Proc. Natl. Acad. Sci. USA
Vol. 94, pp. 137–141, January 1997
Biochemistry

Ligand-induced overexpression of a constitutively active β_2-adrenergic receptor: Pharmacological creation of a phenotype in transgenic mice

PHILIPPE SAMAMA*†, RICHARD A. BOND‡, HOWARD A. ROCKMAN§, CARMELO A. MILANO*, AND ROBERT J. LEFKOWITZ*

*Howard Hughes Medical Institute, Departments of Medicine, Surgery and Biochemistry, Duke University Medical Center, Box 3821, Durham, NC 27710; ‡Department of Pharmacological and Pharmaceutical Sciences, University of Houston, Houston, TX 77204; and §Department of Medicine, University of California at San Diego, La Jolla, CA 92093

Contributed by Robert J. Lefkowitz, November 12, 1996

ABSTRACT **Transgenic overexpression (40- to 100-fold) of the wild-type human β_2-adrenergic receptor in the hearts of mice leads to a marked increase in cardiac contractility, which is apparently due to the low level of spontaneous (i.e., agonist-independent) activity inherent in the receptor. Here we report that transgenic mice expressing a mutated constitutively active form of the receptor (CAM) show no such phenotype, owing to its modest expression (3-fold above endogenous cardiac β-adrenergic receptor levels). Surprisingly, treatment of the animals with a variety of β-adrenergic receptor ligands leads to a 50-fold increase in CAM β_2-adrenergic receptor expression, by stabilizing the CAM β_2-adrenergic receptor protein. Receptor up-regulation leads in turn to marked increases in adenylate cyclase activity, atrial tension determined *in vitro*, and indices of cardiac contractility determined *in vivo*. These results illustrate a novel mechanism for regulating physiological responses, i.e., ligand-induced stabilization of a constitutively active but inherently unstable protein.**

Several naturally occurring and artificially created point mutations of G protein-coupled receptors lead to an abrogation of intramolecular interactions, which normally constrain receptors to an inactive state (1, 2). This results in constitutive or agonist-independent activity of the receptors. Studies of such constitutively active mutant (CAM) receptors have revealed that in many respects their properties mimic those of the activated state of the normal receptors. In several cases they initiate human diseases (3–7). It was also shown *in vitro* that such a CAM β_2-adrenergic receptor possesses increased conformational flexibility, which results not only in constitutive activity of the receptor but in marked instability (8). To assess the *in vivo* consequences of these unique properties, we created transgenic mice overexpressing the CAM β_2-adrenergic receptor in the heart.

EXPERIMENTAL PROCEDURES

Construction of the Transgenic Mouse Line. The cDNA encoding the CAM β_2-adrenergic receptor (1) was spliced to the α-myosin heavy chain gene promoter and the simian virus 40 intron/termination sequences, as described (9). Mouse eggs were injected with the linearized DNA construct and implanted in pseudo-pregnant females (strain C57 Black; genotype B6SJF1/J1). Offspring were selected for the presence of the transgene-specific simian virus 40 sequence.

***In Vitro* Assays of Receptor Function.** Particulate fractions were prepared from the hearts as described (9). The hearts were homogenized in ice-cold buffer (50 mM Hepes, pH 7.2/150 mM NaCl/5 mM EDTA). The 800 g supernatants were spun twice at 38,000 × *g*. The final pellet was resuspended in 50 mM Hepes (pH 7.2)/12 mM $MgCl_2$. Total β-adrenergic receptor binding was measured at 37°C for 45 min using 300 pM ^{125}I-CYP in 50 mM Hepes (pH 7.2), 5 mM EDTA, 0.1 mM ascorbic acid, and included 2 μM propranolol for nonspecific binding (1). β_1 and β_2 subtype proportions were determined by competition with varying doses of the β_2-selective ligand ICI 118,551 (10).

Adenylate cyclase activity was determined as described (9). Heart particulate preparations were pre-incubated with β-adrenergic ligands for 5 min at 37°C in 20 mM Hepes (pH 7.2), 4.5 mM $MgCl_2$, 2.7 mM phospho*enol*pyruvate, 53 μM GTP, 0.1 mM cAMP, 150 mM ATP, 20 units/ml myokinase, 4 units/ml pyruvate kinase, 0.1 mM ascorbic acid. The radioactive tracer ([α-^{32}P]ATP, ≈1 μCi per sample, 1 Ci = 37 GBq; New England Nuclear) was added and the reactions allowed to proceed for another 10 min at 37°C, after which they were quenched on ice. Total adenylate cyclase activity was determined using 10 mM NaF. [^{32}P]cAMP was separated as described (9).

Determination of Atrial Tension. Two- to four-month-old animals were treated for 91 hr with ICI 118,551 (0.7 mg/kg/hr) or vehicle (50% dimethylsulfoxide/50% saline) using osmotic minipumps (Alzet, Palo Alto, CA) implanted subcutaneously under anesthesia (10). The hearts were removed 6–12 hr after the pumps had run out, to allow for four biological half-lives of the ligand to elapse. Left atria (2–4 mg) were excised from the hearts and suspended in carbogenated modified Kreb's bicarbonate solution supplemented with 0.1 mM ascorbic acid, 0.1 μM cocaine, 0.4 μM corticosterone, and 3 μM phentolamine (9). They were paced at optimal frequency for isometric tension development (3.2 Hz), 3 msec pulse duration, voltage at threshold +20%, as described (9). Agonist-induced responses were measured after tension reached a plateau (2–4 min).

Determination of *in Vivo* Left Ventricular (LV) Function. Twelve hours after the pumps had run out (see above), hemodynamic parameters were measured in live anesthetized mice (10). After anesthesia, mice were intubated and connected to a rodent ventilator. Either carotid artery was cannulated with a flame-stretched PE-50 catheter connected to a pressure transducer. Following bilateral vagotomy, the chest was opened and a 1.8 Fr high-fidelity micromanometer cath-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

0027-8424/97/94137-5$2.00/0
PNAS is available online at **http://www.pnas.org**.

Abbreviations: CAM, constitutively active mutant; LV, left ventricular.

†Present address: Pharmacopeia, Inc., 3000 Eastpark Boulevard, Cranbury, NJ 08512.

eter was inserted into the left atrium, advanced through the mitral valve, and secured in the left ventricle. Hemodynamic measurements were recorded at baseline and 45–60 sec after injection of incremental doses of isoproterenol in a cannulated jugular vein. Doses of isoproterenol were specifically chosen to maximize the contractile response but limit the increase in heart rate. Continuous high fidelity LV and fluid filled aortic pressure were recorded simultaneously at baseline and 45–60 sec after each dose of agonist on an 8-channel chart recorder and in digitized form at 2000 Hz for later analysis. Parameters measured were heart rate, aortic pressure, LV systolic and end diastolic pressure, and the maximal and minimal first derivative of LV pressure (LV dP/dtmax per min). Ten sequential beats were averaged for each measurement.

RESULTS AND DISCUSSION

The CAM β_2-adrenergic receptor (1) was expressed under the control of the α-myosin heavy chain promoter. This promoter leads to expression of the transgene in the atrium during embryonic and adult life, and expression in the ventricular myocardium after birth (9). Descendants of two founders were studied between 2 and 4 months of age, when expression of the transgene was constant (data not shown). β-adrenergic receptors are expressed at 59 ± 12 fmol/mg membrane protein (mean ± SEM) in membranes prepared from the hearts of nontransgenic animals (n = 8), with the β_1 and β_2 subtypes accounting for ≈75% and ≈25%, respectively (10). In transgenic animals, total myocardial receptor density was 209 ± 11 fmol/mg (n = 12, Fig. 1*A*), and the increase was accounted for by the exogenous β_2-adrenergic receptor. Expression of the transgene did not affect the expression of the endogenous β_1-adrenergic receptor (nontransgenic littermates: 45 ± 11 fmol/mg membrane protein, n = 6; transgenic animals: 53 ± 6 fmol/mg membrane protein, n = 6).

In nontransgenic mice, the agonist isoproterenol stimulates plasmalemmal adenylate cyclase 2- to 3-fold. Expression of the transgenic CAM β_2-adrenergic receptor leads to an increase in maximal agonist-evoked adenylate cyclase activity in cardiac particulate fractions (Fig. 1*B*). Thus, both expression of the transgene and functional coupling of the exogenous β_2-adrenoreceptors can be demonstrated in these transgenic animals. However, no increase in agonist-independent adenylate cyclase activity was observed, presumably because of the low expression level of the transgene.

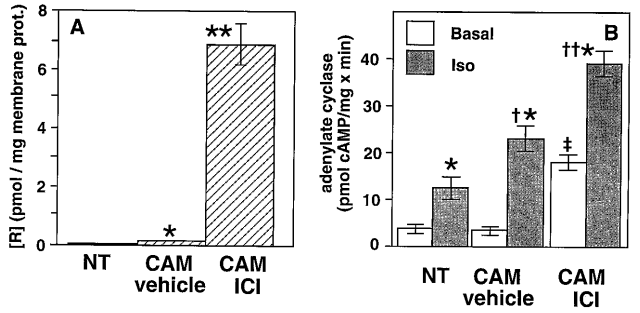

FIG. 1. (*A*) β_2-adrenergic receptor density in the myocardium. NT, nontransgenic littermates (n = 8); CAM vehicle, vehicle-treated CAM β_2-adrenergic receptor transgenic animals (n = 12); CAM ICI, ICI 118,551-treated transgenic animals (n = 8). Shown are means ± SEM. **, $P < 0.01$, CAM ICI vs. CAM vehicle; *, $P < 0.05$, CAM vehicle vs. NT (Student's t test). (*B*) Adenylate cyclase activity in myocardial particulate fractions. Basal, no added drug; Iso, 100 μM isoproterenol. NT, n = 6; CAM vehicle, n = 6; CAM ICI, n = 5. *, $P < 0.05$, Iso vs. Basal; †, $P < 0.05$, Iso CAM vehicle vs. Iso NT; ††, $P < 0.05$, Iso CAM ICI vs. Iso CAM vehicle; ‡, $P < 0.05$, Basal CAM ICI vs. Basal CAM vehicle; Iso CAM vehicle vs. Basal CAM ICI, P = n.s.

Biological Effects of *in Vivo* Antagonist Infusion. Previous studies in cultured cells have shown that the CAM β_2-adrenoreceptor is poorly expressed and spontaneously down-regulated. This down-regulation can be alleviated by inclusion of β-adrenergic ligands in the culture medium, leading to an ≈2-fold increase in expression levels (8, 11). To examine whether such a regulatory effect could be observed *in vivo*, we infused transgenic animals with the inverse agonist ICI 118,551 for 7 or 4 days using subcutaneous osmotic minipumps (11). After the pumps had run out, β-adrenergic receptor function was assessed *in vitro* and *in vivo*. Receptor expression was dramatically increased, to 6.9 ± 0.8 pmol/mg (n = 8, Fig. 1*A*), an ≈50-fold overexpression. This increase was due entirely to up-regulation of the transgene, and the endogenous β_1-adrenoreceptor was not modulated (54 ± 12 fmol/mg membrane protein, n = 5). ICI 118,551 treatment of nontransgenic animals did not result in any change of β-adrenergic receptor density in the myocardium or the lungs (data not shown).

ICI 118,551 treatment also led to an increase in basal adenylate cyclase, to levels comparable with that of agonist-activated cyclase in vehicle-treated transgenic animals (Fig. 1*B*). However, there was no change in either total NaF-stimulated adenylate cyclase activity (nontransgenic: 70 ± 8 pmol cAMP per mg membrane protein per min, n = 6; vehicle-treated transgenic: 68 ± 10 pmol cAMP per mg membrane protein per min, n = 6; ICI 118,551-treated transgenic: 88 ± 24 pmol cAMP per mg membrane protein per min, n = 5), or G protein-coupled receptor kinase 2 immunoreactivity (data not shown) (10).

Atrial tension was measured on excised left atria. In nontransgenic and vehicle-treated transgenic animals, baseline tension was comparable, and was stimulated ≈2-fold by a maximal concentration of the agonist isoproterenol (100 nM; Fig. 2). By contrast, the infusion of ICI 118,551 in transgenic animals produced a significant elevation of baseline tension when compared with nontransgenic and vehicle-treated transgenic animals. This elevated baseline tension was not significantly increased further by the addition of isoproterenol, and was comparable with the agonist-stimulated tension of nontransgenic and vehicle-treated transgenic preparations (Fig. 2). ICI 118,551 treatment of nontransgenic animals did not alter baseline or isoproterenol-stimulated tension (data not shown).

To determine whether treatment with ICI 118,551 alters the *in vivo* cardiac phenotype in transgenic mice overexpressing the mutant β_2-adrenergic receptor, cardiac catheterization was used to measure contractile function in live anesthetized mice. Continuous measurements of LV pressure and the maximal first derivative of LV pressure (dP/dtmax) were recorded

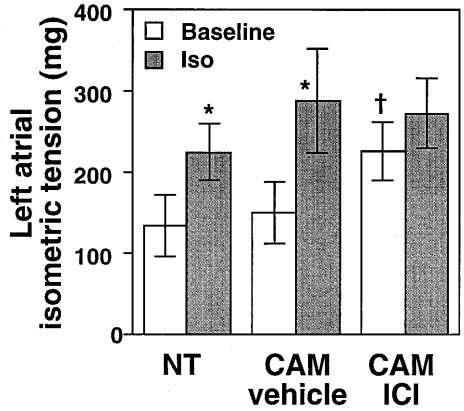

FIG. 2. Left atrial tension. Baseline, saline. Iso, 100 nM isoproterenol. NT, CAM vehicle, CAM ICI as in Fig. 1. *, $P < 0.05$, Iso vs. Baseline. †, $P < 0.05$, CAM ICI Baseline vs. CAM Baseline (Student's t test).

before and after progressive injections of isoproterenol. CAM β_2-adrenergic receptor mice pre-treated with ICI 118,551 showed a 45% increase in basal dP/dtmax, which was accentuated with a low-dose isoproterenol injection. Myocardial relaxation as measured by LV dP/dtmin was also significantly enhanced by treatment in the transgenic mice (data not shown). ICI 118,551 treatment did not alter baseline LV systolic pressure. However, the marked increase in contractile function with isoproterenol (50 pg) in the treated CAM β_2-adrenergic receptor mice was associated with a significantly higher LV systolic pressure (Fig. 3*B*). In contrast, treatment with ICI 118,551 had no significant effect in nontransgenic mice (Fig. 3 *C* and *D*).

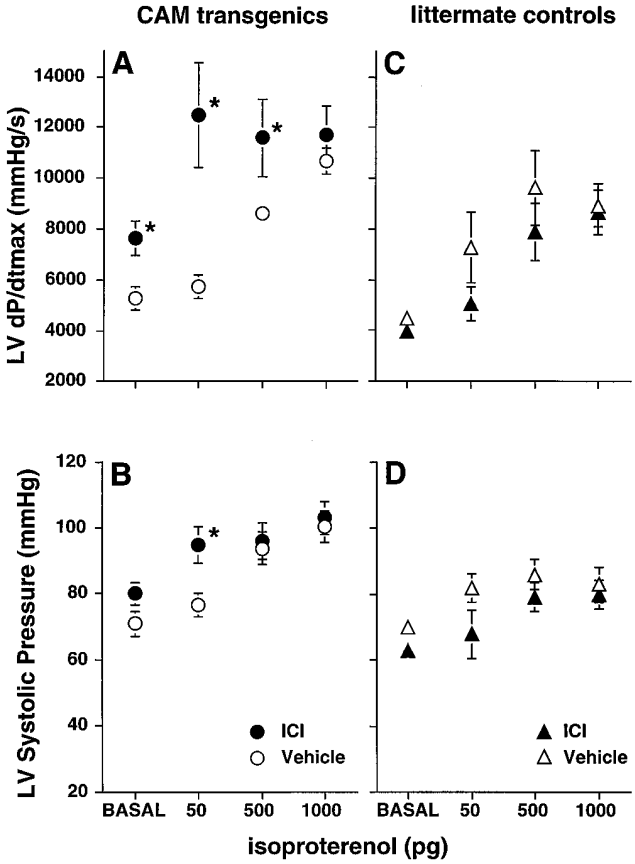

FIG. 3. *In vivo* assessment of LV contractile function. Measured parameters are shown at baseline and after progressive injections of isoproterenol. Hemodynamic measurements were performed in transgenic mice overexpressing the CAM β_2-adrenergic receptor (*A* and *B*: ICI 118,551 treated, $n = 11$; vehicle treated, $n = 10$) and nontransgenic littermate controls (*C* and *D*: ICI 118,551 treated, $n = 11$; vehicle treated, $n = 9$). Data are expressed as mean ± SEM. ICI 118,551-treated vs. vehicle-treated data were compared using a two-way repeated-measure ANOVA. Post-hoc comparison of differences in mean values between the groups at a specific dose were conducted with a Newman–Keuls test; *, $P < 0.01$. A significant between-group main effect in response to isoproterenol was found for LV dP/dtmax (*A*; $P < 0.05$). The pattern of change between groups was statistically different for LV dP/dtmax (*A*; $P < 0.0001$) and LV systolic pressure (*B*; $P < 0.05$). ICI 118,551 treatment of animals did not alter the measured hemodynamic parameters in nontransgenic littermate controls (*C* and *D*). LV end diastolic pressure was not statistically different between groups at baseline (4.4 ± 0.8 vs. 5.3 ± 0.6 mmHg in ICI 118,551-treated vs. vehicle-treated transgenic animals, respectively; 4.9 ± 0.6 vs. 4.0 ± 0.7 mmHg in ICI 118,551-treated vs. vehicle-treated littermate controls, respectively). Pretreatment with ICI 118,551 did not alter heart rate in transgenic mice (484 ± 33 vs. 525 ± 24 beats per min mmHg in ICI 118,551-treated vs. vehicle-treated animals, P = ns), or in nontransgenic littermate controls (383 ± 21 vs. 370 ± 25 beats per min in ICI 118,551-treated vs. vehicle-treated animals, P = ns).

Thus, ligand-induced up-regulation of the CAM β_2-adrenoreceptor leads to constitutive activation of the adrenergic signaling cascade in the heart. This constitutive activation is observed at lower expression levels than those previously obtained with the wild-type β-adrenergic receptor (20–40 pmol/mg) (9), consistent with the higher basal activity of the mutated receptor.

Biochemical Basis of the Receptor Up-Regulation. We infused transgenic animals with a variety of β-adrenergic receptor ligands, ranging in efficacy from inverse agonists to full agonists. Inverse agonists (nadolol, ICI 118,551) and antagonists (propranolol, alprenolol, pindolol) were found to up-regulate the transgenic receptor to a similar extent (Fig. 4*A*, compounds 1–5). Atenolol (2 mg/kg/hr), a preferential β_1-adrenoreceptor antagonist, produced negligible up-regulation ([R] = 315 ± 40 fmol/mg, $n = 4$), indicating that the up-regulation of the transgenic β_2- adrenoreceptor is not mediated by the endogenous β_1-subtype adrenoreceptor. A single bolus dose of ICI 118,551 did not result in receptor up-regulation (data not shown), and the continued presence of ICI 118,551 in the circulation was necessary for the maintenance of receptor overexpression. Indeed, when the 4-day infusion with ICI 118,551 was followed by a 4-day "recovery" period, myocardial receptor density was down to 1 ± 0.5 pmol/mg ($n = 4$).

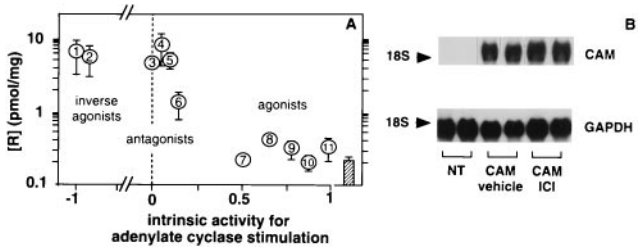

FIG. 4. Effect of *in vivo* treatment with adrenoreceptor ligands on CAM β_2-adrenergic receptor levels and transgene expression. (*A*) Receptor levels in cardiac particulate preparations. The intrinsic activity of drugs at the CAM receptor was assessed *in vitro* at the level of adenylate cyclase in myocardial particulate preparations. Intrinsic activity (IA) was defined using untreated CAM transgenic animals ($n = 2$–4) as the fraction of adenylate cyclase activity evoked by the drug, compared with the reference full agonist isoproterenol (IA = 1) and the basal activity (IA = 0). Negative intrinsic activities (inverse agonists) were assessed with ICI 118,551-treated up-regulated CAM animals ([R] = 4 ± 1 pmol/mg, $n = 4$), with IA = 0 for basal and IA = −1 for maximal inhibition of basal, to the level of nontransgenic basal activity (see Fig. 1*B*). Nadolol and ICI 118,551 were used at 1 μM, and all other drugs were used at 20 × K_d (1). All reactions included 300 nM CGP 20712A, which blocks the endogenous β_1-adrenergic receptor subtype selectively. 1, ICI 118,551 (0.7 mg/kg/hr); 2, nadolol (1.2 mg/kg/hr); 3, propranolol (0.4 mg/kg/hr); 4, alprenolol (0.4 mg/kg/hr); 5, pindolol (0.4 mg/kg/hr); 6, dichloroisoproterenol (1.2 mg/kg/hr); 7, ephedrine (0.3 mg/kg/hr); 8, dobutamine (1 mg/kg/hr); 9, norepinephrine (0.3 mg/kg/hr); 10, epinephrine (0.1 mg/kg/hr); 11, isoproterenol (0.1 mg/kg/hr). Crosshatched bar, untreated CAM transgenic. Shown are mean receptor densities ± SEM of 4–10 animals. (*B*) mRNA levels following drug treatment. NT, CAM vehicle, and CAM ICI as in Fig. 1. Total RNA was extracted from myocardial tissue (Biotecx Laboratories, Houston) and analyzed by Northern blotting. Shown are two representative lanes for each animal group (5 μg RNA per lane). The blots were probed with a simian virus 40 sequence, which is unique to the transgenic DNA construct (CAM, *Upper*), stripped and reprobed with the cDNA encoding glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an internal quantity control (*Lower*) (12). Arrowheads, relative location of the 18S ribosomal RNA. Northern blots (3–4 animals per group) were also quantified on a PhosphorImager (Molecular Dynamics). When normalized to the amount of GAPDH mRNA, the transgene mRNA was not significantly increased by the ICI 118,551 treatment (data not shown).

In contrast to inverse agonists and antagonists, agonists did not cause significant up-regulation (compounds 7–11), whereas dichloroisoproterenol, a weak partial agonist (compound 6), led to intermediate receptor levels. Thus, the magnitude of the up-regulation *in vivo* seems inversely related to drug efficacy, although it does not discriminate between inverse agonists (e.g., nadolol) and antagonists (e.g., alprenolol).

Reserpine perfusion was previously shown to deplete >98% of catecholamine stores in the heart (13). Animals were treated with reserpine (0.3 mg/kg intraperitoneally every 24 hr) prior to and during the 4-day antagonist infusion. Reserpine treatment did not prevent up-regulation of the transgene by either ICI 118,551, alprenolol, or propranolol, and had no effect on receptor levels per se (data not shown). Thus, catecholaminergic tone is not responsible for the low expression of the mutated receptor in transgenic animals.

Total RNA was extracted from the hearts of vehicle- and ICI 118,551-treated transgenic animals, and assayed for transgene expression. ICI 118,551 treatment did not significantly alter steady-state levels of transgene mRNA (Fig. 4*B*). Thus, ligand effects on gene regulation, if any, do not account for the measured increase in binding sites (≈50-fold).

Increases of membrane β-adrenergic receptor density following treatment with β-adrenergic receptor antagonists have been observed in laboratory animals and in patients, albeit of much lower magnitude (≈2-fold) (14, 15). In some cases this increase was shown to be accounted for by the rapid (≈60 min) externalization of an intracellular vesicular pool of receptors (16). We found, however, that transgenic myocardial preparations did not contain an appreciable vesicular receptor population (data not shown), and that the large increase in membrane receptor density observed in our model cannot be ascribed to the externalization of a large pool of intracellular receptors.

We examined whether the effect of drugs on the CAM β-adrenergic receptor *in vivo* might be ascribed to some molecular property of this receptor. In agreement with findings by Gether *et al.* (8), we found that the CAM β-adrenergic receptor exhibits a greatly enhanced lability as compared with the wild-type β_2-adrenergic receptor, in particulate preparations derived from either Sf9 cells (data not shown) or myocardial tissue from transgenic animals (Fig. 5). This instability is consistent with previous observations that the CAM β_2-adrenergic receptor is expressed at lower levels than the wild-type receptor in transfected cells (1, 8), and that it is quite unstable during purification (ref. 8 and unpublished observations). We thus hypothesized that this instability accounts for its low steady-state expression levels, both *in vivo* and *in vitro*. Correspondingly, β-adrenergic receptor ligands might be expected to protect the CAM receptor against decay. In myocardial membranes we found that a representative inverse agonist (nadolol), a representative antagonist (alprenolol), and a full agonist (isoproterenol) were all capable of preventing the loss of binding sites (Fig. 5). Thus, in agreement with findings in purified Sf9 preparations (8), occupancy of the receptor by a ligand, irrespective of its efficacy is sufficient to inhibit or substantially retard the decay of the CAM β_2-adrenergic receptor. This protection presumably causes the receptor to accumulate in the plasmalemma of myocytes, to levels similar to what we have previously observed for the transgenic wild-type β-adrenergic receptor [pmol/mg range (9)]. The much greater receptor up-regulation observed *in vivo* (50-fold) than *in vitro* (2- to 3-fold) presumably relates to the much higher basal receptor expression levels in the isolated cells (several pmol/mg) compared with the *in vivo* heart (≈100 fmol/mg).

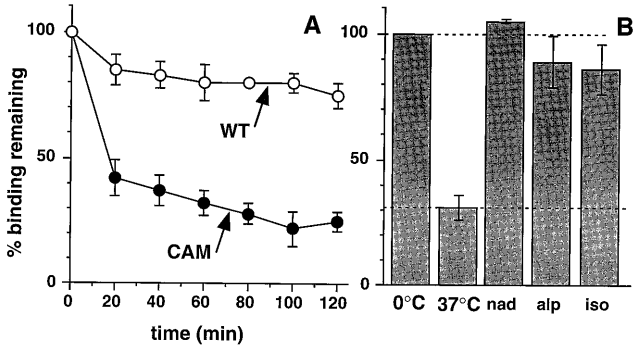

FIG. 5. Stability of wild-type and CAM β_2-adrenergic receptor in Sf9 and myocardial particulate preparations. (*A*) Particulate fractions derived from Sf9 cells harboring either the wild-type or the CAM β_2-adrenergic receptor (10 pmol/mg and 2 pmol/mg, respectively) were prepared like myocardial samples (see Fig. 1). These preparations were incubated for various times at 37°C in ligand-binding buffer prior to the addition of radioligand (see Fig. 1). Shown is the average ± one-half of the difference of two experiments. (*B*) Myocardial particulate preparations of up-regulated CAM transgenic animals (≈10 pmol/mg) were incubated for 1 hr on ice (0°C) or at 37°C with no ligand (37°C) or in the presence of either 2 μM nadolol (nad), 100 nM alprenolol (alp), or 2 μM isoproterenol (iso). Membranes were pelleted and resuspended for 1 hr at 4°C in drug-free binding buffer, pelleted again, and assessed for binding as above. Shown are means ± SEM of 3–6 experiments.

Intriguingly, the full agonist isoproterenol is capable of protecting the receptor *in vitro*, although it does not up-regulate it *in vivo*. Several nonexclusive explanations are possible. First, the stabilizing effect of agonist ligands on the receptor may be offset by the well-documented *in vivo* effects of agonists to down-regulate the receptors by a variety of mechanisms. Because this down-regulatory effect of agonists requires efficacy, the net effect of drug infusion would decrease as the efficacy of the drug increases past a threshold level (marked by pindolol in Fig. 4*A*). Indeed, isoproterenol infusion in transgenic animals harboring high levels of the wild-type receptor [TG33 line (9)] leads to a marked reduction in receptor levels (data not shown). Second, the dose of agonist administered *in vivo* may simply be too low to achieve the maximal receptor occupancy required for full receptor protection. Indeed, unlike antagonists that must occupy a large fraction of receptors to be effective, agonists often require only a small fractional receptor occupancy for maximum biological effects. Testing this hypothesis, however, would require lethal doses of agonists. Third, neuronal and extraneuronal uptake mechanisms may lower the actual concentration of agonists substantially.

We demonstrate here that ligand-induced stabilization of a constitutively active mutant receptor is a novel mechanism for regulating cellular signaling *in vivo*. In a sense, the CAM β_2-adrenergic receptor is a molecular switch that is permanently "on" but expressed at such low levels, due to its inherent instability, that it functions minimally. However, when expression levels are increased by administration of receptor ligands that stabilize the protein, cognate physiological pathways become activated. Thus, signaling is induced by ligands (e.g., antagonists), which do not conformationally activate the receptors, but rather increase their concentration in cell membranes. This unique mechanism for regulating receptor signaling *in vivo* essentially creates a conditional phenotype and might be applicable to a broad array of biologically important molecules. It provides an alternative means for regulating gene expression, which is often focused at the level of gene transcription (e.g., inducible promoters).

We thank Drs. Alexander Macrae and Richard T. Premont for their insightful comments on the manuscript, Dr. J. Robbins for the gift of the α-myosin heavy chain promoter, and Mary Holben and Donna Addison for excellent secretarial assistance. This work was supported

in part by Grants 94R458 American Heart Association Texas affiliate to R.A.B. and HL16037 from the National Institutes of Health to R.J.L.

1. Samama, P., Cotecchia, S., Costa, T. & Lefkowitz, R. J. (1993) *J. Biol. Chem.* **268,** 4625–4636.
2. Lefkowitz, R. J., Cotecchia, S., Samama, P. & Costa, T. (1993) *Trends Pharmacol. Sci.* **14,** 303–306.
3. Robinson, P. R., Cohen, G. B., Zhukovsky, E. A. & Oprian, D. D. (1992) *Neuron* **9,** 719–725.
4. Drya, T. P., Berson, E. L., Rao, V. R. & Oprian, D. D. (1993) *Nat. Genet.* **4,** 280–282.
5. Parma, J., Duprez, L., Van Sande, J., Cochaux, P., Gervy, C., Mockel, J., Dumont, J. & Vassart, G. (1993) *Nature (London)* **365,** 649–651.
6. Duprez, L., Parma, J., Van Sande, J., Allgeier, A., Leclère, J., Schvartz, C., Delisle, M.-J., Decoulx, M., Orgiazzi, J., Dumont, J. & Vassart, G. (1994) *Nat. Genet.* **7,** 396–401.
7. Shenker, A., Laue, L., Kosugi, S., Merendino, J. J., Jr., Minegishi, T. & Cutler, G. B., Jr. (1993) *Nature (London)* **365,** 652–654.
8. Gether, U., Ballesteros, J. H., Seifert, R., Sanders-Bush, E., Weinstein, H. & Kobilka, B. K. (1996) *J. Biol. Chem.*, in press.
9. Milano, C. A., Allen, L. F., Rockman, H. A., Dolber, P. C., McMinn, T. R., Chien, K. R., Johnson, T. D., Bond, R. A. & Lefkowitz, R. J. (1994) *Science* **264,** 582–586.
10. Koch, W. J., Rockman, H. A., Samama, P., Hamilton, R., Bond, R. A., Milano, C. A. & Lefkowitz, R. J. (1995) *Science* **268,** 1350–1353.
11. Pei, G., Samama, P., Lohse, M., Wang, M., Codina, J. & Lefkowitz, R. J (1994) *Proc. Natl. Acad. Sci. USA* **91,** 2699–2702.
12. Bond, R. A., Leff, P., Johnson, T. D., Milano, C. A., Rockman, H. A., McMinn, T. R., Apparsundaram, S., Hyek, M. F., Kenakin, T. P., Allen, L. F. & Lefkowitz, R. J. (1995) *Nature (London)* **374,** 272–276.
13. Glaubiger, G. & Lefkowitz, R. J. (1977) *Biochem. Biophys. Res. Commun.* **78,** 720–725.
14. Maisel, A. S., Motulsky, H. J. & Insel, P. A. (1987) *Circ. Res.* **60,** 108–112.
15. Maisel, A. S., Motulsky, H. J. & Insel, P. A. (1985) *Science* 230, 183–186.
16. Milano, C. A., Dolber, P. C., Rockman, H. A., Bond, R. A., Venable, M. E., Allen, L. F. & Lefkowitz, R. J. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 10109–10113.