We examined the role of cyclic ADP-ribose (cADP-ribose) as a second messenger downstream of adrenergic receptors in the heart after excitation of sympathetic neurons. To address this question, ADP-ribosyl cyclase activity was measured as the rate of \([{}^3H]cADP-ribose\) formation from \([{}^3H]NAD^+\) in a crude membrane fraction of rat ventricular myocytes. Isoproterenol at 1 mM increased ADP-ribosyl cyclase activity by 1.7-fold in ventricular muscle; this increase was inhibited by propranolol. The stimulatory effect on the cyclase was mimicked by 10 nM GTP and 10 mM guanosine 5’-O-(thio)triphosphate, whereas 10 mM GTP inhibited the cyclase. Cholera toxin blocked the activation of the cyclase by isoproteranol and GTP. The above effects of isoprotenerol and GTP in ventricular membranes were confirmed by cyclic GDP-ribose formation fluorometrically. These results demonstrate the existence of a signal pathway from β-adrenergic receptors to membrane-bound ADP-ribosyl cyclase via G protein in the ventricular myocyte cells and suggest that increased cADP-ribose synthesis is involved in up-regulation of cardiac function by sympathetic stimulation.

Sympathetic nerve excitation stimulates β-adrenergic receptors on cardiac myocytes by release of noradrenaline, leading to an increase in the contractility. This cardiostimulant effect is traditionally thought to be mediated by an increase in Ca\(^{2+}\) permeation resulted from cyclic AMP-dependent phosphorylation of voltage-gated ion channels (1, 2). Opening of phosphorylated L-type Ca\(^{2+}\) channels (3) and tetrodotoxin-sensitive Na\(^{+}\) channels (4) results in a transient intracellular Ca\(^{2+}\) concentration increase ([Ca\(^{2+}\)]\(_i\), transient) that is greater than that without sympathetic stimulation. The increased [Ca\(^{2+}\)]\(_i\) is further amplified by Ca\(^{2+}\)-induced Ca\(^{2+}\) release from the sarcoplasmic reticulum ryanodine receptor Ca\(^{2+}\) release channels (5–10), leading to strengthened contraction. In Ca\(^{2+}\)-induced Ca\(^{2+}\) release in the heart, both cyclic ADP-ribose (cADP-ribose)\(^1\) and Ca\(^{2+}\) cooperatively activate type-II ryanodine receptors to release Ca\(^{2+}\) (11–14). However, no information on the concentration of cADP-ribose after β-adrenoreceptor stimulation has yet been reported.

Membrane-bound and cytosolic ADP-ribosyl cyclases constitutively synthesize cADP-ribose from β-NAD\(^+\) (15–21). Formation of cADP-ribose is increased or decreased by stimulation of muscarinic acetylcholine receptors in a subtype-specific manner, and this is mimicked by GTP and blocked by bacterial toxins in NG108-15 neuronal cells (22). ADP-ribosyl cyclase thus seems to be coupled directly with neurotransmitter or hormone receptors via different G proteins in the surface membrane of these cells (23). The same control of cADP-ribose formation could be carried out by ventricular adrenergic receptors. To address this question, we measured ADP-ribosyl cyclase activity in crude membrane fractions of rat ventricular myocytes in the presence or absence of an adrenergic agonist and GTP.

**EXPERIMENTAL PROCEDURES**

**Materials**—β-[2,8-adenine-\(^3H\)]NAD\(^+\) (30.5 Ci/mmol) and [adenylate-\(^32P\)]NAD\(^+\) (800 Ci/mmol) were purchased from NEN Life Science Products. Cyclic ADP-ribose was obtained from either Yamasa Shoyu (Choshi, Japan) or Sigma, and nicotinamide guanine dinucleotide\(^+\) (NDG\(^+\)) was from Sigma. Azide-free cholera toxin (CTx) was purchased from Funakoshi (Tokyo, Japan). Silica Gel 60 F\(_{254}\) plastic TLC sheets were obtained from Merck.

**Membrane Preparation**—Wistar rats used were new born to 4 weeks old. Ventricular heart muscles from cold-anesthetized rats were washed once in ice-cold phosphate-buffered saline. 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. 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 105,000 × g for 15 min. The supernatant was removed, and the final pellet was dispersed in 10 mM Tris-HCl solution, pH 6.6. In each experiment, membranes were freshly prepared and used immediately for enzymatic reactions. In some experiments, rats were intraperitoneally injected with CTx (100 ng/g of body weight) 16 h before sacrifice.

In addition, membranes were prepared from Chinese hamster ovary (CHO) cells stably transfected. To establish these cell lines, pZHCDS8 (22) and pZeoSV (Invitrogen, San Diego, CA) were introduced into CHO dhfr\(^-\) cells using Lipofectin (Life Technologies, Inc.). Cells were selected in the presence of Zeocin (250 μg/ml), and the expression of human CD38 mRNA was verified by RNA blot hybridization analysis.

**ADP-ribosyl Cyclase Assay**—Each 20-μl reaction mixture contained 50 mM Tris-HCl, pH 6.6, 100 mM KCl, 10 mM CaCl\(_2\), 2 μM β-NAD\(^+\), 0.11 μM β-[2,8-adenine-\(^3H\)]NAD\(^+\) (0.06 μCi), and 1.2–11.5 μg of membrane proteins, according to a formula reported previously (22). Reaction mixtures were incubated for 0.5–4 min at 37 °C. Reactions were stopped by adding 2 μl of 10 or 48% trichloroacetic acid, and aliquots were centrifuged for 1 min at 2100 × g, and 2 μl of the supernatant were spotted on silica gel plastic TLC sheets (20 × 10 cm). The layers were developed in U.S.A. and The American Society for Biochemistry and Molecular Biology, Inc.

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developed in the ascending direction for 40–70 min at 23 °C with a mixture of water/ethanol/ammonium bicarbonate (in the ratio 30%: 70%:0.2%), as reported previously (22). The positions of authentic cADP-ribose, ADP-ribose, and NAD$^+$ after UV detection were confirmed in each run. Corresponding areas (about 1 × 0.7 cm) were cut out, and the radioactivity was counted in a liquid scintillation counter. In heat inactivation experiments of cADP-ribose as the enzymatic product, the reaction mixtures, which had been terminated after the 2-min incubation by trichloroacetic acid and adjusted to neutral pH, were either treated at 100 °C or kept on ice for 20 min and then chromatographed.

 Autoradiography of TLC with $[^3H]$NAD$^+$—The same reaction mixture used for the ADP-ribosyl cyclase assay containing 0.06 or 0.36 μCi of $[^3H]$NAD$^+$ was incubated with membranes of rat ventricular muscle. 2 μl of reaction mixture were spotted on TLC sheets and developed. Autoradiography was carried out after exposure on a Fuji BAS 1000 $^3$H imaging plate for 9–27 h.

 ADP-ribosylation—The membranes prepared from CTx-treated or untreated rats (5.6–10 μg of protein) were subsequently incubated at 37 °C for 20–60 min in 100 μl of the membrane buffer containing 25 mM Tris-HCl, pH 7.5, 15 mM thymidine, 2 mM MgCl$_2$, 10 μM CaCl$_2$, 1 mM EDTA, 6 mM diethiothreitol, 2 mM GTP, and 50 μM NAD$^+$, and 2.0 or 8.3 μCi of $[^3H]$NAD$^+$ with or without CTx, according to a method described elsewhere with a slight modification (24). CTx (20 μg) was preactivated by incubation with 25 mM diethiothreitol in 25 mM Tris-HCl, pH 7.4, at 37 °C for 30 min. Incubation for ADP-ribosylation was terminated by dilution of 1 ml of ice-cold 10 mM Tris-HCl, pH 6.6 or 10 μl of 48% trichloroacetic acid, followed by centrifugation at 2100 × g for 5 min. The pellet was washed five times with 10 mM Tris-HCl, pH 6.6, and resuspended in Laemmli gel loading buffer for electrophoresis or in 10 mM Tris-HCl, pH 6.6, for ADP-ribosyl cyclase assay. The proteins were separated by means of SDS-polyacrylamide gel electrophoresis, and the gels were dried and autoradiographed with a Fuji BAS 1000 (Tokyo, Japan).

 Fluorometrical Measurement of ADP-ribosyl Cyclase—ADP-ribosyl cyclase activity was also determined fluorometrically by utilizing a technique based on the measurement of the conversion of NGD$^+$ into the fluorescent product cyclic GDP-ribose (cGDP-ribose), as described (29). Reaction mixtures, which had been terminated after the 2-min incubation, were treated at 100 °C or kept on ice for 20 min and then chromatographed. Autoradiograms (Fig. 1). The activity increased at a constant rate for at least the first 1 min of incubation with 2.11 nM GTP, a value which was similar to that of rat cardiac myocyte membranes (1.25–145 mM Tris-HCl, pH 6.6, for ADP-ribosyl cyclase assay. The proteins were resuspended in Laemmli gel loading buffer for electrophoresis or in 10 mM Tris-HCl, pH 6.6, and resuspended in Laemmli gel loading buffer for electrophoresis or in 10 mM Tris-HCl, pH 6.6, for ADP-ribosyl cyclase assay. The proteins were separated by means of SDS-polyacrylamide gel electrophoresis, and the gels were dried and autoradiographed with a Fuji BAS 1000 (Tokyo, Japan).

 RESULTS

 ADP-ribosyl Cyclase Activity in Cardiac Myocytes Measured by Thin Layer Chromatography—$[^3H]$ADP-ribosyl and $[^3H]$ADP-ribose were produced from $[^3H]$NAD$^+$ by the crude membrane preparation of rat ventricular myocytes. During an incubation period of 4 min the majority of NAD$^+$ was converted to either ADP-ribose or cADP-ribose or both, judging from autoradiograms (Fig. 1). The activity increased at a constant rate for at least the first 1 min of incubation with 2.11 μM NAD$^+$ as substrate (Fig. 2A). The average specific activity of ventricular myocytes was 3.70 ± 0.65 nmol/min/mg of protein (mean ± S.E., n = 7).

 To verify that the above $^3$H accumulation in cADP-ribose fractions is mainly due to accumulation of $[^3H]$cADP-ribose produced by ventricular ADP-ribosyl cyclase, the reaction mixtures were either boiled or kept on ice. $^3$H counts collected in the cADP-ribose fractions from the heat-inactivated product were reduced to 8.7 ± 0.29% (n = 3) of that of non-heat-treated ones. Simultaneously, the decrease of $^3$H counts in the cADP-ribose fraction was confirmed autoradiographically. These results suggest that the enzyme product recovered in the cADP-ribose fraction is heat-labile, a property it shares with cADP-ribose (15, 26).

 In separate experiments, optimal conditions for the assay were examined in more detail. Production of cADP-ribose was optimal at pH 6.5, as shown in Fig. 3. The reduced activity in the alkaline range (pH 7.8) accords well with the membrane form of sea urchin egg ADP-ribose cyclase (20). The effects of varying Ca$^{2+}$ and Mg$^{2+}$ concentrations on basal ADP-ribosyl cyclase activity are shown in Fig. 4. ADP-ribosyl cyclase activity was inhibited by the addition of higher concentrations (>10 mM) of CaCl$_2$. At 1–10 mM MgCl$_2$, cyclase activity increased. The inhibitory effect of Ca$^{2+}$ and the stimulatory effect of Mg$^{2+}$ at the millimolar range rather resemble those of adenylyl cyclase (27). Because addition of 0.01 mM EGTA or EDTA to the assay medium to remove residual cations did not occlude the activity, membrane-bound ADP-ribosyl cyclase seems to require no divalent cations; in this respect, it differs substantially from adenylyl cyclase (28). No requirement for Mg$^{2+}$ has also been reported for ADP-ribosyl cyclase in recombinant human CD38 (29).

 Effects of Isoproterenol on ADP-ribosyl Cyclase Activity—Addition at zero time of 2 μM isoproterenol, a β-adrenergic agonist, increased the rate of $[^3H]$ADP-ribosyl production (Fig. 2A), with a mean value of 5.62 ± 0.97 nmol/min/mg of protein (n = 7). The effect of different ligand concentrations on ADP-ribosyl cyclase activity is shown in Fig. 2B. The maximum activation found was 165.8 ± 14.3% (n = 8) of the control activity at 1 μM isoproterenol (p < 0.002). Propranolol (10 μM), the β-adrenergic receptor antagonist, inhibited the stimulation of ADP-ribosyl cyclase by isoproterenol.

 The effects of varying Ca$^{2+}$ or Mg$^{2+}$ concentrations on isoproterenol-stimulated activity were also examined. Addition of 20 μM isoproterenol resulted in an increase over the basal activity in the concentration ranges tested (Fig. 4). Therefore, optimum concentrations of Ca$^{2+}$ and Mg$^{2+}$ for stimulated activation were identical to those of basal activities.

 Effects of GTP on ADP-ribosyl Cyclase Activity—We determined whether or not β-adrenergic receptor-mediated activation of ventricular ADP-ribosyl cyclase is mimicked by GTP, its analog, and other nucleotides. Addition of 10 mM GTP resulted in a detectable increase in enzyme activity at each time tested between 0.5–4 min, whereas 10 μM GTP inhibited it. The relationship between GTP concentration and the reaction rate is shown in Fig. 5A. The stimulatory effect in ventricular muscle required as little as 1–100 μM GTP. The maximum stimulation was obtained at 10 mM GTP with an average increase of 168.3 ± 10.7% (n = 15) of the control value (p < 0.001). This low concentration is the same dose at which GTP effectively binds on G$_s$ or G$_i$, types of G proteins (30, 31). On the other
Effect of isoproterenol and propranolol on ADP-ribosyl cyclase activity in rat cardiac myocytes. A, time course of ADP-ribosyl cyclase activity in membranes prepared from ventricular myocytes. Reaction mixtures were incubated with [3H]NAD for the indicated time periods. B, relationship between isoproterenol concentration and ADP-ribosyl cyclase activity of ventricular cell membranes with (●, + prop) or without (□, – prop) 10 μM propranolol. Each 20-μl reaction mixture containing components as in A, with various isoproterenol concentrations as indicated, was incubated for 1 min. 100% refers to the activity assayed in the absence of both reagents: 2.23 ± 0.33 and 2.01 ± 0.47 nmol/min/mg of protein for experiments in the absence and presence of propranolol. The values are the means of six and four measurements for A and B of duplicate determinations, respectively. Bars indicate S.E. *, significantly different from control activity (100% without isoproterenol) and from activity with propranolol at the indicated isoproterenol concentrations at p < 0.005 and 0.05; **, at p < 0.002 and 0.001; and ***, at p < 0.005 and 0.005, respectively.

Effect of pH on ADP-ribosyl cyclase activity. Ventricular myocyte membranes were incubated with [3H]NAD for 2 min at various pH. The activity of 1.83 ± 0.17 nmol/min/mg of protein is represented by 100%. Values represent the means of six experiments. Bars indicate S.E.

hand, GTP at higher concentrations (100 μM) produced inhibition (30.5 ± 9.4% (n = 7) of the control level (p < 0.001)), although we could not identify agonists that induce inhibition of the cyclase in rat heart.

GTP-γ-S proved to be a more effective ligand than GTP and produced only an increase in ADP-ribosyl cyclase activity in ventricular myocytes (Fig. 5B). Increases to 238.6 ± 45.5% (n = 6) and 270.3 ± 15.1% (n = 3) of the control were produced at, respectively, 10 and 100 μM GTP-γ-S (p < 0.001); these effective concentrations are the same as those determined in neuroblastoma cells for activation of adenylyl cyclase (32).

Next, we examined the effect of 100 nM GTP in the presence of various concentrations of isoproterenol. The ADP-ribosyl cyclase activities measured with GTP and isoproterenol (10 and 100 nM) were higher (≥150% of the control values without GTP and isoproterenol or isoproterenol alone), as shown in Fig. 5C. The activities in the presence of GTP together with 1 and 10 μM isoproterenol were also high and slightly exceeded those with isoproterenol alone. These results show that both isoproterenol and GTP are stimulatory but are neither additive nor synergistic.

Furthermore, the effect of other nucleotides were examined. ADP-ribosyl cyclase activities in the presence of 10 nM ATP, UTP, GTP, and GDP were 97.8 ± 14.3% (n = 4), 107.8 ± 10.3% (n = 4), 100.5 ± 9.9% (n = 4), and 113.0 ± 5.2% (n = 4) of the control value, respectively, indicating that the stimulatory effect is GTP-specific.

Effects of CTx on Isoproterenol- and GTP-induced Stimulation of ADP-ribosyl Cyclase Activity—The stimulation of ADP-ribosyl cyclase by isoproterenol and GTP was markedly inhibited in ventricular membranes isolated from rats pretreated with 100 ng CTx/g of body weight for 16 h (Fig. 6A). The GTP-induced inhibition, however, was unaffected.

To detect target proteins for CTx, ventricular membranes prepared from both CTx-treated and untreated rats were incubated with [32P]NAD<sup>+</sup> in the presence or absence of CTx. Control membranes showed several ADP-ribosylation substrates for CTx, including a major band at a molecular mass of about 43 kDa produced by CTx (Fig. 6B), as described previously (24, 33–35). In vivo pretreatment of rats with CTx for 16 h resulted in the elimination of label incorporation into this substrate. These results suggest that treatment with CTx of rat ventricular membranes effectively ADP-ribosylates G<sub>S</sub> and other proteins (25, 33).

Effects of Isoproterenol and GTP on Cyclic GDP-ribose Formation—Detection of ADP-ribosyl cyclase activity and its regulation was further pursued by means of fluorometrical assay of accumulated cGDP-ribose from a hydrolysis-resistant substrate, NGD<sup>−</sup> (20, 21). First we measured cGDP-ribose formation with human CD38, which has been shown to possess ADP-ribosyl cyclase activity (15, 17), 22. Incubation of 60 μM NGD<sup>−</sup> with cell membranes prepared from CD38-overexpressing CHO cells but not from mock-transfected CHO cells resulted in a progressive increase in the fluorescence (Fig. 7A).

The cGDP-ribose fluorescence clearly increased after addition of 0.1–100 μM isoproterenol to the reaction mixtures, as shown in Figs. 7B and 8A. The maximal increase to 149.8 ± 10.9% (n = 16) of the pre-exposure level was obtained by 10 μM isoproterenol (p < 0.01). Administration of 1–100 nM GTP enhanced cGDP-ribose formation (Figs. 7C and 8B), with the maximum value of 154.8 ± 5.0% (n = 6) at 10 nM (p < 0.001). Simultaneous addition of 10 nM GTP with 1 nM to 100 μM isoproterenol resulted in higher activities than the control.
value in the absence of GTP at all concentration ranges of isoproterenol tested (Fig. 8A). The activity (156.4 ± 9.9% (n = 3) to 179.0 ± 9.6% (n = 3)) obtained in the presence of both drugs, however, was the level similar to that (155.8 ± 7.4% (n = 18)) with GTP alone.

In contrast, higher concentrations of GTP inhibited the reaction (Figs. 7D and 8); 10 and 100 μM GTP resulted in a reduction to 63.2 ± 4.5% (n = 9) and 67.4 ± 2.8% (n = 9; p < 0.002), respectively. GTP-γS at 0.1–1 μM had no effect on the cGDP-ribose fluorescence, but 10 and 100 μM GTP-γS rather inhibited the reaction. The reason for the discrepancy in the effect of GTP-γS on ADP-ribosyl cyclase measured by the TLC and spectroscopic methods is not clear at this moment. However, it may reside on the difference in ADP-ribosyl cyclase activities because of two different substrates.

Finally, the effects of various nucleotides were examined fluorometrically. The GDP-ribose fluorescence after addition of 10 mM or 100 μM ATP was 105.8 ± 3.1% (n = 5) of the control (preaddition) level; after UTP, 107.5 ± 5.0% (n = 3) or 117.6 ± 10.7% (n = 5); after GTP, 107.3 ± 5.0% (n = 3) or 107.8 ± 7.7% (n = 5); and after GDP, 106.6 ± 6.5% (n = 3) or 111.6 ± 3.6% (n = 5), respectively. The result suggests that the stimulatory and inhibitory effects of GTP on GDP-ribose formation are unique among five nucleotides tested.

**DISCUSSION**

The results show that the adrenergic agonist activates ADP-ribosyl cyclase activity in crude membrane preparation of rat ventricular myocytes, which were measured by both radioisotopic and fluorometric assay. It appears that GTP reproduces the stimulatory effect of isoproterenol on ventricular ADP-ribosyl cyclase. Interestingly, the concentration response curves for isoproterenol and GTP using the two assay systems are very similar. Our results thus provide the first evidence for the role of cADP-ribose as a second messenger downstream of β-adrenoceptors in the mammalian heart cells after sympathetic excitation. It has been reported that two distinct types of ADP-ribosyl cyclase are present in rat cardiac muscle (36). One is confined to the plasma (sarcolemma) membrane and is colocalized with dihydropyridine-sensitive Ca2+ channels, and the other is associated with the sarcoplasmatic reticulum membrane in which type-II ryanodine receptors are located. Our ADP-ribosyl cyclase had the same order of specific activity as that reported in partially purified sarcolemma (1.22 nmol of cyclic GDP-ribose formed/min/mg of protein). The enzyme in rat cardiac sarcolemma is believed to be an ectoenzyme, which in common with CD38 can be inhibited by dithiothreitol (36). However, the ventricular membrane-bound enzyme is not greatly susceptible to 4 mM dithiothreitol in our preliminary
experiments (reduction by 16.6%), when it is included from the start of incubation. These results suggest that ADP-ribosyl cyclase in our preparations is distinct from the CD38-like ecto-enzyme (15) and the cytosolic isotype (19, 20) but is the membrane-bound form that has its catalytic domain on the inside of the membrane, as estimated in NG108-15 cells (22, 23).

Because we did not test any subtype-specific agonists or antagonists, which subtype(s) of β-adrenergic receptors are responsible for the activation of ventricular ADP-ribosyl cyclase is not yet clear. In the rat heart, four subtypes have been identified genetically and pharmacologically (37). β1-, β2-, and β3-adrenoreceptors have been shown to couple to the stimulatory G protein (Gs) pathways and cause cardio-stimulation, whereas β4-adrenoreceptors appear to couple to the Gq type of G protein and mediate cardiodepressant effects (37). Adrenergic stimulation of ADP-ribosyl cyclase may be mediated by either one or two or all three types of β-receptor subtypes that cause cardiostimulation.

Although isoproterenol, GTP, and GTP-γ-S similarly stimulated ventricular ADP-ribosyl cyclase activity, simultaneous application of GTP together with isoproterenol was not additive nor synergistic. Therefore, it seems that the signal pathway from isoproterenol and GTP stimulation is rather shared. The fact for the stimulatory effect obtained by isoproterenol alone without added GTP can be explained by the fact that isoproterenol may utilize a small amount of endogenous GTP in crude membrane fractions for its stimulation.
adenyl cyclase in fat cell membranes (38), in which two distinct regulatory processes are estimated. The lack of inhibitory effect of GTP-γ-S on ADP-ribosyl cyclase is not surprising, because it has been reported that another stable GTP analog, Gpp(NH)p, only activates adenyl cyclase in NG108-15 cells (32, 39), in which morphine and epinephrine inhibit adenyl cyclase. These results on guanine nucleotides strongly suggest the involvement of G proteins in the signal pathway from β-adrenergic receptors to membrane-associated ADP-ribosyl cyclase in rat ventricle, in parallel with the well known pathway to adenyl cyclase (40).

The isoproterenol- and GTP-induced stimulation of ADP-ribosyl cyclase was eliminated by pretreatment of rats with CTx. This action of the toxin is similar to the one where the m1 and m3 muscarinic acetylcholine receptor-induced activation of ADP-ribosyl cyclase was found to be sensitive to CTx in NG108-15 cells (22). Because we observed CTx-specific ADP-ribosylation in one substrate at around 43 kDa in ventricular membranes and elimination of its labeling by pretreatment of rats with CTx, it can be concluded that CTx caused mono-ADP-ribosylation of its target G proteins in rat heart in vivo. These results raise the possibility that CTx-sensitive G proteins might be involved in regulating the cyclase (41). To test this possibility in a more direct way, we performed preliminary experiments by measuring ADP-ribosyl cyclase activity in ventricular membranes treated with CTx in vitro. In such membranes, the same substrates were ADP-ribosylated and the hormonal response was eliminated, being associated with a decrease or no increase in basal activity of ADP-ribosyl cyclase in the TLC or fluorometrical measurements, respectively. Further validation will be necessary for determining the exact species of G proteins involved.

The uncoupling between adrenoreceptors and ADP-ribosyl cyclase after in vivo treatment with CTx can be explained by processes other than the direct effect of G protein discussed above. One such process is the auto-ADP-ribosylation of ADP-ribosyl cyclase, as demonstrated in soluble recombinant CD38 (42). Although NAD glycohydrolase activity is reduced in such ADP-ribosylated CD38 (42), no studies on the cyclase activity have yet been reported. If we assume that Gs is constitutively activated by CTx, and CTx does not chemically modify the cyclase, it can be expected that the cyclase activity is kept at a high level. The second possible explanation can be found in the phosphorylation of ADP-ribosyl cyclase as a result of changes in downstream signaling systems regulated by CTx-sensitive G proteins. Phosphorylation may well have stimulated the cyclase activity in our study in a manner similar to that shown in cytosolic ADP-ribosyl cyclase activity stimulated by cGMP- (20) and cAMP-dependent (21) kinases. In our experiments, however, ADP-ribosyl cyclase activity in CTx-treated membranes was not always high. These results suggest that in rats treated with CTx, many other changes in addition to the single modification on Gs occur, resulting in alterations in the cyclase activity.

Recently, a new physiological effect of β-adrenergic receptor stimulation has been reported, namely the regulation of [Ca2+]i transient by activation of Na+ channels phosphorylated by cyclic AMP-dependent protein kinase, which is referred to as slip mode conductance (4). The Ca2+ influx through phosphorylated Na+ channels (30% of the total content) as well as through the classical pathways of phosphorylated Ca2+ channels (70%) can increase intracellular Ca2+ and thereby activate sarcoplasmic reticulum Ca2+ release, Ca2+ sparks, and the [Ca2+]i transient (5–11, 43). On the cADP-ribose action site, it has recently been shown that FKB12.6 is its binding protein (44), which would result in a larger [Ca2+]i transient by an as yet unclear mechanism. Thus, we hypothesize that Ca2+ and cADP-ribose levels, both of which are increased by adrenergic stimulation in the ventricle by sympathetic nerve excitation, function cooperatively at the ryanodine receptor level. These changes may contribute significantly to local and global cardiac Ca2+ signaling, which controls the force of contraction. In conclusion, our results suggest that stimulation of β-adrenergic receptors in the ventricle can up-regulate cardiac function through cADP-ribose as a second messenger, probably in concert with cytosolic Ca2+ promoting Ca2+-induced Ca2+ release via ryanodine receptors.

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ADP-ribosyl Cyclase Coupled with Adrenoceptors in Heart

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Sympathetic Potentiation of Cyclic ADP-ribose Formation in Rat Cardiac Myocytes
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