The Effect of Diabetes on Expression of β₁-,
β₂-, and β₃-Adrenoreceptors in Rat Hearts
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Diabetic hearts exhibit decreased responsiveness to stimulation by β-adrenoreceptor (β-AR) agonists. This decrease in activity may be due to changes in expression and/or signaling of β-AR. Recently we showed that right atrial strips from 14-week streptozotocin (STZ)-induced diabetic rat hearts exhibit decreased responsiveness to β₁-AR agonist stimulation, but not to β₂-AR agonist. In the present study, we investigated the effects of long-term diabetes on the expression of cardiac β₁, β₂, and β₃-ARs and looked at whether these changes could be restored with insulin treatment. Using reverse transcription-polymerase chain reaction (RT-PCR), PAGE, and Western blot analysis, we found that β₁-AR mRNA and protein levels decreased by 34.9 ± 5.8 and 44.4 ± 5.8%, respectively, in 14-week-STZ-treated diabetic rat hearts when compared withagematched controls. On the other hand, mRNA levels encoding β₂- and β₃-ARs increased by 72.5 ± 16.6 and 97.3 ± 26.1%, respectively. Although the latter translated into a proportional increase in β₂-AR protein levels (100.0 ± 17.0%), β₃-AR protein levels decreased to 82.6 ± 1.1% of control. Insulin treatment for 2 weeks, after 12 weeks of untreated diabetes, partially restored β₁-AR mRNA and protein levels to 60.1 ± 8.4 and 83.2 ± 5.0%, respectively, of control. Although insulin treatment minimally attenuated the rise in mRNA levels encoding β₂- and β₃-ARs, the steady-state levels of these proteins returned to near control values. These data suggest that the decreased responsiveness of diabetic hearts to stimulation of β-AR agonists may be due to a decrease in β₁-AR and an increase β₃-AR expression. Diabetes 50:455–461, 2001

As is well known, cardiac disease is one of the most important complications associated with chronic diabetes. Alloxan and streptozotocin (STZ) are two drugs that are commonly used to experimentally reproduce this pathology in animals, including rats. Among the prominent defects observed in these models are the decreased responsiveness of cardiac preparations to the inotropic and chronotropic effects of β-adrenoreceptor (β-AR) agonist stimulation (1–3). Although the mechanisms underlying depressed cardiac responses to stimulation of β-AR agonists are poorly understood, of interest are studies demonstrating that the density of cardiac β-ARs decreases in diabetic rats (4–7). Almost 20 years ago, Savarase and Berkowitz (8) reported a 28% reduction in the number of β-ARs accompanied by a 24% decrease in the heart rate of STZ-induced diabetic rats when compared with controls. However, in these studies, no attempt was made to distinguish between the various subtypes of β-ARs. In a recent study, we demonstrated that β₁-AR-mediated chronotropic responses in right atria of STZ-induced diabetic rats are impaired, but those mediated via β₂-ARs are preserved (9). It has also been demonstrated that in the human atrium, both β₁- and β₂-ARs are functionally coupled to the adenylate cyclase system (10), and both subtypes of β-ARs are reported to contribute to the cardiac responses of β-AR agonists (11). Thus it has been suggested that, in the human right atrium, both β₁- and β₂-ARs are involved in the physiological regulation of the force of contraction and/or heart rate.

On the other hand, evidence has been provided for the functional expression of β₂-ARs in the human heart, stimulation of which, in contrast to β₁- or β₂-ARs, decreases contractile force (12). Thus we have become interested in the role of β-AR subtypes in diabetes-induced cardiac problems. Our interest stems from reports demonstrating that several pathological states, including diabetes and heart failure, alter the density, sensitivity, and responsiveness of ARs in the heart (13–14). Moreover, no data, to our knowledge, are available on the influence of diabetes on β₁-AR expression. Therefore, in this study we investigated the effects of long-term diabetes on the expression of the three subtypes of cardiac β-ARs in rats and looked at whether changes could be restored and/or reversed with insulin treatment.

RESEARCH DESIGN AND METHODS
Chemicals and drugs. Thiopental sodium was purchased from Abbott Laboratories (Indianapolis, IN), STZ and ethidium bromide were obtained from Sigma-Aldrich (St Louis, MO), and NPH Iletin II (long-acting insulin) was obtained from Eli Lilly (Indianapolis, IN). Antibodies against rat β₁-AR (sc 568), β₂-AR (sc 570), and β₃-AR (sc 1473) were purchased from Santa Cruz Biotechnology (Dallas, CA). All other reagents and solvents used were of the highest grade commercially available.

Induction and verification of experimental diabetes. This study was approved by the Ankara University Animal Care and Use Committee. Male Wistar rats weighing 200–250 g were purchased from Baskent University Animal Care Unit (Ankara, Turkey). The rats were housed two animals per cage in a room with controlled temperature (22°C) and 12-h light:12-h dark cycles. Diabetes was induced with 45 mg/kg STZ (Sigma-Aldrich) dissolved in citrate buffer (pH 4.5) administered as single intravenous tail-vein injection under...
light ether anesthesia. Control rats were injected with an equivalent volume of the vehicle only. Rats were checked for glycosuria semiquantitatively using Urine-Glucostix reagent strips (Diastix; Bayer Diagnostics, U.K.), read by a Glucometer II (Accu-Chek; Boehringer Mannheim). Those with a blood glucose level ≥ 200 mg/dl were considered diabetic.

**Inulin treatment protocol.** After 12 weeks of STZ injection, diabetic rats were randomly divided into treated and nontreated groups. Treated rats were given daily subcutaneous insulin injections (NPH Iletin II) for 2 weeks. Insulin dosages were individually adjusted based on each animal’s blood glucose level to maintain the euglycemic state (8–15 U·kg⁻¹·day⁻¹), given once per day between 9:00 and 10:00 A.M. For this, blood glucose levels were monitored every 2 days using Glucostix reagent strips. Then 4 weeks after the induction of diabetes, control, STZ-induced diabetic, and insulin-treated diabetic rats were killed under thiopental sodium (60 mg/kg, i.p) anesthesia. Final blood glucose levels were measured on samples taken when the rats were killed.

**Sample collection.** After rats were killed, their abdomens were opened and 4–5 ml blood was collected via the left renal artery (15). Blood samples were centrifuged at 3000 g for 20 min, and plasma fractions were removed and stored at −20°C. Plasma glucose and insulin levels were later determined using Peridochrom Glucose GOD-PAP Assay Kit; Boehringer Mannheim, Indianapolis, IN), read by a Glucometer II (Accu-Chek; Boehringer Mannheim). Those with a blood glucose level ≥ 300 mg/dl were considered diabetic.

**Isolation and quantitation of total RNA.** Total RNA was harvested from killed rats, quick-frozen by embedding in dry ice, and stored at −80°C. Total RNA was extracted from whole hearts using a Quick Prep total RNA extraction kit (Amersham Pharmacia Biotech, Piscataway, NJ). At the end of the isolation, RNA samples were dissolved in 1-μl diethylpyrocarbonate (DEPC)-treated water (pH 7.5). The optical density (OD) values of each sample were determined spectrophotometrically using ultraviolet (UV)-visible spectrophotometer (UV-1601, Shimadzu) at wavelength 260 nm (Abs₂₆₀). The amount of RNA in each sample was then determined using the following formula: [RNA] = OD₂₆₀ × dilution factor × 40 μg/ml. OD values of RNA samples were also determined at Abs₃₂₀ and the OD₂₆₀/OD₃₂₀ ratio was used as a cursory estimation of RNA quality. Formamide/formaldehyde agarose gels were later used to evaluate RNA quality. At this time the study was being conducted, we did not have cRNA probes designed based on published sequences in the National Center for Biotechnology Information GenBank database (http://www.ncbi.nlm.nih.gov/entrez/); β₁-AR (accession number NM-012701.1); β₂-AR (accession number NM-012492.1); β₃-AR (accession number NM-013108.1); β-actin (accession number VO-1217-300691). Subscript numbers refer to positions of bases within the published cDNA sequences.

**Amplification of cDNA encoding β₁-, β₂-, and β₃-ARs.** Segments of the cDNA encoding each of the three major subtypes of rat β-ARs were amplified in PCR reactions using gene-specific primers as a way of determining the amount of transcripts present in each sample. For this, 5 μl cDNA polymerase 10× reaction buffer; 2 μl 25 mmol/l MgSO₄; 1 μl 100 mmol/l dNTP; 0.2 μl cDNA polymerase (5 U/μl) (Promega, Madison, WI); 2 μl control, diabetic, or insulin-treated diabetic heart cDNA; and 2 μl (from 25 μmol/l stocks) of respective sense and antisense primers were added to PCR tubes (Table 1). DEPC water was then added to each tube for a final volume of 50 μl. The samples were then mixed, placed in the thermocycler, and denatured for 3 min at 94°C. Thereafter, segments of β-AR cDNAs were amplified using the sequence 45-s denaturation (94°C) followed by 45-s annealing (56°C) and 2-min extension (72°C); this sequence was repeated for a total of 35 cycles, except for β₂-AR samples, which were amplified using 40 cycles. β-Actin was amplified in each set of PCR reactions, and these genes served as internal references during quantitation to correct for operator and/or experimental variations. At the end of the reactions, 5 μl of each PCR product were mixed with 5 μl 2× blue/orange loading dye. The samples were then loaded onto a 2% agarose gel containing ethidium bromide and electrophoresed for 2 h at 100 V (Sci-Plus, U.K.). The resulting gels were then visualized using an UV transilluminator (Viber Loumat TFX 20M UV) and photographed using UV gel camera (Polaroid GH 10; U.K.). Images of the gels were scanned into Adobe Photoshop 3.0 (Adobe Systems, Mountain View, CA) and then imported into Scion Imaging Software (Version 1.62; Frederick, MD). Areas under the curves were measured and used as mRNA concentrations.

**Confirmation of the identities of PCR products.** The products obtained from the PCR reactions were experimentally verified using restriction endonucleases to digest them into specific fragments. For this, banked nucleotide sequences for β₁-, β₂-, and β₃-ARs were digested in silico using a Web-based restriction analysis program (http://darwin.bio.geneseo.edu/~yin/WebGene/RE.html). The endonucleases AccI and SacII were selected for β₁-ARs, PstI and NcoII for β₂-ARs, and SmaI and AccI for β₃-ARs. Restriction maps are shown in Fig. 1.

**Preparation of membrane vesicles from rat hearts.** Membrane vesicles (MVs) were prepared from rat hearts using procedures previously described (16,17), except that the tissue was homogenized for 6 min in 10 mmol/l KCl. MVs were assessed using SDS-PAGE and Western blot analysis. Briefly, 75 μg MV from each sample were dissolved in 20 μl gel dissociation medium (62.5 mmol/l Tris base, 6% SDS, 20% glycerol, and 0.002% bromophenol blue) and electrophoresed for 3.5 h using 4–20% linear gradient gels (BioRad, Burlingame, CA). At the end of this time, the gels were stained with Coomassie solution and destained overnight. Images of destained gels were captured using the Kodak Digital Science Electro-phoresis Documentation and Analysis System 120 (Eastman-Kodak, Rochester, Rochester, NY); intensities of β₁-, β₂-, and β₃-AR protein bands were used as indices of protein amount.

**Western Blot analysis.** Linear gradient gels (4–20%) containing samples of interest were run as described above. At the end of electrophoresis, separated proteins were transferred onto polyvinylidene difluoride (PVDF) membranes.

### TABLE 1

<table>
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<th>Primer</th>
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<th>Annealing temperature (°C)</th>
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</table>

Primers were designed based on published sequences in the National Center for Biotechnology Information GenBank database (http://www3.ncbi.nlm.nih.gov/entrez/); β₁-AR (accession number NM-012701.1); β₂-AR (accession number NM-012492.1); β₃-AR (accession number NM-013108.1); β-actin (accession number VO-1217-300691). Subscript numbers refer to positions of bases within the published cDNA sequences.
RESULTS

Induction of experimental STZ-induced diabetes. All rats injected with STZ had high blood glucose levels within 3 days. At the end of the in vivo experimental protocol, analysis of plasma from diabetic rats showed increases in glucose levels compared with controls (348 ± 14 vs. 105 ± 5 mg/dl; \( P < 0.001 \)) and parallel decreases in insulin levels (26.4 ± 4 vs. 103 ± 10 pmol/l; \( P < 0.01 \)). Insulin treatment for 2 weeks returned plasma glucose levels to near control values (102 ± 18 mg/dl). Mean body weights were significantly lower in diabetic rats than in controls (197 ± 7 vs. 347 ± 8 g; \( P < 0.001 \)). These results were expected, as loss of body mass is a characteristic feature of type 1 diabetes. Insulin treatment of diabetic rats partially corrected the changes in body weight (them to 197.3 ± 26.1% of age-matched control levels (Fig. 2A)). Insulin treatment did not reverse this increase in \( \beta_3 \)-AR mRNA (168.8 ± 13.9% of controls). We also found that 14 weeks of untreated diabetes almost doubled \( \beta_3 \)-AR mRNA levels in heart rat cells, increasing them to 197.3 ± 26.1% of age-matched control levels (Fig. 2B). Insulin treatment of diabetic rats only partially attenuated this rise in \( \beta_3 \)-AR mRNA levels, lowering it to 162.2 ± 18.9% of control levels.

Characterization of PCR products. Specific restriction endonucleases were used to confirm the identities of the PCR products obtained. PCR products generated for each subtype were digested completely and resulted in fragments of predicted sizes (data not shown). These data thus confirm that the products generated in the PCR reactions resulted from specific amplification of cDNA encoding \( \beta_1 \), \( \beta_2 \), and \( \beta_3 \)-ARs. Quantitation of \( \beta_1 \), \( \beta_2 \), and \( \beta_3 \)-proteins in plasma membrane preparations from control, diabetic, and insulin-treated diabetic rat hearts. SDS-PAGE as well as Western blot analysis was used to quantify the amount of \( \beta_1 \), \( \beta_2 \), and \( \beta_3 \)-proteins in MVs from control, diabetic, and insulin-treated diabetic rat hearts. As shown in Fig. 3A, the density of \( \beta_1 \)-AR protein in plasma membrane fraction prepared from diabetic rat hearts was significantly less than that from age-matched controls (44.5 ± 5% of control; \( P < 0.001 \)).

Quantitation of total RNA isolated from rat hearts. OD values at 260 nm and ratios of OD260/OD280, were used to quantify as well as estimate the quality of total RNA isolated from the three groups of rat hearts. With similar overall quality (OD260/OD280 Ratios ~ 1.7), total RNA isolated from diabetic hearts was less than that of control animals (536 ± 80 vs. 992 ± 160 µg). These data are consistent with the observations that several proteins are downregulated in diabetic hearts (19). When RNA samples were electrophoresed using formamide/formaldehyde agarose gels, two distinct bands representing 28S and 18S ribosomal RNA were observed (data not shown). The former suggests that minimal degradation of RNA occurred during the isolation procedure.

Quantitation of \( \beta \)-AR transcripts. After converting the mRNAs into more stable cDNAs, PCRs were used to determine the amounts of \( \beta \)-AR transcripts in hearts of control, diabetic, and insulin-treated diabetic rats. As shown in Fig. 2A, chronic diabetes significantly decreased mRNA levels of \( \beta_1 \)-ARs to 34.9 ± 5.9% of control levels (\( P < 0.001 \)). All data points were normalized to \( \beta_2 \)-actin, as its mRNA levels did not change significantly in this experimental paradigm (data not shown). Treatment of diabetic rats with insulin partially restored mRNA levels of \( \beta_3 \)-AR, to 60.1 ± 8.4% of control levels. In contrast to \( \beta_1 \)-ARs, chronic diabetes significantly increased mRNA levels of cardiac \( \beta_2 \)-ARs to 173.5 ± 16.6% of control levels (\( P < 0.001 \)) (Fig. 2B). Insulin treatment did not reverse this increase in \( \beta_2 \)-AR mRNA (168.8 ± 13.9% of controls). These results were expected, as loss of body mass is a characteristic feature of type 1 diabetes. Insulin treatment of diabetic rats partially corrected the changes in body weight (them to 197.3 ± 26.1% of age-matched control levels (Fig. 2C)). Insulin treatment of diabetic rats only partially attenuated this rise in \( \beta_3 \)-AR mRNA levels, lowering it to 162.2 ± 18.9% of control levels.

Data analysis and statistics. Differences among group values were evaluated by one-way analysis of variance followed by a Newman-Keuls test. Data are presented as means ± SE. Results were considered significantly different at \( P < 0.01 \).
Plasma membrane fractions from diabetic rat hearts showed a smaller but still significant decrease in β2-AR protein content (17.4 ± 1.1% of control; *P < 0.01) (Fig. 3B). On the other hand, untreated diabetes doubled protein levels of β3-AR, to 200.0 ± 17% of control (Fig. 3C). Insulin treatment partially restored expression of β1-AR to 84.4 ± 4.8% of controls and completely restored expression of β2-AR to 100.9 ± 4.8% of controls. Insulin-treatment also significantly attenuated the increase in the expression of β3-AR induced by long-term diabetes (118.9 ± 18.7% of controls).

Based on the intensity of Coomassie-stained proteins corresponding to β1-AR, β2-AR, and β3-AR, we estimated the ratio of...
DISCUSSION

One of the major causes of morbidity and mortality in diabetic patients is cardiovascular disease. Diabetes is also frequently associated with the development of heart failure, even in the absence of coronary artery complications or hypertension (20). The mechanisms involved in diabetes-induced cardiac problems are not clearly understood, but bradycardia (21) and decreased chronotropic and inotropic responses to β-AR–agonist stimulation (1–3) have been demonstrated. Thus a reduction in the number of cardiac β-ARs has been questioned as one of the causative factors for diabetic cardiomyopathy. In fact, reduced density of cardiac β-ARs in diabetic rats has been demonstrated in previous studies (4–7). In those studies, however, β-ARs were not differentiated according to their subtypes. β-ARs of both the β₁ and β₂ subtypes have been reported to coexist in certain cardiac tissues (10,11). Human cardiac β₁- and β₂-ARs are coupled to adenylate cyclase through the Gₛ protein. In the human heart, both receptors mediate the increases in contractile force and sinoatrial rate (14). A growing body of recent evidence, on the other hand, suggests that β₂-ARs that couple to the Gₛ protein and mediate mediate cardiac responses are also present in human heart (12,14,22). Therefore, in the present study we investigated the influence of diabetes on mRNA and protein levels of selective β₁, β₂, and β₂-ARs in rat heart.

A principal finding of the present study was that the complement of β-ARs expressed in the heart is altered with long-term STZ-induced diabetes. These levels were restored to close to those of age-matched controls with 2 weeks of insulin treatment, begun after 12 weeks of untreated diabetes. We observed that the levels of mRNA encoding β₁-AR decreased by 65% in hearts of diabetic rats, and the density of β₁-AR protein on the plasma membrane decreased by 55%. Our findings are in accordance with the those of Matsuda et al. (23), who reported a parallel decrease in myocardial β₁-AR density and β₁-AR mRNA levels in 6-week diabetic rats. Although those investigators did not detect β₂-ARs in their experiments, they suggested that the reduced number of myocardial β₁-ARs that they observed in diabetes could be due to a downregulation of β₁-AR mRNA levels. Matsuda et al. (23) also stated that β₂-AR mRNA was undetectable in control or diabetic ventricular myocardium, at least by Northern blot using the specific oligonucleotide probe. Indeed, downregulation of myocardial β₁-AR mRNA has been demonstrated in human failing hearts (24,25). β₂-ARs, on the other hand, appear to be somewhat uncoupled from adenylate cyclase (26), with no detectable change in β₂-AR mRNA levels in patients with advanced heart failure (24). Moreover, heterogeneous β₁ plus β₂-AR populations have been identified by radioligand binding in rat heart (27), but only β₁-ARs appear to mediate the rate and tension responses in rat atria (28). Hence, under normal physiological conditions, it seems unlikely that β₂-ARs have a functional role in the regulation of heart rate and contractility in this species, in contrast to the situation in humans. Based on these findings, a decrease in the density of cardiac β-ARs in diabetic rats might be attributable to a decrease in the number of β₁-ARs only. In the presence of a pathological state, however, the function of the β₂-AR stimulation might be changed.

Indeed, in the present study, we found that levels of mRNA encoding β₂-ARs were increased 74% in the hearts of diabetic rats, but that the density of this protein on the plasma membrane was decreased by 17.4% when compared to controls. It should be pointed out that the failure of Matsuda et al. (23) to detect β₂-AR mRNA in diabetic ventricular myocardium might have been attributable in part to the use of the less-sensitive Northern blot analysis. However, the reasons for diversity between our mRNA and protein findings of β₂-ARs in diabetic rats are uncertain. An increase in mRNA levels is not necessarily associated with an increase in steady-state levels of protein. Therefore, the decrease in protein levels, despite the increase in mRNA, raises the possibility that the rate of β₂-AR protein degradation might be elevated in diabetes. It is also possible that there could be some post-translational modifications in diabetic heart so that all of the β₂-AR protein is not being delivered from the endoplasmic reticulum to the membrane fraction, which we analyzed.

Because diabetes frequently leads to cardiac pump failure, further leading to congestive heart failure (29), the changes in cardiac β-AR levels in heart failure might be comparable with those of the heart in diabetes. The alterations in failing human hearts have been suggested to be, at least in part, a consequence of the increased stimulation of β₁-ARs by noradrenaline released from the sympathetic nerves in an attempt to restore cardiac function (30). Similarly, it has been demonstrated that cardiac noradrenaline content is increased in diabetic rats (31). In addition, it was reported that noradrenaline turnover, uptake, synthesis, and release are all enhanced in diabetic cardiomyopathy (32,33). Therefore, chronically high concentrations of noradrenaline in diabetes may contribute to the selective downregulation of β₁-ARs, as the affinity of noradrenaline is lower for β₂-ARs. From these data, in contrast to β₁-ARs, one would not expect a decrease in the expression of cardiac β₂-ARs in diabetic rats. As a matter of fact, in paced pigs and dogs, β₂-AR protein and mRNA levels were found to be unchanged despite the reduced number of β₁-ARs and mRNA content (34,35). Therefore, the decrease that we observed in β₂-AR protein levels in diabetic rat hearts is somewhat surprising. However, this slight decrease does not necessarily result in a significant decrease in functional responses mediated by this β-AR subtype, because cardiac β₂-ARs are more effectively coupled to adenylate cyclase than are β₁-ARs. Indeed, cross-regulation between G protein–coupled receptors through G protein function has been demonstrated (36). Strips of right atrial appendage from patients treated with β₁-AR blockers have been reported to exhibit sensitization of β₂-AR–mediated inotropic responses (36). Interestingly, it has been reported that single contracting ventricular myocytes from patients with severe heart failure responded to noradrenaline predominantly through β₂-ARs (37). Thus the subtype density and coupling of cardiac β-ARs might be complicated in certain disease states, such as heart failure and diabetes. These studies lead us to hypothesize that the stimulation of cardiac responses might be brought about predominantly through β₂-AR stimulation when the functional responses mediated by β₁-ARs are depressed. Thus, in an attempt to gain insight into the role of β₂-ARs in diabetes, we previously studied the effect of diabetes on selective β₁- and β₂-AR stimulation in the
right atria of STZ-induced diabetic rats (9). We found a significant decrease in the chronotropic responses of the right atria from 14-week diabetic rats to noradrenaline, although the responsiveness to fenoterol was similar to that of controls. These findings suggest that $\beta_1$-AR but not $\beta_2$-AR–mediated chronotropic responses were reduced in the right atria of diabetic rats. On the other hand, it is important to note that in 14-week–STZ-induced diabetic rats, $\beta_2$-AR protein in heart was decreased by almost 50% in the present study, whereas the decrease in maximum chronotropic response of the right atria to $\beta_2$-AR stimulation was only 29% in our previous study (9). Taken together, these results suggest an abundance of spare receptors in this system. Therefore, by analogy, it might be speculated that the 17.4% decrease in $\beta_2$-AR protein observed in this study was not sufficient to cause a significant change in $\beta_2$-AR–mediated cardiac responses. As is well known, abnormal $\beta$-AR signal transduction appears to be one of the major causes of systolic and diastolic dysfunction in humans with heart failure (38) or diabetes (39). Thus our previous findings demonstrating that there are defective $\beta_1$-AR–mediated chronotropic responses yet preserved $\beta_2$-AR–mediated chronotropic responses, coupled with data from the present study demonstrating an increase in mRNA levels encoding $\beta_2$-ARs, may have some physiological importance. However, at present our results do not allow us to determine whether $\beta_2$-ARs might compensate the decrease in the heart rate of rats when $\beta_1$-AR–mediated responses are impaired to a certain extent in those pathological states. This point remains to be determined.

In the present study, we found a 97% increase in mRNA–encoding $\beta_1$-ARs and a 100% increase in plasma membrane density of $\beta_2$-ARs when compared with controls. $\beta_1$-AR expression has recently been detected in human heart ventricular myocytes (12,14,22). It has also been shown in human ventricular endomyocardial biopsies that isoprenaline produces consistent negative inotropic effects in the presence of the $\beta_1$- and $\beta_2$-AR antagonist nadolol (12). The negative inotropic effect is antagonized by the nonselective $\beta$-AR antagonist bupranolol. A similar negative inotropic effect elicited by $\beta_2$-AR selective agonist BRL 37344 is sensitive to treatment of the preparations with pertussis toxin. These results indicate the involvement of inhibitory G proteins in the $\beta_2$-AR signaling pathway, thereby producing negative inotropic effects. $\beta_2$-ARs differ from $\beta_1$- and $\beta_3$-ARs in that they lack the phosphorylation sites for the $\beta$-AR kinases and the cAMP-dependent protein kinase (40), and may not be down-regulated in heart failure. Thus it has been proposed that cardiodepressant effects mediated through $\beta_2$-ARs contribute to the impaired cardiac function in patients with heart failure (12). To our knowledge, we are the first to demonstrate an increased expression of $\beta_2$-AR mRNA in diabetic rat hearts. Because the contribution of $\beta_2$-ARs to the cardiac responses of $\beta$-AR agonists in rat heart is not clear at present, questions arise as to the pathophysiological implications of our data. Thus, to comment on the role of increased expression of cardiac $\beta_2$-AR mRNA in diabetes-induced cardiac dysfunction, these results should be confirmed in the human heart in future studies.

In the present study, we also found that 2-week insulin treatment of diabetic rats increased the expression of $\beta_1$-AR mRNA, but to a level that was still lower than that of controls. No significant effect of insulin, on the other hand, was observed on the expression of $\beta_2$- and $\beta_3$-AR mRNAs. Insulin appears to be the most effective compound not only in preventing but also reversing the diabetes-induced cardiac changes. In addition, it was reported that the decreased number of cardiac $\beta$-ARs returned to normal in diabetic rats after insulin treatment (4). Insulin treatment, however, seems to be less effective at the more chronic stages of the disease (41). In this regard, one would conclude that the inability of insulin to normalize completely decreased $\beta_1$-AR mRNA in diabetic rats may be due to the duration of diabetes. Alternatively, 2-week treatment of insulin may not be enough for complete normalization. However, our findings that insulin reverses the changes in $\beta$-AR protein levels do not support these possibilities. Studies of the heart in vitro demonstrate that insulin stimulates protein synthesis and inhibits protein degradation (42,43). The effect of insulin, hence, could be more prominent at the level of protein turnover.

In conclusion, the present study demonstrated that the expression of $\beta_1$-ARs decreases, whereas that of $\beta_2$-ARs increases, in hearts of long-term diabetic rats. Our results thus suggest that a decrease in $\beta_1$-AR together with an increase in $\beta_2$-AR expression might be involved in the development of diabetes-induced cardiac dysfunction. The physiological relevance of our findings demonstrating a decrease in cardiac $\beta_3$-AR protein despite the increased $\beta_2$-AR mRNA in diabetic rats is not clear. Further studies are needed to delineate the precise role of cardiac $\beta$-ARs in health and disease.

ACKNOWLEDGMENTS
This work was supported in part by grants from Ankara University Research Committee (U.D.D.) and the Biomedical Research Committee, Indiana University School of Medicine (K.R.B.).

A preliminary report of this study was presented at the International Conference on Diabetes and Cardiovascular Disease, Winnipeg, Canada, 3–6 June 1999.

The authors would like to thank Dr. Henry R. Besch, Jr., for helpful comments on the manuscript.

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