cheneval, J. P. (1971) *Experientia* **27**, 356–358
- 22 Borner, C. S., Guadagno, S. N., Fabbro, D. and Weinstein, I. B. (1992) *J. Biol. Chem.* **267**, 12892–12899
- 23 Stein, O. and Stein, Y. (1980) *Biochim. Biophys. Acta* **620**, 631–635
- 24 Vallotton, M. B., Gerber-Wicht, C., Dolci, W. and Wüthrich, R. P. (1989) *Am. J. Physiol.* **257**, E617–E624
- 25 Chardonnens, D., Lang, U., Rossier, M. F., Capponi, A. M. and Vallotton, M. B. (1990) *J. Biol. Chem.* **265**, 10451–10457
- 26 Shenker, Y., Sider, R. S., Ostafin, E. A. and Grekin, R. J. (1985) *J. Clin. Invest.* **76**, 1684–1687
- 27 Lang, U. and Vallotton, M. B. (1989) *Biochem. J.* **259**, 477–484
- 28 Meyer, T., Regenass, U., Fabbro, D., Alteri, E., Rösel, J., Müller, M., Cravatti, G. and Matter, A. (1989) *Int. J. Cancer* **43**, 851–856
- 29 Matsubara, H., Hirata, Y., Yoshimi, H., Takata, S., Takagi, Y., Umeda, Y., Yamane, Y. and Inada, M. (1988) *Am. J. Physiol.* **255**, H405–H409
- 30 Crozier, I. G., Richards, A. M., Nicholls, M. G., Espiner, E. A., Ikram, H. and Yandle, T. G. (1988) *Clin. Exp. Pharmacol. Physiol.* **15**, 173–183
- 31 Naruse, M., Obana, K., Naruse, K., Yamaguchi, H., Demura, H., Inagami, T. and Shizume, K. (1987) *J. Clin. Endocrin. Metab.* **64**, 10–16
- 32 Richards, A. M. and Nicholls, M. G. (1993) in *The Renin–Angiotensin System* (Iän, J., Robertson, S. and Nicholls, M. G., eds.), vol. 1, pp. 36.1–36.17. Gower Medical Publishing, London
- 33 Ruskoaho, H., Toth, M., Ganten, D., Unger, T. and Lang, R. E. (1986) *Biochem. Biophys. Res. Commun.* **139**, 266–274
- 34 Metsä-Ketelä, T. (1991) *Acta Physiol. Scand.* **223**, 481–485
- 35 Morgan, R. (1991) *N. Engl. J. Med.* **325**, 625–632
- 36 Allouati, G., Serazzi, L. and Levi, R. (1991) *J. Mol. Cell. Cardiol.* **23**, 851–860
- 37 Dösemeci, A., Dhallan, R. S., Cohen, N. M., Lederer, W. J. and Rogers, T. B. (1988) *Circ. Res.* **62**, 347–357
- 38 Church, D. J., Lang, U. and Vallotton, M. B. (1992) *J. Hypertension* **10** (suppl. 4), S51