Desensitization and Selective Down-Regulation of Rat Cardiac β_1-Adrenoceptors by Prolonged In Vivo Infusion of T-0509, a β_1-Adrenoceptor Full Agonist

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ABSTRACT—We studied the effects of prolonged infusion of a selective β_1-adrenoceptor (β_1AR) 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 βAR density. The results were compared with those for isoproterenol. Continuous infusion of isoproterenol at doses of 2.5 − 40 μg/kg/hr, s.c. for 6 days shifted the dose-response curves of isoproterenol (i.v.) for $LVdP/dt_{max}$ to the right and increased the ED_{50} values up to fourfold. Isoproterenol infusion at 40 μg/kg/hr reduced the density of both β_1- and β_2ARs 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 μg/kg/hr), the ED_{50} value of T-0509 (i.v.) for $LVdP/dt_{max}$ was also increased up to fourfold. In contrast to isoproterenol, infusion of T-0509 caused selective down-regulation of β_1ARs by 30% without changing the number of β_2ARs. These results indicate that long-term application of a selective β_1AR full agonist causes desensitization to its inotropy in vivo, with subtype-selective down-regulation of β_1ARs in cardiac ventricles.

Keywords: T-0509, β-Adrenoceptor, Inotropic effect, Desensitization, Down-regulation

In the mammalian heart, β-adrenoceptor (βAR) stimulation elicits a positive inotropic effect (PIE) through activation of adenylate cyclase pathways. However, treatment of whole animals with a βAR agonist usually leads to the development of tolerance (1 − 3). Several mechanisms, such as uncoupling of the receptor-adenylate cyclase complex, decrease in the βAR number and change in the amount of G-proteins, are thought to be responsible for the loss of responsiveness (4 − 7). Down-regulation of βARs 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: β_1-, β_2- and α_1ARs (8, 9). The β_1AR 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 βAR agonist is not clear.

Several β_1AR agonists have been developed during the last two decades. It is still debatable whether selective β_1AR stimulation in vivo elicits tolerance in cardiac tissues. Some cardiotonic agents with partial β_1AR agonist activities, such as denopamine and xamoterol, are reported to have less of a tendency to cause βAR desensitization than isoproterenol in rat myocardium (11 − 13). It is not known whether a selective stimulatory effect on the β_1AR 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 βAR desensitization, norepinephrine is used for chronic treatment (15 − 18). Although dobutamine and norepinephrine are thought to have full β_1AR agonist activity on cardiac contractility, the compounds also have α_1AR agonist activity (19). In addition, dobutamine is equally potent and effective on β_1- and β_2ARs in sarcolemmal membranes (20). Thus, stimuli through AR subtypes other than β_1AR might account for the tolerance to dobutamine and norepinephrine (21 − 23).

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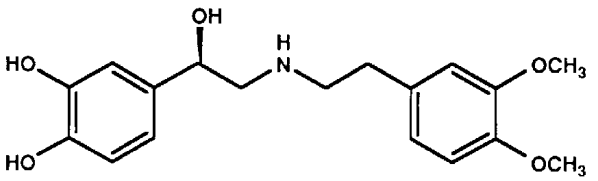

Fig. 1. The chemical structure of T-0509.

The advent of a highly selective β_1AR full agonist has helped to clarify the nature of β_1AR desensitization in vivo. T-0509 (Fig. 1), which is a catechol derivative of denopamine, is considered to be a highly selective full β_1AR agonist in vitro (24–26). Yabana et al. (25) reported that T-0509 was a βAR full agonist with potency at least 150 times higher on β_1ARs than on β_2ARs in isolated tissues. In the same study, T-0509 was also a less potent αAR agonist than isoproterenol.

In this study, we examined whether T-0509 promotes homologous desensitization of its PIE in vivo, and down-regulation of βAR subtypes in cardiac muscles. The results were compared with those for the non-selective βAR 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 ($LVdP/dt_{max}$) was obtained with a differentiator amplifier (EQ-600G, Nihon Kohden). Blood pressure was measured with a pressure transducer (TNF; 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 recticorder (WR-3701; Graphtec, Tokyo).

Chronic treatment with β-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 μg/kg/hr) or T-0509 (5, 10, 40 μg/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 βARs were assessed 16 hr after removal of the pump.

Effects and plasma levels of the agonists during infusion

The pharmacological effects of the β-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 μl) 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 μl) 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 $LVdP/dt_{max}$, 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 β-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 × 2)

in 20 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.5, 37℃) 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 [^{125}I]iodocyanopindolol ([^{125}I]CYP, specific activity: ~74 TBq/mmol) in a final volume of 300 μl assay buffer for 90 min at 37℃. 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 [^{125}I]CYP binding in the presence or absence of a given concentration (500 nM) of the highly β_1-selective antagonist CGP20712A (29). The concentration of CGP20712A used in this study was confirmed from graphical analysis of preliminary displacement experiments of [^{125}I]CYP (total 50 pM) binding to rat ventricular membranes, as that quantity of the compound which would occupy more than 98% of the β_1ARs in the membranes. Equilibrium dissociation constants (K_d) and maximal binding capacities (B_{max}) for total βARs and β_2 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 B_{max} for β_1-subtypes was calculated as the difference between the B_{max} for total βARs and that for β_2-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-dimethoxyphenethyl)amino]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)phenoxy]-2-propanol methanesulfonate] was provided by Ciba-Geigy, Basel, Switzerland. [^{125}I]Iodocyanopindolol (Amersham Japan, Tokyo) and other chemicals were

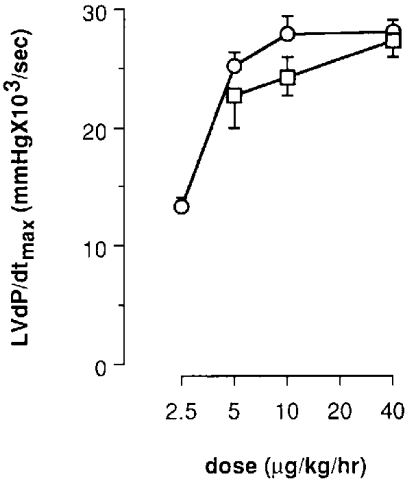

Fig. 2. Influence of continuous infusion on $LVdP/dt_{max}$. Rats were subjected to s.c. infusions of isoproterenol (○) and T-0509 (□). The PIEs were determined on day 2 under pentobarbital anesthesia. Values are expressed as the arithmetic means of 4 to 6 observations with S.E.M. Some of the S.E.M. lie within the symbols. The control value of $LVdP/dt_{max}$ was 9.63±0.71 mmHg×10^3/sec (n=6).

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 $LVdP/dt_{max}$ in anesthetized rats are shown in Fig. 2.

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

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 μg/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 μg/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 μg/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.

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 μg/kg elicited the maximal responses in $LVdP/dt_{max}$ (173% increase), HR (41% increase) and MAP (54% decrease). The ED_{50} values for isoproterenol were 20.5 ± 5.5 ($LVdP/dt_{max}$), 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 μg/kg also produced maximal effects on $LVdP/dt_{max}$ (181% increase), HR (33% increase) and MAP (42% decrease) (Fig. 4). The ED_{50} values for T-0509 were 24.0 ± 3.5 ($LVdP/dt_{max}$), 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.

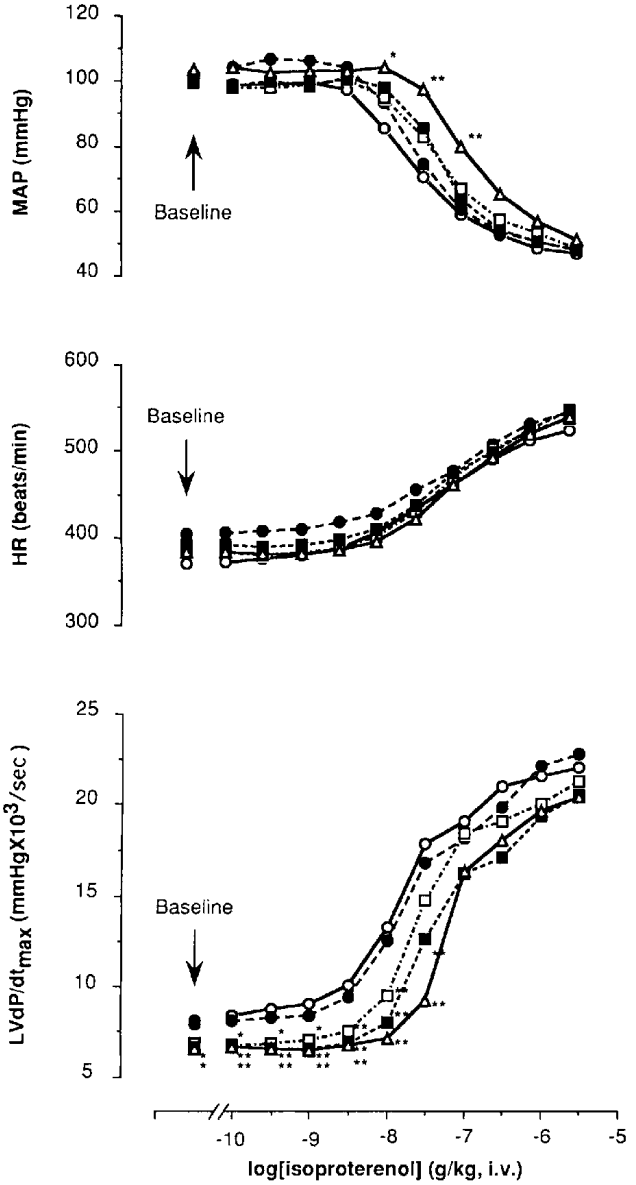

Fig. 3. Effects of intravenously administered increasing doses of isoproterenol on $LVdP/dt_{max}$, heart rate (HR) and mean arterial blood pressure (MAP) in anesthetized rats continuously infused with isoproterenol for 6 days. Values are the mean of 4 or 5 experiments. ○: control group; ●: 2.5, □: 5.0, ■: 10, △: 40 μg/kg/hr, s.c. group. Each standard error of the mean was less than 15% of the respective mean value. *P<0.05, **P<0.01 cf control group. The basal levels of $LVdP/dt_{max}$, HR and MAP in the control group were 8.06 ± 0.35 mmHg $\times 10^3$/sec, 371 ± 14 beats/min and 100 ± 3 mmHg, respectively. The control ED_{50} values for isoproterenol (i.v.) were 20.5 ± 5.5 ng/kg ($LVdP/dt_{max}$), 69.3 ± 26.5 ng/kg (HR) and 33.8 ± 11.0 ng/kg (MAP).

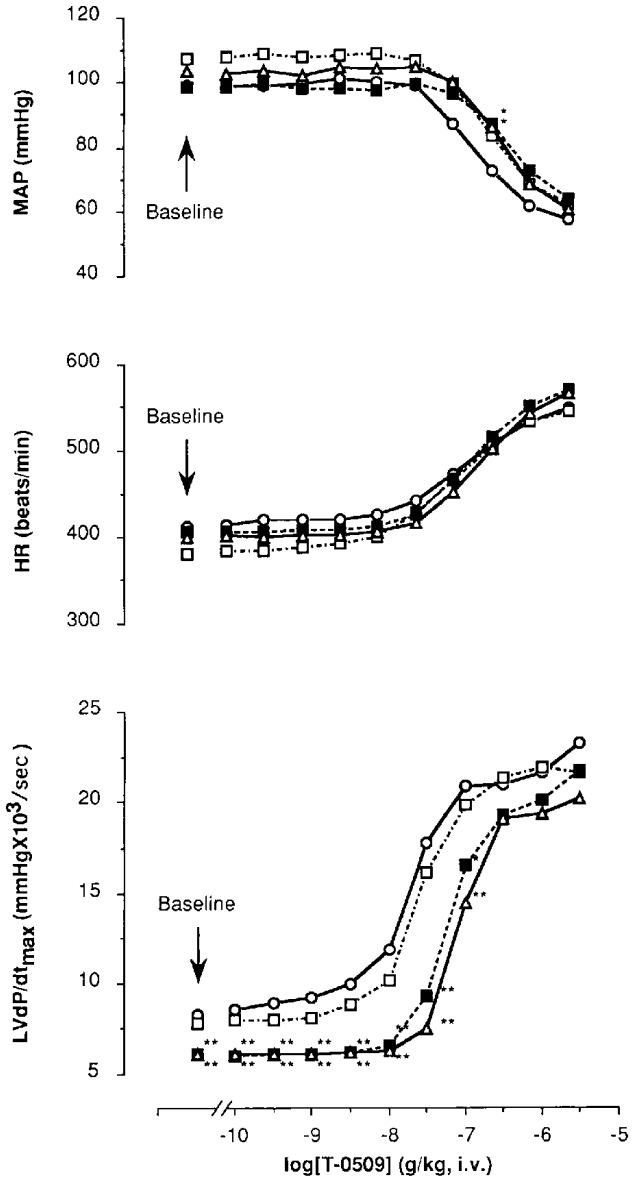

Fig. 4. Effects of intravenously administered increasing doses of T-0509 on $LVdP/dt_{max}$, heart rate (HR) and mean arterial blood pressure (MAP) in anesthetized rats continuously infused with T-0509 for 6 days. Values are each the mean of 5 or 6 experiments. ○: control group; □: 5.0, ■: 10, △: 40 μg/kg/hr, s.c. group. Each standard error of the mean was less than 15% of the respective mean value. $^*P<0.05$, $^{**}P<0.01$ cf control group. The basal levels of $LVdP/dt_{max}$, HR and MAP in the control group were 8.25 ± 0.42 mmHg $\times 10^3$/sec, 412 ± 16 beats/min and 99 ± 6 mmHg, respectively. The control ED_{50} values for T-0509 (i.v.) were 24.0 ± 3.5 ng/kg ($LVdP/dt_{max}$), 115 ± 17 ng/kg (HR) and 223 ± 49 ng/kg (MAP).

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 $\times 10^3$/sec)		ED_{50} (ng/kg, i.v.)
		Baseline	Maximum	
Isoproterenol				
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.

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 ED_{50} 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 [^{125}I]CYP to rat ventricular membranes was monophasically saturable and of high affinity (B_{max}=15.1±0.8 fmol/mg protein and K_d=6.0±0.5 pM (n=10), in the control group). In the preliminary displacement experiments, equilibrium dissociation constants (K_i) of CGP20712A for β_1- and β_2ARs 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 β_1-antagonist CGP20712A (500 nM) also indicated monophasically saturable binding of [^{125}I]CYP to β_2ARs with an affinity the same as that to total βARs (B_{max}=4.4±0.2 fmol/mg protein and K_d =5.0±0.4 pM (n=10), in the control group), indicating a heterogeneous population of βAR-subtypes. Pretreatment with agonists did not change the wet weight of the left ventricles. K_d values for [^{125}I]CYP were not changed in any group either in the absence or presence of CGP20712A (Table 3).

Table 4 shows the maximal binding capacities (B_{max}) of [^{125}I]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 B_{max} 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 B_{max} values of both total βARs and β_1-subtypes by 23% and 30% of the control, respectively, without alteration of the β_2 B_{max}.

Table 3. Effects of continuous infusion of β-agonists in vivo on the wet LV weight and K_d values of [^{125}I]CYP for β-adrenoceptors in rat ventricular membranes

Infusion	wet LV wt. (g)	$K_{d,total}$ (pM)	$K_{d,\beta 2}$ (pM)
Control	0.74±0.01	6.0±0.5	5.0±0.4
Isoproterenol			
10 μg/kg/hr, s.c.	0.80±0.03	5.8±0.4	6.1±0.7
40	0.79±0.04	5.5±0.6	6.2±0.6
T-0509			
10 μg/kg/hr, s.c.	0.69±0.02	5.5±0.5	5.6±0.4
40	0.79±0.03	5.5±0.4	5.7±0.4

Each value represents the mean±S.E.M. of 10 observations. $K_{d,total}$, $K_{d,\beta 2}$: equilibrium dissociation constant in the absence and presence of 500 nM CGP20712A, respectively.

DISCUSSION

We studied the influence of β_1AR 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 β_1AR full agonist induced tolerance to its PIE in vivo and selective loss of β_1AR subtypes on rat cardiac ventricular membranes. Moreover, in the present model, prolonged infusion of isoproterenol produced desensitization to its PIE in vivo

Table 4. Effects of continuous infusion of β-agonists in vivo on the maximal binding capacity (B_{max}) of [^{125}I]CYP to β-adrenoceptor subtypes in rat ventricular membranes

Infusion	B_{max} (fmol/mg protein)		
	Total [% change]	β_1 [% change]	β_2 [% change]
Control	15.1±0.8	10.7±0.7	4.4±0.2
Isoproterenol			
10 μg/kg/hr, s.c.	11.6±0.9* [−23%]	8.6±0.7 [−20%]	3.0±0.3** [−32%]
40	9.4±1.0** [−38%]	6.9±0.8** [−36%]	2.5±0.3** [−43%]
T-0509			
10 μg/kg/hr, s.c.	12.7±0.9 [−19%]	8.1±0.8 [−24%]	4.6±0.2 [+5%]
40	11.6±1.0* [−23%]	7.5±0.8* [−30%]	4.1±0.3 [−7%]

Each value represents the mean±S.E.M. of 10 observations. *P<0.05, **P<0.01 cf control group.

and non-selective down-regulation of myocardial βARs, even at doses lower than those reported previously.

Although T-0509 was described as a non-selective βAR partial agonist, Compound XVI (32), it appeared later to be a highly selective β_1AR full agonist with weak β_2- and little α_1-activity on isolated mammalian tissues (25). We therefore characterized the β_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 $LVdP/dt_{max}$, compared with 1.6-fold in the case of isoproterenol. These data are consistent with the β_1-selectivity and full agonist activity of T-0509 reported by Yabana et al. (25).

We also found that the extent of desensitization to β-agonists varied among different parameters. The acute effects of the β-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 β-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 β-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 βARs, i.e., the β_1- and β_2-subtypes, in rat cardiac homogenates and membranes (8). Granneman et al. (33) demonstrated the absence of β_3AR mRNA in rat heart, suggesting no expression of β_3AR on rat myocardium. In the present study, the density of total βAR was lowered on left ventricular membranes prepared from animals treated with isoproterenol at 10 and 40 μg/kg/hr. Isoproterenol infusion did not cause subtype selective down-regulation. In contrast to the case of isoproterenol, T-0509 infusion at 40 μg/kg/hr selectively reduced the β_1AR density on rat ventricular membranes without affecting the density of β_2ARs.

There is now a body of evidence indicating the differential regulation of cardiac βAR subtypes by catecholamines (34). In explanted human hearts with dilated cardiomyopathy, β_1ARs are significantly and selectively more down-regulated than the β_2-subtype (35, 36). It is plausible that the increased level of plasma norepinephrine in patients with heart failure (37) selectively stimulates and decreases β_1ARs. Norepinephrine has selective β_1-full agonist activity as well as potent α_1AR agonist activity. α_1-Adrenergic stimuli have been shown to cause a PIE and cardiac hypertrophy (9). Thus, occupancy of α_1ARs by norepinephrine may cause some stimulation with a concomitant modulating effect on the function of βARs along with homologous desensitization by βAR stimulation (21–23). However, the present data obtained by long-term infusion of T-0509 suggest that potent β_1AR stimulation by itself causes selective down-regulation of β_1ARs on the myocardium without a change in β_2AR density. Of course, it is possible that β_2ARs 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 βAR subtypes would have to depend on the selectivity of the β-agonist because down-regulation of β_2ARs by isoproterenol, in contrast to that by T-0509, remained at 16 hr after the infusion.

In this study, isoproterenol at 5 μg/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 μg/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 β_2ARs. Therefore, selective stimulation of β_1ARs may be less prone to the development of tolerance than simultaneous stimulation of β_1 and β_2ARs.

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