Angiotensin II stimulates cyclic ADP-ribose formation in neonatal rat cardiac myocytes

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To examine the role of cyclic ADP-ribose (cADP-ribose) as a second messenger downstream of angiotensin II (Ang II) receptor activation in the heart, ADP-ribosyl cyclase activity was measured in a crude membrane fraction of ventricular myocytes. Ang II at 10–100 nM increased ADP-ribosyl cyclase activity by 40–90 % in the ventricular muscle of neonatal (2–4-day-old) rats, but not in fetal or adult hearts. This increase was inhibited by the Ang II antipeptide. Stimulation of ADP-ribosyl cyclase was reproduced by GTP and guanosine 5′-[γ-thio]triphosphate, and prevented by guanosine 5′-[β-thio]diphosphate. Prior treatment of the rats with cholera toxin A and B subunits also blocked the Ang II-induced activation. The density of Ang II receptors detected as [^{3}H]Ang II binding was higher in neonatal than adult rats. These results demonstrate the existence of a signalling pathway from Ang II receptors to membrane-bound ADP-ribosyl cyclase in the ventricular muscle cell and suggest that the Ang II-induced increase in cADP-ribose synthesis is involved in the regulation of cardiac function and development.

Key words: ADP-ribosyl cyclase, angiotensin II receptor, NAD^+, signal transduction, ventricular muscle.

INTRODUCTION

Angiotensin II (Ang II) is important in blood pressure control and cardiac hypertrophy in response to mechanical stress [1–3]. The effect of Ang II is exerted through the Ang II receptors AT_1 and AT_2, whereas most of the known functions of Ang II in the cardiovascular system are mediated through AT_1. AT_2 expression is abundant during the fetal stage but rapidly decreases soon after birth to the level seen in adult organs. Interestingly, the genes encoding AT_1 and AT_2 are induced after injury [4,5]. Stimulation with Ang II leads to the activation of phospholipase C [1–3], resulting in the production of diacylglycerol and inositol trisphosphate, which in turn results in the mobilization of intracellular Ca^{2+} and the activation of protein kinase C [6]. Subsequently, the Ras and mitogen-activating protein kinase pathway is activated [6,7]. Recently, it has been reported that Ang II activates calcineurin, a Ca^{2+}-dependent phosphatase, which dephosphorylates the nuclear factor NF-AT3, and then accelerates the translocation of NF-AT3 into the nucleus [8,9]. This process involving Ang II has been shown to be highly critical for the activated transcription of fetal genes, which leads to cardiac hypertrophy [8–10]. Interestingly, this process is inhibited by the immunosuppressants cyclosporin A or FK506 [9,10], whose endogenous ligand might be cyclic ADP-ribose (cADP-ribose) [11–13]. In the heart, cADP-ribose and Ca^{2+} co-operate in the activation of type II ryanodine receptors to release Ca^{2+} [14–17], which is called Ca^{2+}-induced Ca^{2+} release [18–20]. It would therefore be of interest to test whether the concentration of cADP-ribose increases after stimulation of the Ang II receptor.

Membrane-bound and cytosolic ADP-ribosyl cyclases that synthesize cADP-ribose from NAD^+ are detected in mammalian tissue [11], including the heart [21–23]. The formation of cADP-ribose is increased by the stimulation of M_1 and M_3 muscarinic acetylcholine receptors or decreased by M_2 and M_4 receptors in a subtype-specific manner in NG108-15 neuronal cells [24], and is stimulated by isoprenaline (isoproterenol) through β-adrenoceptors in the rat heart [23]. ADP-ribosyl cyclase thus seems to be coupled directly to neurotransmitter or hormone receptors via different G-proteins in the surface membrane of these cells [25]. The same control of cADP-ribose formation might be effective by ventricular Ang II receptors. To address this question, we measured ADP-ribosyl cyclase activity in crude membrane fractions of ventricular myocytes of rats of different ages in the presence or absence of Ang II, or its analogues, an Ang II receptor-subtype-specific agonist and an antagonist, or GTP. We also examined this activity in rats treated with holo-cholera toxin (CTx).

EXPERIMENTAL

Materials

β-[2,8-*adenine*-^{3}H]NAD^+ (30.5 Ci/mmol) and [*tyrosyl*-3,5-^{3}H(N)]Ang II (5-L-isoleucine) ([^{3}H]Ang II; 50 Ci/mmol) were purchased from New England Nuclear (Boston, MA, U.S.A.). cADP-ribose was obtained from either Yamasa Shoyu (Choshi, Japan) or Sigma (St Louis, MO, U.S.A.). Oxidized β-nicotinamide guanine dinucleotide (β-NGD^+), human Ang II, human Ang I, [sarcosine^1,Ala^8]Ang II and Ang II antipeptide (Glu-Gly-Val-Tyr-Val-His-Pro-Val) were obtained from Sigma, and human Ang II and CGP 42112A [*N*-α-nicotinoyl-Tyr-Lys(benzyloxycarbonyl-Arg)-His-Pro-Ile] from the Peptide Institute (Osaka, Japan). Azide-free CTx was obtained from Funakoshi (Tokyo, Japan). Candesartan (CV-11974) was a gift from Takeda Chemi-

Abbreviations used: Ang II, angiotensin II; AT_1 and AT_2, Ang II receptors; cADP-ribose, cyclic ADP-ribose; CTx, cholera toxin; β-NGD^+, oxidized β-nicotinamide guanine dinucleotide.

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cal Industries (Osaka, Japan). Silica Gel 60 F254 plastic TLC sheets were obtained from Merck (Darmstadt, Germany).

Membrane preparation

Wistar rats of various ages were used. Ventricular heart muscles from cold-anaesthetized rats were washed once in ice-cold PBS. Minced myocytes were suspended in 10 mM Tris/HCl solution, pH 7.3, with 5 mM $MgCl_2$ (5 ml for each ventricle) at 4 °C for 30 min [26]. The suspension was homogenized in a Teflon–glass homogenizer; the resultant homogenate was centrifuged at 4 °C for 5 min at 1000 ***g*** to remove unbroken cells and nuclei. Crude membrane fractions were prepared by centrifugation (twice) of homogenates at 105000 ***g*** for 15 min. The supernatant was removed and the final pellet was dispersed in 10 mM Tris/HCl solution, pH 6.6 [24]. For each experiment, membranes were freshly prepared and used immediately for enzymic reactions. In some experiments, rats were injected intraperitoneally with CTx (100 ng/g body weight) 8 h before being killed.

ADP-ribosyl cyclase assay

Each 20 μl reaction mixture contained 50 mM Tris/HCl, pH 6.6, 100 mM KCl, 10 μM $CaCl_2$, 2 μM NAD^+, 0.1 μM [2,8-*adenine*-^{3}H]NAD^+ (0.06 μCi) and 0.40–7.16 μg of membrane proteins, in accordance with a formula reported previously [24]. The optimal conditions for measuring ADP-ribosyl cyclase activity have been reported elsewhere [23]. Reaction mixtures were incubated for 0.5–4 min at 37 °C; the reactions were stopped by the addition of 2 μl of 10 % or 48 % (w/v) trichloroacetic acid. Aliquots were then centrifuged for 1 min at 2100 ***g***; 2 μl of the supernatant was spotted on silica-gel plastic thin-layer sheets (20 cm × 10 cm), which were developed in the ascending direction for 40–70 min at 23 °C with a mixture of water/ethanol/NH_4HCO_3 (30 % : 70 % : 0.2 M or 36 % : 64 % : 0.3 M), as reported previously [24]. The positions of authentic cADP-ribose, ADP-ribose and NAD^+ were detected by means of UV illumination and those of ^{3}H-labelled products were confirmed autoradiographically in each run, as reported previously [23]. Corresponding areas (approx. 1 cm × 0.7 cm) were removed and the radioactivity was counted in a liquid-scintillation counter.

Fluorimetric measurement of ADP-ribosyl cyclase

ADP-ribosyl cyclase activity was also determined fluorimetrically by using a technique based on the measurement of the conversion of β-NGD^+ into the fluorescent product cGDP-ribose, as described previously [27,28]. In brief, 2.5 ml of reaction mixtures containing 60 μM β-NGD^+, 50 mM Tris/HCl, pH 6.6, 100 mM KCl, 10 μM $CaCl_2$ and membranes (1.5–20 μg of protein) were maintained at 37 °C under constant stirring. The samples were then excited at 300 nm and fluorescence emission was continuously monitored at 410 nm in a Shimadzu (Kyoto, Japan) RF-5300PC spectrofluorophotometer.

Assay of [^{3}H]Ang II binding

Cell membrane fractions were suspended in 50 mM Tris/HCl buffer, pH 7.4, to a protein concentration of approx. 0.5 mg/ml. Aliquots (0.2 ml) of the final binding mixture consisting of 50 mM Tris/HCl, pH 7.4, 0.5 % BSA, various concentrations of [^{3}H]Ang II, and membrane fraction (usually 0.01 mg of protein) in plastic tubes were incubated for 5 min at 25 °C by a method reported previously [29]. The incubation was terminated by adding 1 ml of ice-cold solution containing 50 mM Tris/HCl, pH 7.4, to each; the reaction mixtures were filtered on Whatman GF/B glass fibre filters. The filters were washed three times and dried, then rinsed in scintillation fluid. After immersion for at least 2 h at room temperature, radioactivity was measured with an Aloka liquid-scintillation spectrometer. Unlabelled Ang II (10 μM) was added to duplicate tubes to estimate non-specific binding. Specific binding was usually approx. 40–60 % of total bound radioactivity.

RESULTS

ADP-ribosyl cyclase activity in cardiac myocytes measured by TLC

[^{3}H]cADP-ribose and [^{3}H]ADP-ribose were generated from [^{3}H]NAD^+ by the crude membrane preparation of ventricular myocytes of neonatal (2–4-day-old) rats. During an incubation period of 4 min, autoradiograms indicated that most NAD^+ was converted into either ADP-ribose or cADP-ribose or both (results not shown). When a large amount of membrane fraction (4.38–7.16 μg/20 μl of reaction mixture) was used as an enzyme source, [^{3}H]cADP-ribose was rapidly formed and converted into [^{3}H]ADP-ribose, probably reflecting the two-step reaction of ADP-ribosyl cyclase and subsequent cADP-ribose hydrolase [23]. However, when membrane proteins (0.90–1.55 μg) in the reaction mixture were reduced, [^{3}H]cADP-ribose formation increased at a constant rate for at least the first 1–2 min of incubation with 2.11 μM NAD^+ as substrate (Figure 1). The average specific activity of ventricular myocytes of neonatal rats calculated over the first 2 min was 3.81 ± 0.39 nmol/min per mg of protein (mean ± S.E.M., $n = 4$).

Effects of Ang II on ADP-ribosyl cyclase activity

The addition of 10 nM Ang II at zero time increased the rate of [^{3}H]cADP-ribose production (Figure 1) to a mean of 5.57 ± 0.48 nmol/min per mg of protein ($n = 4$). The effect of different ligand concentrations on ADP-ribosyl cyclase activity is shown in Figure 2. The maximum activation found was 185.7 ± 23.3 % ($n = 4$) of the control activity at 10 nM Ang II ($P < 0.002$, Student's t test).

Stimulation of ADP-ribosyl cyclase was not specific to Ang II; it was also obtained with 10 nM Ang I (167.0 ± 10.1 %, $n = 3$). The same concentrations of an Ang II antagonist, [Sar^1,Ala^8]Ang II, or an Ang II receptor antagonist, Ang II antipeptide, had

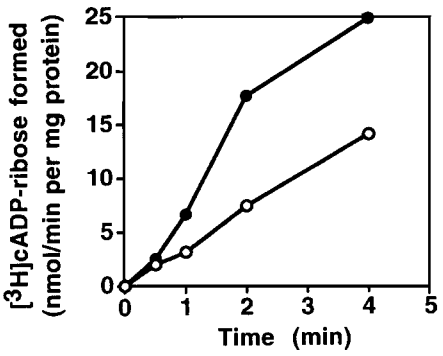

Figure 1 Effect of Ang II on ADP-ribosyl cyclase activity in rat cardiac myocytes

Time course of ADP-ribosyl cyclase activity in membranes prepared from ventricular myocytes (1.59 μg/20 μl of sample). Reaction mixtures were incubated with (●) or without (○) 10 nM Ang II for the indicated periods. Results are means for duplicate determinations of one representative experiment of five with similar results.

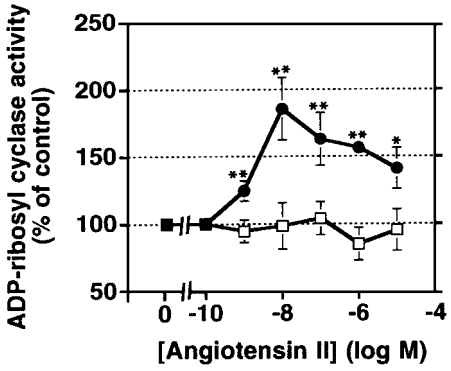

Figure 2 Effect of Ang II and Ang II antipeptide on ADP-ribosyl cyclase activity in rat cardiac myocytes

Relationship between Ang II concentration and ADP-ribosyl cyclase activity of ventricular cell membranes with (□) or without (●) 10 μM Ang II antipeptide. Each 20 μl reaction mixture containing components described in the Experimental section, with various Ang II concentrations as indicated, was incubated for 1 min. Values are percentages of the activity assayed in the absence of either reagent (6.16 $\pm$ 2.66 and 5.66 $\pm$ 2.69 nmol/min per mg of protein). Results are means $\pm$ S.E.M. for four measurements of duplicate determinations. *Significantly different ($P < 0.005$ and $P < 0.05$) from control activity (100% without Ang II) and from the activity with propranolol at the indicated isoprenaline concentrations; **$P < 0.002$ and $P < 0.002$ respectively.

essentially no effect on the cyclase activity: 96.5 $\pm$ 5.1 % ($n = 8$) and 97.9 $\pm$ 7.8 % ($n = 5$) of the control activity respectively.

Simultaneous application of 10 μM Ang II antipeptide with various concentrations (0.1 nM to 10 μM) of Ang II inhibited the stimulation of ADP-ribosyl cyclase by Ang II (Figure 2). Similarly, [Sar^1,Ala^8]Ang II (10 μM) suppressed the effect of Ang II (results not shown).

The addition of 10 nM GTP resulted in a detectable increase in enzyme activity. The maximum stimulation was obtained at 10 nM GTP, with an average increase to 168.3 $\pm$ 10.7 % ($n = 15$) of the control value ($P < 0.001$). The same degree of stimulation was obtained by the simultaneous addition of 10 nM GTP and Ang II, which was neither additive nor synergistic. Thus the Ang II-receptor-mediated activation of ventricular ADP-ribosyl cyclase seems to share the G-protein-mediated pathway.

Effects of Ang II on the formation of cGDP-ribose

ADP-ribosyl cyclase activity can be detected by the fluorimetric assay of accumulated cGDP-ribose from a hydrolysis-resistant substrate, β-NGD$^+$ [27,28]. With this method it was possible to measure more precisely the mechanism of coupling of Ang II receptors to the activation of ADP-ribosyl cyclase. Incubation of 60 μM β-NGD$^+$ with cell membranes (1.5–20 μg/2.5 ml of reaction mixture) prepared from neonatal (2–4-day-old) rat hearts resulted in a progressive and linear increase in fluorescence (Figure 3), as demonstrated previously in cardiac muscle [23]. The increase in cGDP-ribose fluorescence depended on the enzyme concentration used. The addition of 10 mM $MgCl_2$ increased the enzyme activity by 29 % ($n = 2$), whereas 10 mM $CaCl_2$ had less effect (94 % of the control value, $n = 2$), indicating that $MgCl_2$, but not $CaCl_2$, has a stimulatory effect, as reported previously [23].

The cGDP-ribose fluorescence increased in the presence of 100 nM Ang II (Figure 3) or in a dose-dependent manner after the addition of 0.1–100 nM Ang II to the reaction mixture (Figure 4). The average increase was to 142.5 $\pm$ 8.5 % ($n = 8$) of the pre-exposure level at 10 nM Ang II, with a significance of

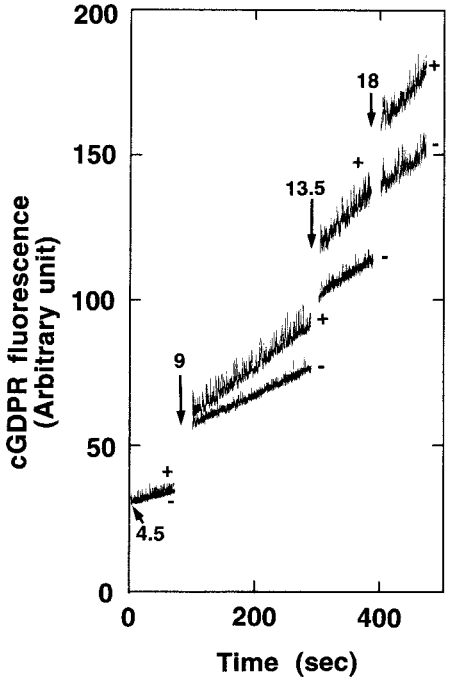

Figure 3 cGDP-ribose-producing activities in membranes prepared from neonatal rat cardiac muscle

Various concentrations of cell membranes prepared from the ventricular muscle of neonatal rats (day 2 after birth) were incubated with 60 μM β-NGD$^+$; fluorescence of the resulting cGDP-ribose was continuously monitored. Time courses of fluorescence were recorded in the presence (+) or absence (−) of 100 nM Ang II. Reaction was initiated by adding 10 μl (4.5 μg) of enzyme. Each arrow indicates the addition of 10 μl of membrane solution to 2.5 ml of the reaction mixture.

$P < 0.001$. The Ang II-induced increase (20–80 % over the control) was observed at all given enzyme concentrations (Figure 3). A similar increase of 36 % ($n = 2$) was generated by 10 nM Ang II in the presence of 10 mM $MgCl_2$, but an increase of only 6 % ($n = 2$) was obtained with the simultaneous addition of 10 nM Ang II and 10 mM $CaCl_2$.

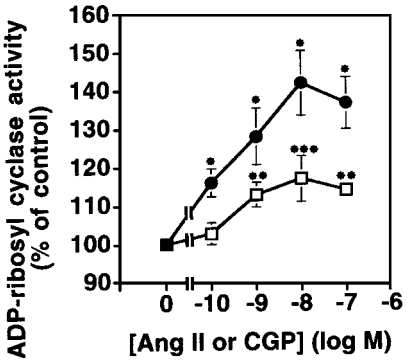

Figure 4 Effects of various concentrations of Ang II and CGP 42112A on ADP-ribosyl cyclase activity

Relationship between Ang II (●) or CGP 42112A (CGP) (□) concentrations and cGDP-ribose formation by ventricular cell membranes of 2–4-day-old rats. Results are means $\pm$ S.E.M. for four to eight measurements. *, **, *** Significantly different ($P < 0.002$, $P < 0.01$ and $P < 0.02$ respectively) from control activity (100% without Ang II or CGP 42112A).

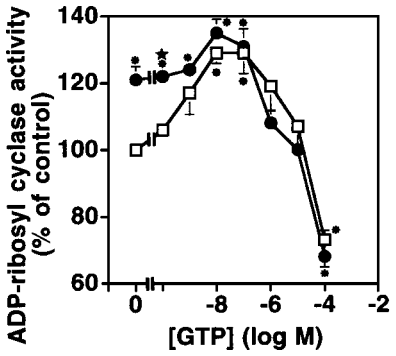

Figure 5 Effects of GTP on ADP-ribosyl cyclase activity in rat ventricular muscle

cGDP-ribose formation was measured with (●) or without (○) 10 nM Ang II and in the presence or absence of various concentrations of GTP. Results are means ± S.E.M. for three experiments. *Significantly different ($P < 0.002$) from control value (100% without GTP and Ang II); ★ $P < 0.01$ compared with the value in the presence of 0.1 nM GTP.

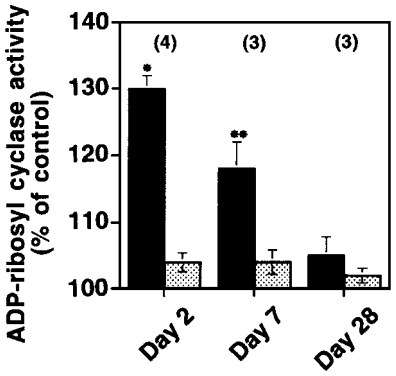

Figure 6 Effect of pretreatment of rats with CTx on Ang II-induced activation of ADP-ribosyl cyclase activity

Rats of three different ages (2, 7 and 28 days after birth) were injected intraperitoneally with CTx (100 ng/g body weight) 8 h before being tested. cGDP-ribose formation was measured in ventricular myocyte membranes of treated rats (grey bars) or untreated rats (black bars) in the presence or absence of 100 nM Ang II. Values are percentages of the control activity and are expressed as means ± S.E.M. for the number of experiments in parentheses. **Significantly different ($P < 0.001$) from the control (without Ang II) value and that of CTx-treated rats. *Significantly different ($P < 0.01$ and $P < 0.05$) from the control and CTx values respectively.

A similar test with various concentrations of CGP 42112A, a potent AT_2-receptor-selective ligand [30,31], also resulted in activation but the response was less than one-third of that generated by Ang II (Figure 4). The activation by Ang II was inhibited by 1–10 nM candesartan, a specific antagonist of the AT_1 subtype [32,33].

The addition of 1–100 nM GTP enhanced cGDP-ribose formation (Figure 5), to a maximum of 130.0 ± 3.2 % ($n = 5$, $P < 0.001$). Higher concentrations of GTP inhibited the reaction, as shown previously [23]. Simultaneous administration of 10 nM Ang II with 0.1–1 nM of GTP resulted in a greater activation than seen in the control value in the presence of GTP alone. However, neither synergistic activation nor inhibition was observed. An increase of 46.2 ± 3.4 % ($n = 3$) over the control value was produced with 10 μM guanosine 5′-[γ-thio]-triphosphate. Guanosine 5′-[β-thio]diphosphate (10 μM) neither stimulated nor inhibited the activity (102.3 ± 0.3 %, $n = 3$) but

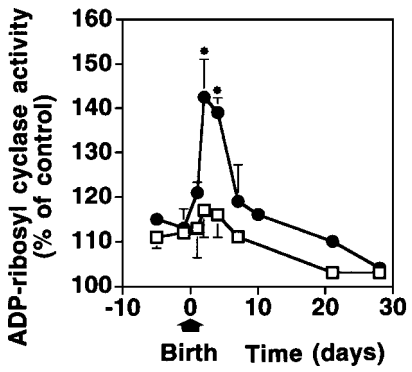

Figure 7 Developmental regulation of Ang II-induced or CGP 42112A-induced activation of ADP-ribosyl cyclase activity

cGDP-ribose formation was measured in ventricular membranes prepared from rats in the developmental stages in the presence or absence of 100 nM Ang II (●) or 10 nM CGP 42112A (□). Results are means ± S.E.M. for three to eight determinations in five separate ventricular samples. *Significantly different from control at $P < 0.001$ and from all other values at $P < 0.05$.

prevented the stimulatory effect elicited by the subsequent administration of 10 nM Ang II.

In previous studies we have suggested that the G-protein-coupling receptors for ADP-ribosyl cyclase activation are CTx-sensitive [23,24]. The effect of this agent on the actions of Ang II receptors was examined in ventricular membranes isolated from rats of three different ages (2, 7 or 28 days old) pretreated for 8 h with 100 ng of CTx/g body weight. The stimulation of ADP-ribosyl cyclase by 100 nM Ang II observed in control rats was completely inhibited in the treated rats (Figure 6). Interestingly, as shown in Figure 6, the stimulation of ADP-ribosyl cyclase by Ang II seems to be age-dependent. We therefore examined the developmental modification of the Ang II effect. A very small increase in activation (approx. 10 %) generated by 10 or 100 nM Ang II was detected in fetal ventricular muscle; however, a sudden increase in cGDP-ribose formation was elicited by Ang II during the 2–5 days after birth (Figure 7) followed by a rapid decline at the end of the first week. A subsequent gradual decline, reaching nearly no activation, was observed over the 2–4 weeks after birth. The low activation (10–20 % over control level) by 10 nM CGP 42112A observed in fetal and neonatal hearts also declined gradually. In the ventricle muscle of normal adult (3–12-month-old) rats, little or no stimulation by either Ang II or CGP 42112A was observed (results not shown).

Ang II binding assay

Previous studies have shown that Ang II receptor densities at mRNA [34] or protein [34,35] levels are developmentally regulated in the rat. In our study, Ang II-binding sites were measured by using a single concentration of 2 μM [^{3}H]Ang II in the rat ventricles. The sites in neonatal (2–4-day-old) rats was 1139 ± 295 fmol/mg of protein ($n = 4$). There were more Ang II-binding sites in newborns than in 4-week-old rats (684 ± 23 fmol/mg of protein, $n = 3$). This result demonstrates that [^{3}H]Ang II-binding sites are also regulated developmentally.

DISCUSSION

The results obtained with the radioisotope method show that Ang II activates ADP-ribosyl cyclase activity in the crude membrane preparation of ventricular myocytes of newborn rats

(Figures 1 and 2). It was previously confirmed with the heat-inactivation method that the ^3H accumulation in cADP-ribose fractions is due to the accumulation of [^3H]cADP-ribose produced by ventricular ADP-ribosyl cyclase [23]. Fluorimetric detection also showed the stimulatory effect of Ang II on cGDP-ribose formation (Figures 3–7). Our results thus provide the first evidence for the role of cADP-ribose as a second messenger downstream of Ang II receptors in mammalian heart cells.

The effect of Ang II on cGDP-ribose formation is developmentally regulated (Figures 6 and 7). cGDP-ribose formation was maximally activated by Ang II during days 2–4 after birth; this activation was less prominent in fetal and adult rats, suggesting an important role for Ang II in myocyte development. The reason for this is not yet clear, but one possible explanation might be found in the number of Ang II receptors expressed. The time course of the Ang II-induced activation of ADP-ribosyl cyclase resembles that of the AT_1 mRNA level during developmental stages [34]. It has been reported that the the density of both AT_1 and AT_2 receptor subtypes doubled immediately after birth, reaching its maximum on day 2 and decreasing toward prenatal values thereafter [34,35].

In this and previous [23] experiments we have established that GTP and guanosine 5′-[γ-thio]triphosphate stimulate ventricular ADP-ribosyl cyclase, and guanosine 5′-[β-thio]diphosphate prevents Ang II-induced activation. The result obtained for GTP and GTP analogues strongly suggests the involvement of G-proteins in the signal pathway from Ang II receptors to membrane-associated ADP-ribosyl cyclase in rat ventricle, paralleling the well-known pathway to phospholipase C [1–4]. It can therefore be concluded that GTP at lower concentrations (approx. 10 nM) duplicates the stimulatory effect of Ang II, whereas higher concentrations of GTP inhibit the ADP-ribosyl cyclase activity. Such dual control of ADP-ribosyl cyclase by different G-proteins might be responsible in part for the lack of additivity between GTP and Ang II.

The Ang II-induced stimulation of ADP-ribosyl cyclase was eliminated by pretreatment of rats with CTx, as reported previously for β-adrenergic receptors [23]. This action of the bacterial toxin is similar to that in which the M_1 and M_3 muscarinic receptor-induced activation of ADP-ribosyl cyclase was found to be sensitive to CTx in NG108-15 cells [24]. The simplest explanation of these results is that the CTx-sensitive G_s protein might be involved in the pathway from Ang II receptors to ADP-ribosyl cyclase. However, this does not exclude the possibility that other, novel G-proteins might affect this process.

The stimulatory effect of $MgCl_2$ and the inhibitory effect of $CaCl_2$ on ADP-ribosyl cyclase were confirmed in the same cardiac muscle as reported previously [23]. Ang II-stimulated activity was higher in the presence of $MgCl_2$ and lower with $CaCl_2$. These findings suggest that bivalent cations have a modulatory effect on basal and agonist-stimulated ADP-ribosyl cyclase but that $MgCl_2$ is not essential for the basal and hormone-stimulated ADP-ribosyl cycling reaction, which makes it different from the formation of cAMP [36].

We determined pharmacologically that the AT_1 rather than the AT_2 subtype is involved in the Ang II-induced activation of cGDP-ribose formation (Figures 4 and 7) but it is important that similar experiments are performed on subtype-specific knock-out mice. Furthermore, it should be of interest, from the viewpoint of hypertrophy as a consequence of interaction with calcineurin, to identify the contribution of AT_1-subtype receptors to the increased rate of cADP-ribose formation in the heart of adult and hypertensive rats. Such experiments are now being conducted with the use of spontaneously hypertensive rats.

In conclusion, the stimulation of Ang II receptors increases the rate of cADP-ribose formation in the rat ventricle, which might in turn, by functioning as the second messenger, enhance the increase in intracellular [Ca^{2+}], which promotes Ca^{2+}-induced Ca^{2+} release via ryanodine receptors.

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