Relationship between Desensitization and Downregulation of β-Adrenoceptors in Cardiac Tissues after Prolonged In Vivo Infusion of T-0509, a β1-Adrenoceptor Agonist

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ABSTRACT—To examine the contribution of β-adrenoceptor (βAR) downregulation to desensitization of βARs by chronic administration of a βAR agonist, we compared the adenylyl cyclase (AC) activities in two kinds of cardiac ventricular membranes with decreased available βARs: one was derived from rats infused with a selective β1AR agonist, T-0509 [(−)-(R)-1-(3,4-dihydroxyphenyl)-2-[(3,4-dimethoxyphenethyl)amino]ethanol hydrochloride], in vivo (40 μg/kg/hr, s.c. for 6 days); and the other was obtained from treatment of control membranes with an irreversible βAR antagonist, bromoacetyl alprenolol methane (BAAM). T-0509 infusion decreased the densities of β1ARs and β2ARs by 26% and 32%, respectively, and reduced the maximal isoproterenol-stimulated AC activity by 53%. The amount of Gsα and Giα proteins in the membranes was not significantly changed by T-0509 infusion. To make preparations that mimic the T-0509-induced downregulation, we treated the control membranes with 100 nM BAAM in vitro. The BAAM treatment decreased the Bmax value of [125I]iodocyanopindolol for β1ARs and β2ARs by 29% and 36%, respectively, whereas it reduced the maximal effect of isoproterenol on AC activity only by 37%. These results suggest that downregulation of βARs cannot fully account for the desensitization by chronic treatment of T-0509 and that other mechanism(s) can play a significant role in the loss of responsiveness.

Keywords: T-0509, Cardiac ventricle, Adenylyl cyclase, Desensitization of β-adrenoceptor, Irreversible β-adrenoceptor antagonist

Prolonged infusion of animals with a β-adrenoceptor (βAR) agonist usually causes the desensitization (loss of responsiveness) and downregulation (loss of receptors) of cardiac βARs. Several experiments in vitro have shown that the downregulation of βARs is a major mechanism for the desensitization caused by a long-term exposure to the β-agonist (1, 2). However, the contribution of the βAR downregulation in myocardium to the desensitization by the in vivo infusion of a β-agonist has not been examined quantitatively. It is possible that the other mechanisms, such as uncoupling in the βAR-adenyl cyclase (AC) complex (3) or change in the amount of G protein α-subunit (4, 5), are also responsible for the desensitization in vivo.

To investigate the relationship between the desensitization and downregulation of the cardiac βARs, we compared the AC activities in two kinds of cardiac ventricular membranes that were decreased in the number of available βARs: one was derived from rats infused with a βAR agonist, and the other was treated with an irreversible βAR antagonist to mimic the decrease in βAR binding sites by chronic infusion of a βAR agonist. It is known that prolonged infusion of isoproterenol is more likely to decrease the number of β2ARs than that of β1 in myocardium (6). However, there is no irreversible β2AR antagonist available to mimic the βAR downregulation by isoproterenol. In this study, we unexpectedly found that prolonged infusion of the selective β1AR agonist T-0509 (7, 8) downregulated both β1ARs and β2ARs in cardiac ventricular membranes to the same extent. Bromoacetyl alprenolol methane (BAAM) is a non-selective and irreversible βAR antagonist at a relatively low concentration (9). Since BAAM irreversibly binds to β1ARs and β2ARs, BAAM can block the further agonist and antagonist binding. We used T-0509 for prolonged infusion and BAAM for irreversible blockage of βARs to examine the
relationship between \( \beta \)AR downregulation and desensitization.

**MATERIALS AND METHODS**

**Chronic treatment and membrane preparation**

Male Sprague-Dawley rats (7–8 weeks of age, 210–320 g) were used for the experiments. Osmotic minipumps (Alzet 2ML1; Alza, Palo Alto, CA, USA) were implanted subcutaneously into the back of the neck of rats under ether anesthesia. The pumps were loaded with either isoproterenol (40 \( \mu \)g/kg/hr, s.c.) or T-0509 (40 \( \mu \)g/kg/hr, s.c.) dissolved in 0.9% NaCl containing 0.1% sodium metabisulphite. Control animals were given a sham-operation. Rats were housed with food and water ad libitum. Under these conditions, plasma concentrations of isoproterenol and T-0509 in blood were sufficient to maintain effective doses on \( \beta \)ARs in cardiac tissues (10). Prolonged infusion of either drug caused no weight changes (10). After 6 days of infusion, the animals were anesthetized with pentobarbital (50 mg/kg, i.p.) and their hearts were rapidly removed. The left ventricles (ventricular free walls and septa) were excised from the atria and right ventricular free walls. The isolated left ventricles were minced finely and homogenized by a Polytron (setting 6, 15 sec \( \times 2 \)) in 20 vol. of ice-cold 50 mM Tris-HCl buffer (pH 7.5) containing 10 \( \mu \)g/ml soybean trypsin inhibitor Type II-S, 5 \( \mu \)g/ml leupeptin, 2 mM EDTA and 7 \( \mu \)g/ml peptatin A. The homogenates were filtered through 4 layers of gauze and centrifuged at 28,000 \( \times g \) for 10 min. The pellets were rehomogenized as described above, aliquoted and then frozen in liquid nitrogen. The samples were stored at \(-80^\circ \)C until use.

**Radioligand binding assay**

Membranes were centrifuged at 28,000 \( \times g \) for 10 min. The resulting pellets were finally suspended in 1 ml of ice-cold 50 mM Tris-HCl buffer (pH 7.5). The membranes (50–100 \( \mu \)g of protein) were incubated with appropriate concentrations (5.0–50 \( pM \)) of \([^{125}I]\)iodocyanopindolol (\([^{125}I\]CYP, specific activity: approximately 74 TBq/mmol) in a final volume of 1 ml of ice-cold buffer for 60 min at 37°C. The reactions were stopped by rapid dilution with 8 ml of ice-cold 50 mM Tris-HCl buffer. The media were immediately filtered through a glass fiber filter (Whatman GF/C) on a Brandel cell harvester. The filter was washed with an additional 8 ml of ice-cold buffer. Specific binding to cardiac ventricular membranes was defined as that displaced by 1 \( pM \) (\( \pm \))-propranolol. Equilibrium dissociation constants (\( K_d \)) and the maximal binding capacities (\( B_{max} \)) for total \( \beta \)ARs were determined by non-linear least-square analysis fitting the data to Michaelian rectangular hyperbolic curves using a computer program, SP123, developed by H. Ono (University of Tokyo) (11). The relative proportions of the \( \beta_1 \)ARs and \( \beta_2 \)ARs in the membrane were defined as the specific binding that was and was not displaced by 300 nM CGP20712A (12), respectively.

**Adenylyl cyclase assay**

Membranes were centrifuged at 28,000 \( \times g \) for 10 min. The resulting pellets were finally suspended in Heps buffer (10 mM, pH 7.4) at a concentration of 2 mg of protein per 1 ml. AC activities were measured by the method of Salomon et al. (13). The assay mixture containing 15 \( \mu l \) of membrane suspension (approx. 30 \( \mu g \) of membrane protein), 0.12 mM ATP, 50 \( \mu M \) GTP, 2.8 mM phosphoenol pyruvate, 0.1 mM cAMP, 20 units/ml myokinase, 4 units/ml pyruvate kinase, 0.8 mM EDTA, 10 mM MgCl\(_2\) and 1 \( \mu Ci \) of \([^{3}P\]ATP in a final volume of 50 \( \mu l \). Enzyme activities were determined in the absence of activators or the presence of increasing concentrations (3 \( nM \)–100 \( pM \)) of isoproterenol, 10 mM NaF or 100 \( \mu M \) forskolin. The reactions were started by the addition of membranes. The assay mixtures were incubated for 30 min at 37°C. The incubation was terminated by the addition of 1 ml of an ice-cold solution containing 0.5 mM ATP, 0.5 mM cAMP and \([^{3}H]cAMP \) (approximately 100,000 cpm). Cyclic AMP in the mixture was isolated by sequential chromatography on Dowex and aluminum oxide columns. Data were fitted to logistic curves by non-linear least square analysis.

**BAAM treatment**

The membranes from the control group were incubated for 1 hr at 0°C with or without 100 nM BAAM in Tris-HCl buffer (60 mM, pH 7.5 at 0°C) containing 0.12 mM ATP, 50 \( \mu M \) GTP, 2.8 mM phosphoenol pyruvate, 0.1 mM cAMP, 20 units/ml myokinase, 4 units/ml pyruvate kinase, 0.8 mM EDTA and 10 mM MgCl\(_2\). The reaction was stopped by adding of 3 vol. of Tris-HCl buffer (10 mM, pH 7.5) containing 5 mM EDTA, 5 mM EGTA, 0.12 mM ATP, 50 \( \mu M \) benzamidine, 10 \( \mu M \)/ml soybean trypsin inhibitor Type II-S and 5 \( \mu g/ml \) leupeptin. The media were rapidly centrifuged at 28,000 \( \times g \) for 10 min. The pellets were resuspended in the supplemented Tris-HCl buffer (10 mM, pH 7.5) and homogenized with a Polytron (setting 6, 15 sec). The suspensions were centrifuged at 28,000 \( \times g \) for 10 min. For the receptor binding assay, the resulting pellets were resuspended in Tris-HCl buffer (50 mM, pH 7.5) at a concentration of 250–500 \( \mu g \) of protein per 1 ml. For the AC assay, the pellets were suspended in Heps buffer (10 mM, pH 7.4) at a concentration of 2 mg of protein per 1 ml. Both the radioligand binding assay and AC assay were performed as described above.
SDS/PAGE and immunoblotting

Gel electrophoresis and immunoblotting were performed using the procedure described by Kimura et al. (5) with some modifications. Briefly, left ventricular membranes were thawed and mixed with 2 vol. of Tris-HCl buffer (10 mM, pH 7.5) containing 5 mM EDTA, 5 mM EGTA, 10 μg/ml benzamidine, 10 μg/ml soybean trypsin inhibitor Type II-S and 5 μg/ml leupeptin. The suspensions were centrifuged at 28,000 x g for 10 min. The pellets were resuspended in the supplemented Tris-HCl buffer (10 mM, pH 7.5) and homogenized by a Polytron (setting 6, 15 sec x 2). The resulting suspensions (7.5 μg of protein) were dissolved in an equal volume of SDS sample buffer containing 50 mM Tris-HCl (pH 6.8), 10% glycerol, 1% SDS, 1% 2-mercaptoethanol and 0.02% bromophenol blue. The solutions were boiled for 5 min before application to SDS/PAGE gels. After electrophoresis, proteins were transferred from the gels to Hybond ECL by the semidry method at 1 mA/cm² for 30 min (14). Membranes were incubated for 1 hr at room temperature in a blocking solution containing 50 mM Tris-HCl (pH 8.0), 2 mM CaCl₂, 80 mM NaCl, 5% nonfat dry milk, 0.2% Nonidet P-40 and 0.02% NaN₃. Immunoblotting was performed at room temperature in TBS-T buffer containing 20 mM Tris-HCl (pH 7.5), 137 mM NaCl and 0.2% Tween-20. To quantify the G protein α-subunits (Gα), we used two antibodies, UI-1 and UI-2. These antibodies were raised against synthetic peptides corresponding to the carboxyl terminus (CKNNLKDCCGLF) of the inhibitory Gα protein (Gia) subtypes, Gia1 and Gia2 (antibody UI-2) and the common amino acid sequence (CGAGESGKSTIVKQMK) of Gα proteins (antibody UI-1). Antibodies were purified with antigen-peptide affinity columns (15, 16). The membranes were incubated with the primary antibody (UI-1 or UI-2), diluted 1/500, for 1 hr and with horseradish peroxidase-conjugated secondary antibody, diluted 1/5000, for 1 hr. Immuno-reactivity was detected with an Enhanced Chemiluminescence (ECL) Western Blot Detection system and Hyperfilm-ECL. The 41-kDa bands detected by UI-2 and the 45-kDa bands by UI-1 were regarded as the signal for Gia₁ (Gα of the stimulatory G protein), respectively. The intensities of the specific bands were determined by a GT-8000 scanner (300 dpi; Epson, Tokyo) and the software NIH Image (W. Rasband, NIH).

Protein assay

Protein content was determined by the method of Lowry et al. (17) using bovine serum albumin as a standard.

Statistical evaluation

All results were expressed as means±S.E.M. from n experiments. Values were examined by one-way analysis of variance (ANOVA). Where a difference was found across the groups, Dunnett's t-test was performed to assess the significance of the difference. The significance level was P<0.05.

Table I. Effects of continuous in vivo infusion of β-adrenoceptor agonists (40 μg/kg/hr, s.c.) on the maximal binding capacity (Bmax) of [125I]CYP to β-adrenoceptor subtypes in rat ventricular membranes

| Infusion      | [125I]CYP Kd (pM) | Bmax (fmol/mg protein) |
|---------------|-------------------|------------------------|
|               |                   | Total                  | G₁      | G₂      |
| Control       | 8.30±0.70         | 36.3±1.6               | 24.6±1.2| 11.7±0.4|
| (−) Isoprenaline [% change] | 10.0±0.50 | 23.5±1.2*** | 19.2±1.0* | 4.3±0.4*** |
|               |                   | [−35%]                 | [−22%]  | [−63%]  |
| T-0509        | 8.65±1.04         | 26.2±1.8**             | 18.2±1.3*| 8.0±0.6***|
|               |                   | [−28%]                 | [−26%]  | [−32%]  |

Each value represents the mean±S.E.M. of 4 observations. *P<0.05, **P<0.01, ***P<0.001 cf. control group.

Drugs

T-0509 [(−)-(R)-1-(3,4-dihydroxyphenyl)-2-[(3,4-dimethoxyphenethyl)amino]ethanol hydrochloride] was generously donated by Tanabe Seiyaku (Osaka). CGP20712A [1-[(2-(3-carbamoyl-4-hydroxy)phenoxy)ethylamino]-3-[4-(1-methyl-4-trifluoromethyl-2-imidazoyl)phenoxy]-2-propanol methanesulfonate] was kindly provided by Ciba-Geigy (Basel, Switzerland). G protein antibodies (UI-1 and UI-2) were generous gifts from Drs. M. Ui and T. Katada (University of Tokyo). Horseradish peroxidase-conjugated goat anti-rabbit IgG, Hybond ECL, ECL Western Blot Detection system and Hyperfilm-ECL were obtained from Amersham (Buckinghamshire, England). [125I]Iodocyanopindolol (New England Nuclear, Boston, MA, USA), [α-32P]ATP (American Radiolabeled...
Chemicals, St. Louis, MO, USA), bromoacetyl alpenolol methane (Research Biochemical, Inc., Natick, MA, USA) and other chemicals were purchased from commercial sources.

RESULTS

Downregulation of βARs by prolonged infusion

Table 1 shows the maximal binding capacities (Bmax) and affinity (Kd) of [125I]CYP to βAR subtypes in left ventricular membranes prepared from sham-operated or βAR agonist-treated rats. Specific binding of [125I]CYP to the ventricular membranes was monophasically saturable and of high affinity (Kd=8.3±0.7 pM (n=4), in the control group). From preliminary experiments, we found that the equilibrium dissociation constants (Kd) of CGP20712A for β1ARs and β2ARs were 7.2±2.8 nM and 4.6±0.7 µM (n=6), respectively. The results of [125I]CYP binding experiments with 300 nM CGP20712A, which can block almost all the β₁AR binding sites, indicated heterogeneous populations of βAR subtypes in the membranes. The populations of β₁AR and β₂AR subtypes in the control group were 69.4±0.7% and 30.6±0.7%, respectively. Kd values for [125I]CYP were not changed by prolonged infusion of the βAR agonists, indicating the remaining βAR agonists were negligible.

Prolonged infusion of isoproterenol at 40 µg/kg/hr resulted in downregulation of total βARs by 35%. When we determined the decrease in βAR subtypes, we found that isoproterenol infusion reduced the density of β₁ARs and β₂ARs by 22% and 63%, respectively. Continuous administration of T-0509 (40 µg/kg/hr) caused reduction of the β₁AR and β₂AR densities by 26% and 32% of the control, respectively.

Desensitization by prolonged infusion

Figure 1 shows effects of the prolonged infusion on isoproterenol-stimulated AC activities in the ventricular membranes. In the control membranes, isoproterenol stimulated the AC activity maximally to 6.70±0.40 pmol/mg/min. The basal activity of AC (3.60±0.17 pmol/mg/min, in control membranes, n=3) tended to be decreased by the prolonged infusion of isoproterenol (3.10±0.36 pmol/mg/min) and T-0509 (3.01±0.21 pmol/mg/min). The prolonged infusion of the β agonists attenuated the isoproterenol-stimulated AC activity. Isoproterenol-infusion decreased the maximal AC activation of isoproterenol by 55%. The EC₅₀ value of isoproterenol was not changed by prolonged infusion of isoproterenol (104±17 nM in control membranes and 184±49 nM in treated membranes). Prolonged infusion of T-0509 reduced the maximal value of isoproterenol-stimulated AC activity by 53% without change in the EC₅₀ value of isoproterenol (113±26 nM in treated membranes).

NaF (10 mM), an activator of G-protein, and forskolin (100 µM), an activator of AC, stimulated the cardiac AC in the control group up to 24.7±1.3 pmol/mg/min and 35.8±2.1 pmol/mg/min, respectively (Fig. 1). Prolonged infusion of isoproterenol or T-0509 tended to decrease NaF- and forskolin-stimulated AC activities (21.9±2.6 [isoproterenol], 21.0±1.2 [T-0509] pmol/mg/min for NaF; 29.5±5.1 [isoproterenol], 22.5±3.3 [T-0509] pmol/mg/min for forskolin), but the reduction was not statistically significant.

Fig. 1. Effects of prolonged in vivo infusion of β-adrenoeceptor agonists on isoproterenol- (upper), NaF- or forskolin-stimulated (lower) adenyl cyclase activity of rat left ventricular membranes. Rats were infused with isoproterenol or T-0509 at a rate of 40 µg/kg/hr, s.c. for 6 days. Control animals were given a sham operation. Mean±S.E.M. of 3 experiments.
Table 2. Effects of BAAM treatment on the maximal binding capacity (B<sub>max</sub>) of [125I]CYP to β adrenoceptor subtypes in rat ventricular membranes

| Treatment   | K<sub>d</sub> (pM) | B<sub>max</sub> (fmol/mg protein) |
|-------------|-------------------|----------------------------------|
|             |                   | Total | β<sub>1</sub> | β<sub>2</sub> |
| Control     | 9.0 ± 1.3         | 31.2 ± 1.2 | 21.4 ± 0.9 | 9.8 ± 0.4 |
| BAAM (100 nM) | 12.0 ± 1.6  | 21.5 ± 1.4 | 15.2 ± 1.7 | 6.3 ± 0.5 |
| [% change] |                   | [−31%] | [−29%] | [−36%] |

Each value represents the mean ± S.E.M. of 4 observations.

Irreversible Inhibition of β<sub>1</sub>ARs

To make preparations that mimic the state of β<sub>1</sub>AR agonist-induced downregulation, we treated membranes from the control group with BAAM. Table 2 shows the effect of BAAM treatment on the B<sub>max</sub> values of [125I]CYP in the ventricular membranes. The binding isotherms of [125I]CYP were monophasic and saturable. Treatment of the control ventricular membranes with BAAM decreased the B<sub>max</sub> values of [125I]CYP for β<sub>1</sub>ARs and β<sub>2</sub>ARs by 29% and 36%, respectively, without change in the K<sub>d</sub> value of [125I]CYP. In the same preparations, isoproterenol activated the AC in a dose-dependent manner (Fig. 2). BAAM treatment reduced the maximal AC activity of isoproterenol stimulation by 37% without changing the basal activity of the AC. The EC<sub>50</sub> value of isoproterenol to stimulate the AC in BAAM-treated membranes was the same as that in the control membranes. BAAM treatment changed neither NaF- nor forskolin-stimulated AC activity.

G protein content

The effects of the prolonged infusion on the G protein expression in ventricular membranes were assessed by immunoblot analysis. The left panel of Fig. 3 shows the representative gels of the immunoblot. UI-2 detected a single antigen (41 kDa) that possibly corresponds to Gi<sub>2α</sub> since expression of Gi<sub>3α</sub> is low in myocardium. UI-1 detected two antigens that correspond to G<sub>iα</sub> (41 kDa) and G<sub>α<i>c</i></sub> (45 kDa). Neither isoproterenol- nor T-0509-infusion changed the densities of bands corresponding to G<sub>α<i>c</i></sub> and G<sub>iα</sub> (Fig. 3).

DISCUSSION

There are heterogeneous populations of βAR subtypes, β<sub>1</sub>ARs and β<sub>2</sub>ARs, in rat cardiac homogenates and membranes (18). Granneman et al. (19) showed the absence of the β<sub>3</sub>AR mRNA in rat hearts, suggesting no expression of β<sub>3</sub>AR in rat myocardium. In the present study, the prolonged in vivo infusion of a non-selective βAR agonist, isoproterenol, caused downregulation of the β<sub>2</sub>ARs to a greater extent than that of the β<sub>1</sub>ARs. These results are consistent with the view that isoproterenol causes downregulation of β<sub>2</sub>ARs, whereas β<sub>1</sub>ARs are relatively resistant to downregulation (6).

T-0509 is a catechol derivative of a selective β<sub>1</sub>AR partial agonist, denopamine, and has been shown to have selective β<sub>1</sub>AR full agonist activity with less potent α<sub>1</sub>-adrenergic activity than isoproterenol (7, 8).

Prolonged infusion of T-0509 caused parallel downregulation of the βAR subtypes (approximately by 30%) in contrast to the case of isoproterenol-infusion. This is presumably due to the weak β<sub>2</sub>-adrenergic activity of T-
Fig. 3. Immunoblot analysis of G protein α-subunit expression in left ventricular membranes from control, isoproterenol- and T-0509-infused rats. Panel A: A representative result of an immunoblot detected by G protein-specific antibodies (UI-1 and UI-2) is shown. Panel B: Densitometric results are expressed as a ratio to the control for each G protein. The 41-kDa bands detected by UI-2 and the 45-kDa bands by UI-1 were regarded as the signal for Gia and Gsa, respectively. C, control; I, isoproterenol (40 μg/kg/hr); T, T-0509 (40 μg/kg/hr). Means ± S.E.M. of 4 experiments.

As βAR-stimulated AC activity of rat left ventricular membranes is dependent on the level of βAR expression (20), we tried to decrease the apparent number of βARs by treating the membranes with an irreversible βAR antagonist BAAM. BAAM treatment decreased the Bmax values of [125I]CYP for β1 and β2ARs by 29% and 36%, respectively, without any change in the Kd value, suggesting the irreversible antagonist activity of BAAM. These results also indicate that the BAAM treatment decreased the numbers of available βAR subtypes to the same (or greater) extent as T-0509 infusion at a dose of 40 μg/kg/hr. T-0509 infusion caused the desensitization of βARs in the ventricular membranes, resulting in reduc-
tion of the maximum value of isoproterenol-stimulated AC activity by 53%. In contrast, BAAM treatment decreased it only by 37%. The weak positive intrinsic activity of BAAM does not explain the smaller decrease in the maximal isoproterenol-stimulated AC activity because BAAM is derived from the βAR antagonist alprenolol and the basal level of the AC activity in the BAAM-treated membranes was not significantly different from that in the control membranes, indicating that the positive intrinsic activity of BAAM was negligible. These results suggest that the downregulation of βARs that could be mimicked by the BAAM treatment is responsible for at most 70% of the desensitization induced by T-0509 infusion.

Cardiac AC is regulated by dual pathways. β-Adrenergic stimulation activates AC through stimulatory G proteins (Gs) and the AC activity is suppressed by inhibitory G proteins (Gi). It is possible that the change in the amount of G proteins is involved in the desensitization to the βAR agonist. Kimura et al. (5) showed a selective reduction of the Gsα in rat cardiac membranes by isoproterenol treatment (4 mg/kg, i.p., b.i.d. for 4 days). In contrast, Eschenhagen et al. (4) reported that prolonged infusion of isoproterenol (2.4 mg/kg/day, s.c. for 8 days) increased the expression of the pertussis-toxin-sensitive G proteins and the amount of Gαi10 mRNA in rat myocardium. However, in the present study, we could not detect any significant changes of Gsα or Gαiα proteins in the membranes prepared from rats infused with βAR agonists. We cannot explain the discrepancy between the experiments. The experimental conditions for administering isoproterenol and its doses were clearly different among the three groups including us, and a higher concentration of isoproterenol may be necessary to observe the change in the amount of G protein. The change in the amount of G protein α-subunits cannot be a mechanism for the desensitization by the prolonged infusion in the present experiments. Thus, another mechanism(s), such as uncoupling between βARs and G proteins, decrease in AC activity by modification or decrease of AC proteins might contribute to the T-0509-induced desensitization, because prolonged infusion tended to decrease the basal and forskolin-stimulated AC activity. In this context, it should be noted that uncoupling of βAR from G protein persisted after prolonged stimulation of C6 cells with salmeterol in vitro (21).

In conclusion, the present data suggest that downregulation of βARs cannot fully account for the βAR desensitization by prolonged infusion of the βAR agonist and that another mechanism(s) can play a significant role in the loss of responsiveness. This is the first study that has quantitatively estimated the contribution of βAR downregulation to desensitization by prolonged infusion of a βAR agonist. The method used in this study may be applicable for examining certain types of heart failure (3, 22) where the desensitization and downregulation of βARs have been observed.

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