Desensitization and Selective Down-Regulation of Rat Cardiac \( \beta_1 \)-Adrenoceptors by Prolonged In Vivo Infusion of T-0509, a \( \beta_1 \)-Adrenoceptor Full Agonist

Yoji Sato\(^1\), Satomi Adachi-Akahane\(^1\), Pablo Prados\(^2\), Kazuhiro Imai\(^2\) and Taku Nagao\(^1,*\)

\(^1\)Department of Toxicology and Pharmacology and \(^2\)Department of Analytical Chemistry, Faculty of Pharmaceutical Sciences, University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan

Received June 13, 1995 Accepted September 18, 1995

ABSTRACT—We studied the effects of prolonged infusion of a selective \( \beta_1 \)-adrenoceptor (\( \beta_1 \)AR) full agonist, T-0509 [(-)-(R)-1-(3,4-dihydroxyphenyl)-2-[(3,4-dimethoxyphenethyl)amino]ethanol hydrochloride], with regard to its inotropic effect in vivo and cardiac \( \beta_1 \)AR density. The results were compared with those for isoproterenol. Continuous infusion of isoproterenol at doses of 2.5 - 40 \( \mu \)g/kg/hr, s.c. for 6 days shifted the dose-response curves of isoproterenol (i.v.) for \( \text{LVdP/dt}_{\text{max}} \) to the right and increased the \( \text{ED}_{50} \) values up to fourfold. Isoproterenol infusion at 40 \( \mu \)g/kg/hr reduced the density of both \( \beta_1 \)- and \( \beta_2 \)ARs by 36\% and 43\% respectively, in left ventricular membranes. Following 6-day infusion of T-0509 at doses sufficient to induce a positive inotropic effect (5 - 40 \( \mu \)g/kg/hr), the \( \text{ED}_{50} \) value of T-0509 (i.v.) for \( \text{LVdP/dt}_{\text{max}} \) was also increased up to fourfold. In contrast to isoproterenol, infusion of T-0509 caused selective down-regulation of \( \beta_1 \)ARs by 30\% without changing the number of \( \beta_2 \)ARs. These results indicate that long-term application of a selective \( \beta_1 \)AR full agonist causes desensitization to its inotropy in vivo, with subtype-selective down-regulation of \( \beta_1 \)ARs in cardiac ventricles.

Keywords: T-0509, \( \beta \)-Adrenoceptor, Inotropic effect, Desensitization, Down-regulation

In the mammalian heart, \( \beta \)-adrenoceptor (\( \beta \)AR) stimulation elicits a positive inotropic effect (PIE) through activation of adenylate cyclase pathways. However, treatment of whole animals with a \( \beta \)AR agonist usually leads to the development of tolerance (1 - 3). Several mechanisms, such as uncoupling of the receptor-adenylate cyclase complex, decrease in the \( \beta \)AR number and change in the amount of G-proteins, are thought to be responsible for the loss of responsiveness (4 - 7). Down-regulation of \( \beta \)ARs has been shown to be an important process for the development of tolerance, especially during long-term administration of the agonist (4, 5).

There are heterogeneous populations of adrenoceptor subtypes in the mammalian myocardium: \( \beta_1 \)-, \( \beta_2 \)- and \( \alpha_1 \)ARs (8, 9). The \( \beta_1 \)AR subtype has been shown to be predominant in cardiac tissues and responsible for the PIE and positive chronotropic effect (10). However, implication of the other AR subtypes in tolerance to the \( \beta \)AR agonist is not clear.

Several \( \beta_1 \)AR agonists have been developed during the last two decades. It is still debatable whether selective \( \beta_1 \)AR stimulation in vivo elicits tolerance in cardiac tissues. Some cardiotonic agents with partial \( \beta_1 \)AR agonist activities, such as denopamine and xamoterol, are reported to have less of a tendency to cause \( \beta \)AR desensitization than isoproterenol in rat myocardium (11 - 13). It is not known whether a selective stimulatory effect on the \( \beta_1 \)AR subtype or partial intrinsic activity is responsible for the weak desensitization in vivo. In contrast, tolerance to dobutamine easily develops in cardiac tissue in vivo (14). In several studies dealing with \( \beta \)AR desensitization, norepinephrine is used for chronic treatment (15 - 18). Although dobutamine and norepinephrine are thought to have full \( \beta \)AR agonist activity on cardiac contractility, the compounds also have \( \alpha_1 \)AR agonist activity (19). In addition, dobutamine is equally potent and effective on \( \beta_1 \)- and \( \beta_2 \)ARs in sarcolemmal membranes (20). Thus, stimuli through AR subtypes other than \( \beta_1 \)AR might account for the tolerance to dobutamine and norepinephrine (21 - 23).
The advent of a highly selective $\beta_1$AR full agonist has helped to clarify the nature of $\beta_1$AR desensitization in vivo. T-0509 (Fig. 1), which is a catechol derivative of denopamine, is considered to be a highly selective full $\beta_1$ AR agonist in vitro (24-26). Yabana et al. (25) reported that T-0509 was a $\beta_1$AR full agonist with potency at least 150 times higher on $\beta_1$ARs than on $\beta_2$ARs in isolated tissues. In the same study, T-0509 was also a less potent $\alpha$AR agonist than isoproterenol.

In this study, we examined whether T-0509 promotes homologous desensitization of its PIE in vivo, and down-regulation of $\beta$AR subtypes in cardiac muscles. The results were compared with those for the non-selective $\beta_3$AR agonist isoproterenol.

MATERIALS AND METHODS

Animals
Male Sprague-Dawley rats (Nippon Bio-Supply Center, Tokyo; 7-8 weeks of age, 210-320 g) were used for the experiment.

Cardiovascular parameters
Cardiovascular effects of agonists were measured without thoracotomy by the previously described method (11). Briefly, rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.). Left ventricular pressure was measured with a high-fidelity pressure transducer (TP-300T; Nihon Kohden, Tokyo) connected with polyethylene tubing to an injection needle (22 gauge, 32 mm) inserted into the left ventricle through the sixth or seventh intercostal space. The maximal first derivative of the left ventricular pressure (LVdP/dtm) was obtained with a differentiator amplifier (EQ-600G, Nihon Kohden). Blood pressure was measured with a pressure transducer (TNS; Gould, Oxnard, CA, USA), which was connected to a polyethylene tube inserted into the left femoral artery. Heart rate (HR) was measured by a cardiotachometer (AT-601G, Nihon Kohden), triggered by the arterial pressure pulse. All the measurements were recorded on a rectiocorder (WR-3701; Graphtec, Tokyo).

Chronic treatment with $\beta$-agonists
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 (2.5, 5, 10, 40 $\mu$g/kg/hr) or T-0509 (5, 10, 40 $\mu$g/kg/hr) dissolved in 0.9% NaCl containing 0.1% sodium metabisulfite. Control animals were given a sham operation. Rats were housed with food and water available ad libitum. After 6 days of infusion, the animals were anesthetized with ether and the pumps were removed. The PIE of the respective agonist in vivo and the amount of ventricular $\beta$ARs were assessed 16 hr after removal of the pump.

Effects and plasma levels of the agonists during infusion
The pharmacological effects of the $\beta$-agonists and their plasma levels during infusion were measured to ensure that the drug doses were sufficient to produce a PIE, as well as to confirm the accuracy of drug delivery. Functional parameters for the groups of animals were determined on day 2. Blood samples (150 $\mu$L) were obtained from the left femoral artery on day 2 just prior to determination of cardiovascular parameters, on day 6, or 16 hr after the end of the 6-day infusion. Isoprenaline and T-0509 in plasma (50 $\mu$L) were determined by an automated high performance liquid chromatography analyzer with chemiluminescence detection as described previously (27). The detection limits for isoproterenol and T-0509 were 1.3 and 0.9 fmol on injection, respectively.

Inotropic effects in agonist-treated rats
Drug-treated animals were anesthetized with pentobarbital sodium (50 mg/kg, i.p.) and LVdP/dtm, HR and mean arterial blood pressure (MAP) were monitored. Drugs dissolved in 0.9% NaCl solution were administered via the right femoral vein, increasing the doses by a factor of three at intervals of 1-5 min. The ED$_{50}$ value was defined as the dose causing a half-maximal fractional increase in a response, and it was estimated by nonlinear least-squares regression analysis, fitting the dose-response relationships to a logistic equation. In this context, a response was defined as the value of an agonist-induced change in a parameter relative to the maximal change in each animal.

Membrane preparation
Cardiac ventricular membranes were prepared by the method of U'Prichard et al. (28) with some modifications. Briefly, the animals treated continuously with $\beta$-agonists as described above were anesthetized with pentobarbital sodium (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 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.5, 37°C) and centrifuged at 28,000 × g for 10 min. The pellet was rehomogenized and centrifuged as described above. The resulting pellet was finally suspended at a concentration of 8.5 mg original wet tissue per 1 ml buffer.

Receptor binding assay

Ventricular membranes (50 to 100 μg of protein) were incubated with appropriate concentrations (1.5 - 50 pM) of [125I]iodocyanopindolol ([125I]CYP, specific activity: ~74 TBq/mmol) in a final volume of 300 μl assay buffer for 90 min at 37°C. After the incubation, the samples were diluted, harvested and poured onto glass fiber filters (Whatman GF/C), which were throughly washed using a Brandel cell harvester. The radioactivity retained on the filters was counted in a gamma counter (ARC-300; Aloka, Tokyo) at an efficiency of 78%. Specific binding to myocardial membranes was defined as that displaced by 1 μM (±)-propranolol. The relative proportions of the βAR subtypes in the tissue were assessed by performing saturation binding assays of specific [125I]CYP binding in the presence or absence of a given concentration (500 nM) of the highly β1-selective antagonist CGP20712A (29). The concentration of CGP20712A used in this study was confirmed from graphical analysis of preliminary displacement experiments of [125I]CYP (total 50 pM) binding to rat ventricular membranes, as that quantity of the compound which would occupy more than 98% of the β1ARs in the membranes. Equilibrium dissociation constants (Kd) and maximal binding capacities (Bmax) for total βARs and β2 subtypes were determined by nonlinear least-squares analysis, fitting the data to Michaelian rectangular hyperbolic curves with a computer program, SP123, developed by H. Ono (University of Tokyo) (30). The Bmax for β1-subtypes was calculated as the difference between the Bmax for total βARs and that for β2-subtypes. Protein content was determined by the method of Lowry et al. (31) using bovine serum albumin as a standard.

Statistical evaluation

All results are 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, Bonferroni’s multiple t-test was performed to assess the significance of the difference. The significance level was P < 0.05.

Drugs

T-0509 ([(−)-(R)]-1-[3,4-dihydroxyphenyl]-2-[(3,4-dimethoxyphenethylamino)ethanol hydrochloride] was kindly donated by Tanabe Seiyaku, Osaka. CGP20712A [1-2-((3-carbamoyl-4-hydroxy)phenoxy)ethylamino]-3-[4-(1-methyl-4-trifluoromethyl-2-imidazoyl)phenoxyl]-2-propanol methanesulfonate] was provided by Ciba-Geigy, Basel, Switzerland. [125I]Iodocyanopindolol (Amersham Japan, Tokyo) and other chemicals were

---

Table 1. Plasma concentration of β-agonists on day 2 or day 6

| Drug          | Concentration (nM) | Dose of chronically infused β-agonist (μg/kg/hr) |
|--------------|--------------------|-----------------------------------------------|
|              |                    | 2.5  | 5    | 10   | 40   |
| T-0509       | day 2              | —    | 2.86±0.53 | 7.33±0.99 | 30.38±2.48 |
|              | day 6              | —    | 2.30±0.44 | 4.20±0.85 | 14.79±2.00 |
| Isoproterenol| day 2              | 0.62±0.04 | 0.77±0.13 | 1.24±0.05 | 3.36±1.10 |
|              | day 6              | 0.42±0.04 | 0.53±0.11 | 1.89±0.99 | 6.65±0.75 |

The plasma concentration of T-0509 or isoproterenol was measured on day 2 or day 6 under pentobarbital anesthesia. Values are expressed as the arithmetic means of 4 to 6 observations with S.E.M.
purchased from commercial sources.

RESULTS

Cardiovascular effects of β-agonists during infusion

Effects of continuous infusion of β-agonists for 2 days on LVdP/dt\text{max} in anesthetized rats are shown in Fig. 2.

\( LVdP/dt_{\text{max}} \): Two-day infusion of isoproterenol at 40 \( \mu \)g/kg/hr produced the maximal response in LVdP/dt\text{max} (28.0±0.9 mmHg \times 10^3/\text{sec}, n=4, \text{Fig. 2}). By infusion of each agonist at doses of 5.0 \( \mu \)g/kg/hr or more, LVdP/dt\text{max} was significantly increased to levels comparable with those for the maximal effect of isoproterenol.

\text{Plasma concentration}: Prolonged administration of β-agonists caused increases in their plasma concentrations on both days 2 and 6 in a dose-dependent manner (Table 1). Plasma levels of isoproterenol infused for 6 days at 10 and 40 \( \mu \)g/kg/hr were higher than those on day 2. Although the levels of plasma T-0509 at any dose on day 6 were lower than on day 2, those in the groups infused for 6 days at 10 and 40 \( \mu \)g/kg/hr were still sufficient to cause the nearly maximal PIE. To confirm sufficient washout of β-agonists, plasma samples were collected after a 16-hr washout period from rats that had been infused with the agonists at 40 \( \mu \)g/kg/hr. One of five samples from the isoproterenol-infused rats contained a low but detectable amount of isoproterenol (0.62 nM), and two of five samples from the T-0509-infused rats had only trace amounts of T-0509 (0.11 and 0.24 nM). Plasma agonist levels in the other seven samples were below the limits of our detection method. These results indicated adequate clearance of the agonists.

\text{Heart rate and blood pressure}: Control values of HR and MAP on the day 2 were 378±18 beats/min and 103±7 mmHg (n=6), respectively. Two-day infusion of β-agonists significantly increased HR in a dose-dependent manner (up to 551±24 beats/min [isoproterenol, n=4] and 582±11 beats/min [T-0509, n=6]). MAP was not significantly changed by subcutaneous infusion of either isoproterenol or T-0509.

Acute effects of β-agonists in control groups

As shown in Fig. 3, intravenous administration of isoproterenol to the sham-operated control rats at a dose of 3 \( \mu \)g/kg elicited the maximal responses in \( LVdP/dt_{\text{max}} \) (173% increase), HR (41% increase) and MAP (54% decrease). The \( ED_{50} \) values for isoproterenol were 20.5±5.5 (\( LVdP/dt_{\text{max}} \)), 69.3±26.5 (HR) and 33.8±11.0 (MAP) ng/kg. Intravenous administration of T-0509 to the control animals at a dose of 3 \( \mu \)g/kg also produced maximal effects on \( LVdP/dt_{\text{max}} \) (181% increase), HR (33% increase) and MAP (42% decrease) (Fig. 4). The \( ED_{50} \) values for T-0509 were 24.0±3.5 (\( LVdP/dt_{\text{max}} \)), 115±17 (HR) and 223±49 (MAP) ng/kg. Thus, the PIE of T-0509 was 9.3 times more potent than its hypotensive effect.
Influence of chronic administration

The cardiovascular effects of isoproterenol in isoproterenol-infused rats are shown in Fig. 3 and Table 2.

Basal levels of HR and MAP in all isoproterenol-pretreated groups were not different from those in the control group. Baselines of LVdP/dt_{max} in the groups pretreated at doses less than 10 μg/kg/hr were not different from those in the control group, whereas they were significantly reduced in the groups pretreated at doses of 10 and 40 μg/kg/hr.

Table 2. Influence of isoproterenol and T-0509 pretreatment (6 days) on basal LVdP/dt_{max} and inotropic responses to respective agonists

| Drug       | n  | LVdP/dt_{max} (mmHg × 10^3/sec) | ED_{50} (ng/kg, i.v.) |
|------------|----|---------------------------------|-----------------------|
| Isoproterenol |    | Baseline                         | Maximum               |
| control    | 5  | 8.06±0.35                       | 22.0±1.7              | 20.5±5.5              |
| 2.5 μg/kg/hr, s.c. | 5  | 7.94±0.25                       | 22.8±1.1              | 27.8±4.1              |
| 5.0         | 4  | 6.88±0.16                       | 21.3±1.2              | 34.2±7.3              |
| 10          | 5  | 6.67±0.56*                      | 20.5±1.4              | 56.9±10.0*            |
| 40          | 5  | 6.59±0.18*                      | 20.4±1.3              | 76.1±11.6**           |
| T-0509     |    |                                 |                       |
| control    | 5  | 8.25±0.42                       | 23.2±1.0              | 24.0±3.5              |
| 5.0 μg/kg/hr, s.c. | 5  | 7.84±0.26                       | 21.9±1.9              | 30.0±8.9              |
| 10          | 5  | 6.08±0.35**                      | 21.7±0.9              | 70.3±3.5**            |
| 40          | 6  | 6.13±0.56**                      | 20.2±1.8              | 95.2±7.9**            |

Baseline values and inotropic responses to isoproterenol and T-0509 were measured after a 16-hr washout period. Values are means±S.E.M. ED_{50} value represents the dose causing a half-maximal fractional increase in LVdP/dt_{max}. *P<0.05, **P<0.01 cf control group.

The maximal effects of acute i.v. infusion of isoproterenol on the parameters tended to decrease with isoproterenol-pretreatment, but were not significantly different from those in the control group.

In the 2.5-μg/kg/hr group, the PIE of isoproterenol (i.v.) was not affected by chronic administration. In the isoproterenol-pretreated groups given doses of 5.0 μg/kg/hr or more, however, the dose-response curves for the PIE of isoproterenol (i.v.) were shifted to the right. The ED_{50} values for the PIE in the 10- and 40-μg/kg/hr groups were significantly higher, being 3- and 4-fold greater than that for the control group, respectively.

On the other hand, the positive chronotropic effect of isoproterenol was not affected by chronic pretreatment. The effect of isoproterenol on MAP was significantly attenuated only in the 40-μg/kg/hr group.

The cardiovascular effects of T-0509 in rats pretreated with T-0509 are shown in Fig. 4 and Table 2.

The basal levels of HR and MAP were not changed by pretreatment with T-0509. Baselines of LVdP/dt_{max} in the groups pretreated at 5 μg/kg/hr, s.c. were not significantly different from those in the control group, whereas they were significantly lowered in the groups pretreated at doses of 10 and 40 μg/kg/hr.

Influence of chronic administration

The cardiovascular effects of isoproterenol in isoproterenol-infused rats are shown in Fig. 3 and Table 2.

Basal levels of HR and MAP in all isoproterenol-pretreated groups were not different from those in the control group. Baselines of LVdP/dt_{max} in the groups pretreated at doses less than 10 μg/kg/hr were not different from those in the control group, whereas they were significantly reduced in the groups pretreated at doses of 10 and 40 μg/kg/hr.

The maximal effects of acute i.v. infusion of isoproterenol on the parameters tended to decrease with isoproterenol-pretreatment, but were not significantly different from those in the control group.

In the 2.5-μg/kg/hr group, the PIE of isoproterenol (i.v.) was not affected by chronic administration. In the isoproterenol-pretreated groups given doses of 5.0 μg/kg/hr or more, however, the dose-response curves for the PIE of isoproterenol (i.v.) were shifted to the right. The ED_{50} values for the PIE in the 10- and 40-μg/kg/hr groups were significantly higher, being 3- and 4-fold greater than that for the control group, respectively.

On the other hand, the positive chronotropic effect of isoproterenol was not affected by chronic pretreatment. The effect of isoproterenol on MAP was significantly attenuated only in the 40-μg/kg/hr group.

The cardiovascular effects of T-0509 in rats pretreated with T-0509 are shown in Fig. 4 and Table 2.

The basal levels of HR and MAP were not changed by pretreatment with T-0509. Baselines of LVdP/dt_{max} in the groups pretreated at 5 μg/kg/hr, s.c. were not significantly different from those in the control group, whereas they were significantly lowered in the groups pretreated at doses of 10 and 40 μg/kg/hr.
Pretreatment with T-0509 tended to decrease but not significantly change the maximal effects of acutely administered T-0509 on the parameters. In the 5.0-μg/kg/hr group, the PIE of T-0509 (i.v.) was not affected by prolonged infusion. In the groups given doses of 10 and 40 μg/kg/hr, s.c., however, the dose-response curves for the PIE of T-0509 (i.v.) were shifted to the right. The ED₅₀ values for the PIE of the 10- and 40-μg/kg/hr groups were significantly 3- and 4-fold greater than that for the control group, respectively.

The positive chronotropic effect of T-0509 was not affected by chronic pretreatment. The effect of T-0509 on MAP was slightly but significantly attenuated in the 10- and 40-μg/kg/hr groups.

Radioligand binding assay

Specific binding of [¹²⁵I]CYP to rat ventricular membranes was monophasically saturable and of high affinity (Bmax = 15.1 ± 0.8 fmol/mg protein and Kd = 6.0 ± 0.5 pM (n=10), in the control group). In the preliminary displacement experiments, equilibrium dissociation constants (Kd) of CGP20712A for β₁ and β₂ARs were 7.2 ± 2.8 nM and 4.6 ± 0.7 μM (n=6), respectively. The results of saturation analysis performed in the presence of the highly selective β₁-antagonist CGP20712A (500 nM) also indicated monophasically saturable binding of [¹²⁵I]CYP to β₂ARs with an affinity the same as that to total βARs (Bmax = 4.4 ± 0.2 fmol/mg protein and Kd = 5.0 ± 0.4 pM (n=10), in the control group), indicating a heterogeneous population of β₂AR-subtypes. Pretreatment with agonists did not change the wet weight of the left ventricles. Kd values for [¹²⁵I]CYP were not changed in any group either in the absence or presence of CGP20712A (Table 3).

Table 4 shows the maximal binding capacities (Bmax) of [¹²⁵I]CYP to βAR-subtypes in left ventricular membranes obtained from pretreated rats. Prolonged infusion with isoproterenol at 10 and 40 μg/kg/hr caused significant down-regulation of total βAR Bmax by 23% and 38% of the control, respectively. Pretreatment with isoproterenol did not cause subtype-selective reduction of βARs.

Continuous administration of T-0509 at a dose of 40 μg/kg/hr resulted in significant reduction of the Bmax values of both total βARs and β₁-subtypes by 23% and 30% of the control, respectively, without alteration of the β₂ Bmax.

DISCUSSION

We studied the influence of β₁AR subtype selectivity of a βAR full agonist on the development of tolerance to its PIE in vivo and on βAR density in the left ventricle. We found that continuous infusion of the β₁AR full agonist induced tolerance to its PIE in vivo and selective loss of β₁AR subtypes on rat cardiac ventricular membranes. Moreover, in the present model, prolonged infusion of isoproterenol produced desensitization to its PIE in vivo.
and non-selective down-regulation of myocardial \(\beta\)ARs, even at doses lower than those reported previously.

Although T-0509 was described as a non-selective \(\beta\)AR partial agonist, Compound XVI (32), it appeared later to be a highly selective \(\beta_1\)AR full agonist with weak \(\beta_2\) and little \(\alpha_1\)-activity on isolated mammalian tissues (25). We therefore characterized the \(\beta_1\)-selectivity of T-0509 in whole animals. Intravenous infusion of T-0509 into control rats produced a maximal PIE equivalent to that caused by isoproterenol. The mean ED\(_{50}\) value of T-0509 on MAP was 9.3 times greater than that on LVD\(_{p/dt_{max}}\), compared with 1.6-fold in the case of isoproterenol. These data are consistent with the \(\beta_1\)-selectivity and full agonist activity of T-0509 reported by Yabana et al. (25).

We also found that the extent of desensitization to \(\beta\)-agonists varied among different parameters. The acute effects of the \(\beta\)-agonists on MAP, as well as the PIE, were significantly attenuated after the prolonged treatment, whereas the positive chronotropic effects were not. These differences suggest that the positive chronotropic effects of \(\beta\)-agonists are more resistant to desensitization than the other effects. The reason for the resistance is not clear. It might be involved in differential neuronal control. Hayes et al. (2) reported desensitization to the positive chronotropic effect of \(\beta\)-agonists with pithed rats instead of measuring the physiological parameters in intact rats, when they were pretreated with a high dose of isoproterenol.

There are heterogeneous populations of \(\beta\)ARs, i.e., the \(\beta_1\) and \(\beta_2\)-subtypes, in rat cardiac homogenates and membranes (8). Granneman et al. (33) demonstrated the absence of \(\beta_2\)AR mRNA in rat heart, suggesting no expression of \(\beta_2\)AR on rat myocardium. In the present study, the density of total \(\beta_2\)AR was lowered on left ventricular membranes prepared from animals treated with isoproterenol at 10 and 40 \(\mu\)g/kg/hr. Isoproterenol infusion did not cause subtype selective down-regulation. In contrast to the case of isoproterenol, T-0509 infusion at 40 \(\mu\)g/kg/hr selectively reduced the \(\beta_1\)-AR density on rat ventricular membranes without affecting the density of \(\beta_2\)ARs.

There is now a body of evidence indicating the differential regulation of cardiac \(\beta\)AR subtypes by catecholamines (34). In explanted human hearts with dilated cardiomyopathy, \(\beta_1\)ARs are significantly and selectively more down-regulated than the \(\beta_2\)-subtype (35, 36). It is plausible that the increased level of plasma norepinephrine in patients with heart failure (37) selectively stimulates and decreases \(\beta_1\)ARs. Norepinephrine has selective \(\beta_1\)full agonist activity as well as potent \(\alpha_1\)AR agonist activity. \(\alpha_1\)-Adrenergic stimuli have been shown to cause a PIE and cardiac hypertrophy (9). Thus, occupancy of \(\alpha_1\)ARs by norepinephrine may cause some stimulation with a concomitant modulating effect on the function of \(\beta\)ARs along with homologous desensitization by \(\beta\)AR stimulation (21–23). However, the present data obtained by long-term infusion of T-0509 suggest that potent \(\beta_1\)AR stimulation by itself causes selective down-regulation of \(\beta_1\)ARs on the myocardium without a change in \(\beta_2\)AR density. Of course, it is possible that \(\beta_2\)ARs might be down-regulated to some extent by T-0509 infusion on day 6 and might have recovered during the wash-out period (16). Even if this had been the case, down-regulation of \(\beta\)AR subtypes would have to depend on the selectivity of the \(\beta\)-agonist because down-regulation of \(\beta_2\)ARs by isoproterenol, in contrast to that by T-0509, remained at 16 hr after the infusion.

In this study, isoproterenol at 5 \(\mu\)g/kg/hr (s.c.) appeared to have a nearly maximal PIE on day 2 and to cause desensitization to its PIE during chronic treatment. In contrast, T-0509 at 5 \(\mu\)g/kg/hr (s.c.) produced a marked PIE on day 2 but did not induce desensitization. In addition, T-0509 did not cause down-regulation of cardiac \(\beta_2\)ARs. Therefore, selective stimulation of \(\beta_1\)ARs may be less prone to the development of tolerance than simultaneous stimulation of \(\beta_1\) and \(\beta_2\)ARs.

Acknowledgments

This study was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan. We would like to thank Tanabe Seiyaku and Ciba-Geigy for generously providing the drugs used in this study. We thank Dr. H. Ono (University of Tokyo) for kindly providing the data analysis program and Dr. H. Kurose (University of Tokyo) for critically reviewing this manuscript.

REFERENCES

1. Chang HY, Delin RM and Kunos G: Selective desensitization of cardiac beta receptors by prolonged in vivo infusion of catecholamine in rats. J Pharmacol Exp Ther 221, 784–789 (1982)
2. Hayes JS, Pollock GD and Fuller RW: In vivo cardiovascular responses to isoproterenol, dopamine and tyramine after prolonged infusion of isoproterenol. J Pharmacol Exp Ther 231, 633–639 (1984)
3. Hayes JS, Wyss VL, Scheneck KS and Cohen ML: Effects of prolonged isoproterenol infusion on cardiac and vascular responses to adrenergic agonists. J Pharmacol Exp Ther 237, 757–763 (1986)
4. Hausdorff WP, Caron MG and Lefkowitz RJ: Turning off the signal: desensitization of \(\beta\)-adrenergic receptor function. FASEB J 4, 2881–2889 (1990)
5. Homcy CJ, Vatin SF and Vatner DE: \(\beta\)-Adrenergic receptor regulation in the heart in pathophysiological states: abnormal adrenergic responsiveness in cardiac disease. Annu Rev Physiol 53, 137–159 (1991)
6. Eschenhagen T, Mende U, Diederich M, Nose M, Schmitz W, Scholz H, Schulte am Esch J, Warnholz A and Schäfer H: Long term \(\beta\)-adrenoceptor-mediated up-regulation of \(G\_o\) and \(G\_s\) mRNA levels and pertussis toxin-sensitive guanine
nucleotide-binding proteins in rat heart. Mol Pharmacol 42, 773–783 (1992)
7 Kimura H, Miyamoto A and Ohshika H: Down-regulation of β-adrenoceptors and loss of Gαs subunit levels in ventricular myocardium of rats treated with isoprenaline. Life Sci 53, 171–176 (1993)
8 Minneman KP, Hegstrand LR and Molinoff PB: Simultaneous determination of beta-1 and beta-2 adrenergic receptors in tissue containing both receptor subtypes. Mol Pharmacol 16, 34–46 (1979)
9 Terzic A, Pucet M, Vassort G and Vogel SM: Cardiac α1-adrenoceptors: an overview. Pharmacol Rev 45, 147–175 (1993)
10 Molenaar P and Summers RJ: Characterization of beta-1 and beta-2 adrenoceptors in guinea pig atrium: functional and receptor binding studies. J Pharmacol Exp Ther 241, 1041–1047 (1987)
11 Yahana H, Naito K and Nagao T: Effect of chronic administration of denopamine (TA-064), a new positive inotropic agent, on cardiac response of rats to denopamine. Jpn J Pharmacol 42, 87–97 (1986)
12 Kowalski MT, Haworth D, Lu X, Thomson DS and Barnett DB: Comparison of the effects of xamoterol and isoprenaline on rat cardiac β-adrenoceptors: studies of function and regulation. Br J Pharmacol 99, 27–30 (1990)
13 Limas CJ and Limas C: Effects of xamoterol on the reversible cycling of cardiac β-adrenoceptors. J Cardiovasc Pharmacol 16, 945–951 (1990)
14 Unverferth DH, Blanford M, Kates RE and Leier CV: Tolerance to dobutamine after a 72 hour continuous infusion. Am J Med 69, 262–266 (1980)
15 Deighton NM, Brown AD, Hamilton CA and Reid JL: Regulation of adrenergic receptor number following chronic noradrenaline infusion in the rabbit. Naunyn Schmiedebergs Arch Pharmacol 338, 517–522 (1988)
16 Snively MD, Ziegler MG and Insel PA: A new approach to determine rates of receptor appearance and disappearance in vivo. Application to agonist-mediated down-regulation of rat renal cortical beta-1 and beta-2-adrenergic receptors. Mol Pharmacol 27, 19–26 (1985)
17 Snively MD, Ziegler MG and Insel PA: Subtype-selective down-regulation of rat renal cortical alpha- and beta-adrenergic receptors by catecholamines. Endocrinology 117, 2182–2189 (1985)
18 Brown L, Sernia C, Newing R and Flether P: Cardiac responses after norepinephrine-induced ventricular hypertrophy in rats. J Cardiovasc Pharmacol 20, 316–323 (1992)
19 Kenakin TP: An in vitro quantitative analysis of the alpha adrenoceptor partial agonist activity of dobutamine and its relevance to isotropic selectivity. J Pharmacol Exp Ther 266, 210–219 (1981)
20 Minneman KP, Hegstrand LR and Molinoff PB: The pharmacological specificity of beta-1 and beta-2 adrenergic receptors in rat heart and lung in vitro. Mol Pharmacol 16, 21–33 (1979)
21 Bouvier M: Cross-talk between second messengers. Ann NY Acad Sci 594, 120–129 (1990)
22 Arner P, Kriengholm E and Engfeldt P: In vivo interactions between beta-1 and beta-2 adrenoceptors regulate catecholamine tachyphylaxis in human adipose tissue. J Pharmacol Exp Ther 259, 317–322 (1991)
23 Houslay MD: 'Crosstalk': a pivotal role for protein kinase C in modulating relationships between signal transduction pathways. Eur J Biochem 195, 9–27 (1991)
24 Kurozawa H, Satoh E, Yanagisawa T and Taara N: Selective β1-receptor full agonists, T-0509 and T-1583, increase the force monophonaphically and cyclic AMP biphasically in canine ventricular muscle. J Cardiovasc Pharmacol 13, 105–117 (1990)
25 Yahana H, Watanabe H, Narita H and Nagao T: Selective and full β1-adrenoceptor agonist action of a catechol derivative of denopamine (T-0509) in the guinea pig cardiac muscle and trachea: comparison with denopamine, xamoterol and isoprenaline. Br J Pharmacol 106, 335–341 (1992)
26 Yahana H, Murata S, Narita H, Shimizu R, Miyagishita T, Takeda M and Nagao T: Selective β1-adrenoceptor agonist activity of denopamine and its derivatives in dogs. Biol Pharm Bull 16, 471–474 (1993)
27 Prados P, Higashidate S, Imai K, Satoh Y and Nagao T: Selective determination of (−)-isoproterenol and (−)-(R)-1-(3,4-dihydroxyphenyl)-2-[3,4-dimethoxyphenoxy]aminomethyl) ethanol (T-0509), a cardioactive agent, in rat plasma utilizing a fully automated catecholamine analyzer. Biomed Chromatogr 8, 49–51 (1994)
28 U'Prichard DC, Bylund DB and Synder SH: (±)[3H]Epinephrine and (−)[3H]dihydroalprenolol binding to β1- and β2- adrenergic receptors in brain, heart and lung membranes. J Biol Chem 253, 5090–5102 (1978)
29 Dooley DJ, Bittiger H and Reymann NC: CGP20712A: a useful tool for quantitating β1- and β2-adrenoceptors. Eur J Pharmacol 130, 137–139 (1986)
30 Ikeda S, Oka J and Nagao T: Effects of four ditiazem stereoisomers on binding of d-cis-[3H]siltiazem and (−)[3H][3H]PN200-110 to rabbit T-tubule calcium channels. Eur J Pharmacol (Mol Pharmacol Section) 208, 199–205 (1991)
31 Lowry OH, Rosebrough NJ, Farr AL and Randall RJ: Protein measurement with the Folin phenol reagent. J Biol Chem 193, 265–275 (1951)
32 Molenaar P, McPherson GA, Malta E and Raper C: The influence of molecular structure on the affinity and efficacy of some β-adrenoceptor agonists. Naunyn Schmiedebergs Arch Pharmacol 331, 240–246 (1985)
33 Granneman JG, Lahners KN and Chaudhry A: Molecular cloning and expression of the rat β3-adrenoceptor receptor. Mol Pharmacol 40, 895–899 (1991)
34 Munzt KH, Zhao M and Miller JC: Downregulation of myocardial β-adrenoceptors. Receptor subtype selectivity. Cire Res 74, 369–375 (1994)
35 Bristow MR, Ginsburg R, Umas V, Fowler M, Minobe W, Rasmussen R, Zera P, Menlove R, Shah P, Jamieson S and Stinson EB: β1- and β2-Adrenergic-receptor subpopulations in nonfailing and failing human ventricular myocardium: coupling of both receptor subtypes to muscle contraction and selective β1-receptor down-regulation in heart failure. Circ Res 59, 297–309 (1986)
36 Brodde OE: β1- and β2-Adrenoceptors in the human heart: properties, function, and alteration in chronic heart failure. Pharmacol Rev 43, 203–242 (1991)
37 Cohn JN, Levine TB, Oblivi MT, Garberg V, Lura D, Francis GS, Simon AB and Rector T: Plasma norepinephrine as a guide to prognosis in patients with chronic congestive heart failure. N Engl J Med 311, 819–823 (1984)