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Long Term β-Adrenoceptor-Mediated Up-regulation of $G_{i\alpha}$ and $G_{o\alpha}$ mRNA Levels and Pertussis Toxin-Sensitive Guanine Nucleotide-Binding Proteins in Rat Heart

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SUMMARY

Chronic stimulation of β-adrenoceptors leads to increased mRNA and protein levels of pertussis toxin (PTX)-sensitive guanine nucleotide-binding proteins (G proteins) in the heart. In the present study the time course is reported of the effect of isoprenaline infusions on myocardial mRNA levels of $G_{i\alpha\text{-}2}$, $G_{i\alpha\text{-}3}$, $G_{o\alpha}$, $G_{s\alpha}$, and G_{β} and myocardial levels of PTX-sensitive G proteins. Rats were treated by subcutaneous infusions, with osmotic minipumps, of 0.9% NaCl, isoprenaline (2.4 mg/kg/day), propranolol (9.9 mg/kg/day), or a combination thereof for 1, 2, 3, 4, or 8 days, and two groups were treated with NaCl or isoprenaline for 13 or 26 days. Additional groups of rats were treated with 0.24 or 0.07 mg/kg/day for 4 days to determine the dose dependency of the effects of isoprenaline. mRNA concentrations were determined by standardized slot blotting with ^{32}P-labeled cDNA or cRNA probes. In isoprenaline-treated rats, mRNA levels of all members of the $G_{i\alpha}/G_{o\alpha}$ family increased gradually in parallel. The increase in $G_{i\alpha\text{-}2}$, $G_{i\alpha\text{-}3}$, and $G_{o\alpha}$ mRNA levels reached significance on days 2–3, reached maximal values on days 3–4, and remained stable over the total time of treatment of up to 26 days. Compared with NaCl, maximal increases were 77% ($G_{i\alpha\text{-}2}$; 16.4 versus 9.3 pg/μg), 58% ($G_{i\alpha\text{-}3}$; 4.65 versus 2.95 pg/μg), and 78% ($G_{o\alpha}$; 0.40 versus 0.22 pg/μg). $G_{s\alpha}$ mRNA levels (about 30 pg/μg) and G_{β} mRNA levels remained unchanged. In isoprenaline-treated rats myocardial levels of PTX-sensitive G proteins increased by maximally 54%, compared with control. The time course differed slightly from the time course of mRNA up-regulation in the first 3 days of treatment and paralleled the increase in mRNA levels from day 4 on. Propranolol had no effect on G_{α} mRNA or protein levels when given alone but abolished all effects of isoprenaline when given in combination. Isoprenaline at a dose of 0.24 mg/kg/day still induced an increase in $G_{i\alpha\text{-}2}$ mRNA levels of 24%, without any effects on histopathology of the myocardium. It is concluded that under *in vivo* conditions (i) chronic β-adrenergic stimulation induces increased mRNA expression of all members of the $G_{i\alpha}/G_{o\alpha}$ family but not of $G_{s\alpha}$ and G_{β} in the heart, (ii) increases in both mRNA protein levels are relatively slow in onset and persist as long as stimulation is present, and (iii) protein levels parallel the increase in mRNA levels, suggesting that regulation of mRNA levels dominates in determining the level of PTX-sensitive G proteins in the heart under long term β-adrenergic stimulation. The effects of isoprenaline were shown to be dose dependent and to occur independently of the induction of fibrotic necrosis. The data provide evidence that enhanced *de novo* synthesis of all members of the $G_{i\alpha}/G_{o\alpha}$ family, without a concomitant increase in $G_{s\alpha}$ and G_{β}, plays an important role in the adaptation of the heart to chronic catecholamine stimulation under *in vivo* conditions.

Cellular responses to chronic hormonal stimulation decrease time and concentration dependently. This desensitization process is composed of two components, i.e., a fast one, occurring in seconds to minutes, which involves phosphorylation, uncoupling from signal-transducing G proteins, and sequestration of the β-adrenoceptor (1), and a delayed one, occurring in hours or days, which involves alterations of the cellular content of β-adrenoceptors and additionally of signal-transducing G proteins. Examples of the slow component of desensitization after β-adrenergic stimulation are decreases in β-adrenoceptor mRNA levels and β-adrenoceptor density (2), increases in PTX-sensitive and immunoreactive $G_{i\alpha}$ in neonatal cardiomyocytes (3), or increases in $G_{i\alpha\text{-}2}$ mRNA and $G_{i\alpha}$ protein levels in

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ABBREVIATIONS: G protein, guanine nucleotide-binding protein; $G_{i\alpha}$, α subunit of inhibitory guanine nucleotide-binding proteins ($G_{i\alpha\text{-}1}$, $G_{i\alpha\text{-}2}$, and $G_{i\alpha\text{-}3}$ subtypes); $G_{o\alpha}$, guanine nucleotide-binding protein structurally and functionally related to $G_{i\alpha}$; $G_{s\alpha}$, stimulatory guanine nucleotide-binding protein; G_{β}, β subunit, common to all heterotrimeric guanine nucleotide-binding proteins; PTX, pertussis toxin; SDS, sodium dodecyl sulfate; bp, base pairs; kb, kilobases; HPLC, high performance liquid chromatography.

S49 cells (4). Likewise, a 4-day infusion of isoprenaline induced specific increases in mRNA levels of $G_{i\alpha\text{-}2}$ and $G_{i\alpha\text{-}3}$ and in the PTX-sensitive amount of $G_{i\alpha}$ in the heart, whereas $G_{s\alpha}$ mRNA levels did not change (5, 6). Increases in G_i proteins probably do not only contribute to desensitization of the stimulatory adenylyl cyclase pathway (3–7) but also lead to sensitization of the inhibitory adenylyl cyclase pathway (4, 6).

Taken together, these data suggest (i) that the expression of β-adrenoceptors and $G_{i\alpha}$ is modulated by chronic β-adrenergic stimulation, (ii) that β-adrenoceptor-mediated increases $G_{i\alpha}$ might at least in part be responsible for a diminished responsiveness to both β-adrenoceptor-dependent and -independent stimulators of the adenylyl cyclase, and (iii) that β-adrenoceptor-mediated increases in $G_{i\alpha}$ lead to facilitated signal transduction in G_i-mediated inhibitory signal transduction pathways.

However, most data were obtained under *in vitro* conditions using high agonist concentrations and might, therefore, be not representative for the situation *in vivo*. It is also not yet clear which of the four main PTX-sensitive G protein α subunits ($G_{i\alpha\text{-}1}$, $G_{i\alpha\text{-}2}$, $G_{i\alpha\text{-}3}$, and $G_{o\alpha}$) might contribute to the increase in PTX-sensitive G proteins under chronic β-adrenergic stimulation and no information exists on whether G_β, which is common to all G proteins and might serve as a scavenger for α subunits (8), is regulated concomitantly with inhibitory α subunits in the heart. Recent data suggest that up-regulation of inhibitory G protein α subunits has no net effect on adenylyl cyclase activity if it is accompanied by similar increases in levels of G_β (9). Little information exists about the mechanisms that determine myocardial levels of G protein α subunits. Investigating the time course of the effect of chronic β-adrenergic stimulation on PTX-sensitive G proteins and their respective mRNAs and on $G_{s\alpha}$ and G_β mRNA levels in the heart under *in vivo* conditions is an important first step in understanding the role of different G protein α subunits in the heart and the possible molecular mechanisms of their regulation.

Materials and Methods

Animal treatment. Male Wistar rats (170–220 g at time of pump implantation) were treated with subcutaneous infusions by means of osmotic minipumps (Alzet osmotic pumps type ML2). Minipumps were implanted subcutaneously in the neck under short term ether anesthesia. Mean rate of infusion was 5 μl/hr (varying between 4.4 and 5.6 μl/hr). Four different groups (n = 9 each) were treated 1, 2, 3, 4, or 8 days with either 0.9% NaCl as control, (±)-isoprenaline-HCl (dissolved in 0.002 M HCl; 2.4 mg/kg/day; Boehringer Ingelheim, Ingelheim, FRG), (±)-propranolol-HCl (9.9 mg/kg/day; Sigma, St. Louis, MO), or a combination thereof (isoprenaline plus propranolol). The animals had free access to food and tap water. Heart rate was measured once each day, by recording electrocardiograms in the conscious rats, 3 days before and during treatment. Body weight was measured daily. Additionally, two groups [control (n = 8) and isoprenaline (n = 12)] were treated for 13 or 26 days, and two additional groups of rats (n = 4) were treated with 0.24 or 0.07 mg/kg/day isoprenaline for 4 days to investigate the dose dependency of the effects of isoprenaline. For the 26-day treatment minipumps were removed after 13 days under short term ether anesthesia, and a new freshly filled pump was implanted. At the end of the respective treatment duration, pumps were removed under short term ether anesthesia directly before sacrifice. Because drug delivery was stable for 14 days, pumps were reimplanted into another rat when total time of drug delivery was 13 days or less. The rats were killed by a blow on the neck and bleeding from the carotid arteries. Hearts were rapidly removed and exsanguinated in ice-cold 0.9% NaCl. Ventricles were freed of fat and connective tissue before further processing.

Determination of serum catecholamines. Determination of myocardial catecholamines was performed using HPLC with electrochemical detection, according to the method of Weicker *et al.* (10). After cervical dislocation of the rats, the blood was collected from the carotid arteries and centrifuged at 14,000 × μg for 10 min at 4°. The supernatant (serum) was stored at −80° until use. Serum (600 μl) and 100 μl of 10 pg/μl dihydroxybenzylamine as internal standard were transferred into sample cartridges (Recipe, Munich, FRG) containing 1 ml of 2 M Tris/2% EDTA buffer (pH 8.7) and 20 mg of alumina. The cartridges were shaken upside down for 10 min. After adsorption of the catecholamines by this procedure, the buffer was removed and the residue was washed three times, using 1 ml of a 0.2% Tris buffer, pH 8.6. For desorption of catecholamines from the alumina, 125 μl of glacial acetic acid were added and after vigorous shaking the supernatant (containing the catecholamines) was centrifuged into a sample vial. Twenty microliters of the sample were injected into the HPLC system, which consisted of a Merck L-6200 Intelligent Pump, a Merck D-2000 Chromato-Integrator (Merck, Darmstadt, FRG), a Waters model 410 electrochemical detector (Waters Millipore, Eschborn, FRG) with a glassy carbon working electrode set at a potential of 0.6 V versus Ag/AgCl, and a reverse phase C_{18} column (Recipe). The eluent consisted of 50 mM sodium acetate, 20 mM citric acid, 2 mM sodium 1-octane-sulfonate (Merck), 1 mM di-*n*-butylamine, and 0.1 mM sodium EDTA (Sigma, Munich, FRG) dissolved in distilled water/methanol (96:4, v/v), with a flow rate of 0.9 ml/min. Before separation of myocardial catecholamines on the HPLC column, a standard solution containing 5 ng/ml norepinephrine, 3 ng/ml epinephrine, 5 ng/ml dihydroxybenzylamine, 3 ng/ml dopamine, and 2.5 ng/ml isoprenaline was injected several times until the retention times were identical and the integrator was calibrated.

Total RNA preparation. Total RNA preparation was performed according to the method of Auffray and Rougeon (procedure C; Ref. 11) with modifications as described previously (5). Total RNA yield varied between 0.88 and 1.34 mg/g of tissue wet weight, depending upon the different treatments (see Fig. 2B). The mean A_{260}/A_{280} was 2.16 ± 0.03 (n = 175). RNA blotting and hybridization were performed as described in detail previously (5). Total RNA preparation from rats treated with different doses of isoprenaline was performed according to the method of Chomczynski and Sacchi (12), which resulted in higher RNA yields than the LiCl/urea method (see Table 2).

cDNA probes. Plasmids (pGEM-2) with cDNA inserts for rat $G_{i\alpha\text{-}1}$, $G_{i\alpha\text{-}2}$, $G_{i\alpha\text{-}3}$, or $G_{s\alpha}$ were kind gifts from Dr. R. R. Reed (13). A plasmid (pUC8) with the full length cDNA of rat $G_{o\alpha}$ was kindly supplied by Dr. Kozasa (14). According to the nomenclature introduced by Hsu *et al.* (15), this cDNA was of the $G_{o\alpha\text{-}1A}$ subtype. A 600-bp *Bam*HI/*Eco*RI fragment of the 3′ coding and noncoding region was subcloned in pGEM-2 (pGEM-2$G_{o\alpha}$) and later used as a template for *in vitro* transcription of radiolabeled single-stranded $G_{o\alpha}$ cRNA probes. A plasmid (pGEM-3Zf$^-$) with the full length cDNA of G_β (16), truncated by restriction with *Xho*I, was constructed by P. Gierschik (Heidelberg, FRG). Transformation in *Escherichia coli*, plasmid preparation, and cDNA purification were performed by standard molecular biology methods (17). Sizes of cDNA inserts were as follows: 1950 bp ($G_{i\alpha\text{-}1}$), 1750 bp ($G_{i\alpha\text{-}2}$), 3070 bp ($G_{i\alpha\text{-}3}$), and 620 and 1120 bp (*Eco*RI fragments of $G_{s\alpha}$). The 1120-bp cDNA fragment was used for $G_{s\alpha}$ probing. Because of 94% nucleotide homology in the coding regions between $G_{i\alpha\text{-}1}$ and $G_{i\alpha\text{-}3}$, we used a 600-bp *Xba*I/*Eco*RI fragment of the 3′ noncoding end of $G_{i\alpha\text{-}1}$ and a 625-bp *Eco*RV/*Eco*RI fragment of the 3′ noncoding end of $G_{i\alpha\text{-}3}$ for hybridization analysis. Comparative probing with the full length cDNA for both $G_{i\alpha\text{-}1}$ and $G_{i\alpha\text{-}3}$ revealed identical results (data not shown). A 840-bp *Bgl*II fragment of G_β was used for hybridization.

cDNAs were ^{32}P-labeled (nick translation kit; Amersham Buchler) with [^{32}P]dCTP (3000 Ci/mmol; NEN-DuPont, Bad Homburg, FRG) to a specific activity of 3.2–8.0 × 10^8 dpm/μg. Unbound radioactivity

was separated by gel filtration with DNA-grade Sephadex G-50 (Pharmacia Fine Chemicals, Uppsala, Sweden). To increase sensitivity, radiolabeled $G_{o\alpha}$ cRNA probes (600 bases) were produced by *in vitro* transcription of 1 μg of pGEM-2$G_{o\alpha}$ using SP_6 polymerase in the presence of 150 μCi of [^{32}P]UTP (3000 Ci/mmol; NEN-DuPont). Specific radioactivity was about 2×10^9 dpm/μg.

Hybridization. Blot membranes were prehybridized at 42° overnight in a solution containing 50% formamide, 5× Denhardt solution (Ficoll, polyvinylpyrrolidone, and bovine serum albumin, 1 mg/ml each), 0.9 M NACl, 0.06 M NaH_2P0_4, 0.006 M EDTA, 0.1% SDS, and 200 μg/ml tRNA from yeast. Denatured radiolabeled cDNA probes (5 min, 100°) or cRNA probes were added to fresh hybridization solution (prehybridization solution) at a concentration of 2×10^6 and 3×10^6 dpm/ml, respectively. Hybridization was performed in 50 μl of buffer/cm^2 of membrane at 42° for 20–48 hr. After hybridization with cDNA probes, blot membranes were washed with a final stringency of 0.2× standard saline citrate, 0.1% SDS, at 65°. The washing protocol for cRNA probes included additive digestion with RNase A (1 μg/ml) and RNase T_1 (10 units/ml) in 2× standard saline citrate for 30 min at 37°. Wet blot membranes were sealed in plastic wrap and exposed to medium-sensitivity medical X-ray film (R2; 3M, Italy) at −80°, using intensifying screens. For subsequent hybridizations with other radiolabeled cDNAs, blots were stripped and exposed to highly sensitive X-ray film (Kodak X-OMAT AR), using intensifying screens, to verify complete removal of the probe.

Quantification of mRNA levels. Hybridization intensity of autoradiographic signals on slot blots was measured by two-dimensional densitometry (TLC II; CAMAG, Berlin, FRG). Ten micrograms of total RNA were applied in triplicate. Slot blots were hybridized subsequently with $G_{i\alpha-2}$, $G_{i\alpha-3}$, and $G_{s\alpha}$ in varying order. For analysis of $G_{o\alpha}$ mRNA levels new slot blots were prepared. To obtain absolute values for specific mRNA levels and to compare densitometric values from different slot blots, cRNA standards in the sense orientation were produced by *in vitro* transcription of rat full length cDNAs using the SP_6 or T_7 RNA polymerase promoter of pGEM-2 (5). The cRNAs were transcribed in microgram quantities and gel purified. Concentration was determined photometrically at 260 nm, in triplicate. Dilutions with Tris/EDTA buffer containing 0.5% SDS were stored in aliquots at −80°. For each slot blot a six-point standard curve from 30 to 600 pg for $G_{i\alpha-2}$, from 20 to 200 pg for $G_{i\alpha-3}$, from 1 to 50 pg for $G_{o\alpha}$, and from 50 to 800 pg for $G_{s\alpha}$ was established in duplicate. Autoradiographic density of hybridization signals was plotted versus the applied amount of cRNA standard and revealed a linear relationship (data not shown).

To determine whether radiolabeled G protein probes exhibited nonspecific binding, each individual slot blot was also loaded with 10 μg of rRNA (Pharmacia Fine Chemicals). Hybridization of the radiolabeled $G_{s\alpha}$ cDNA probe with 10 μg of rRNA amounted to 4–11% of hybridization with 10 μg of cardiac total RNA. This signal was substracted from all other sample values on the blot. There was no nonspecific binding of $G_{i\alpha-2}$ cDNA, $G_{i\alpha-3}$ cDNA, or $G_{o\alpha}$ cRNA to rRNA.

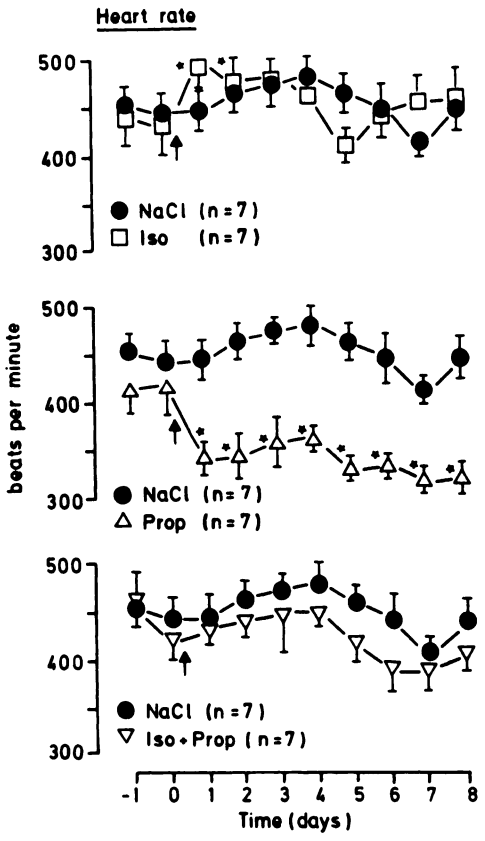
PTX-catalyzed ADP-ribosylation. Homogenization of ventricular tissue (100 mg), PTX-catalyzed ADP-ribosylation, SDS-polyacrylamide gel electrophoresis, and quantification of autoradiographic signals were performed as described in detail previously (6).

Protein measurement. Heart tissue protein concentration was measured to determine whether changes in heart weight were accompanied by changes in the absolute amount of protein or were due to edema. Measurement of protein concentration was performed in tissue homogenate in 4 M LiCl/8 M urea after overnight incubation at 4° (see Total RNA preparation). Determinations were done in triplicate, according to the method of Bradford (18). LiCl (4 M)/8 M urea did not interfere with the measurements.

Statistics. All values presented are arithmetic means ± standard errors. Statistical significance was estimated using Student's *t* test for paired (heart rate) or unpaired observations. A *p* value of <0.05 was considered significant.

Results

Physiological parameters and serum catecholamine concentrations. Heart rate, body weight, heart weight, total RNA, protein content, and serum catecholamine levels were measured to ensure adequate drug delivery by the minipumps and accurate drug dosages, as well as to characterize the model. Isoprenaline infusion induced an increase in heart rate by maximally 56 beats/min on day 1, which was only transient; heart rate reached predrug values after 3–4 days of continuous treatment (Fig. 1). Body weight was not influenced by isoprenaline, propranolol, or the combination thereof in the first 8 days and was slightly increased by isoprenaline after 13 and 26 days of treatment (data not shown). The most pronounced effect of isoprenaline was an increase in ventricular wet weight, both absolute and relative to body weight. Interestingly, heart/body weight ratio (Fig. 2A) increased very rapidly (by 30 ± 4% of control values) after the first day of treatment, reached a maximal increase on day 3 (42 ± 3%), and then slowly declined almost to control values after 4 weeks of treatment. The isoprenaline-induced cardiac enlargement was accompanied by an increase in total RNA yield, i.e., total RNA concentration in myocardial tissue, which amounted to maximally 52 ± 5% on day 3 (Fig. 2B). Taking into account the increase in ventricular



* $p < 0.05$ vs. values before treatment

Fig. 1. Influence of treatment on heart rate. Rats were treated by 1–8-day infusions of 0.9% NaCl (*NaCl*), isoprenaline (*Iso*) (2.4 mg/kg/day), propranolol (*Prop*) (9.9 mg/kg/day), or isoprenaline plus propranolol (*Iso + Prop*) (same doses). Heart rate was measured by electrocardiogram recordings on conscious nonanesthesized rats. Heart rate is given for 2 days before implantation of the minipumps (days −1 and 0) and for days 1–8 of treatment. *Arrows*, time of implantation of minipumps. *, $p < 0.05$ versus the arithmetic mean of values on days −1 and 0.

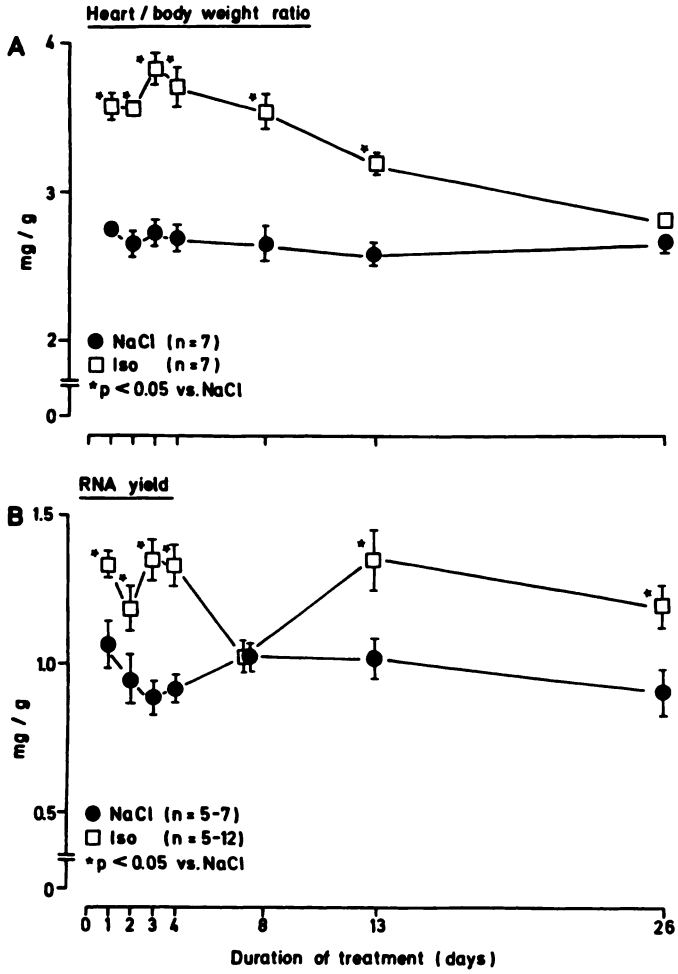


Fig. 2. Influence of treatment on heart/body weight ratio (A) and total RNA yield (B). Rats were treated by 1–26-day subcutaneous infusions of 0.9% NaCl (*NaCl*) or isoprenaline (*Iso*) (2.4 mg/kg/day). Heart/body weight ratio is the biventricular wet weight (mg) per body weight (g) at the day of sacrifice. RNA yield is the amount of total RNA (mg) extracted per biventricular wet weight (g). RNA concentration was determined photometrically at 260 nm. *, $p < 0.05$ versus NaCl.

weight, the absolute amount of total RNA extracted from both ventricles had more than doubled after 3 days of isoprenaline infusion. Total RNA yield in the isoprenaline-treated rats declined after longer treatment time, reached control values on day 8, and was again increased by 33 ± 8% and 33 ± 8% on day 13 and 26, respectively. In contrast, ventricular total protein concentration (ranging between 138 and 165 mg/g of wet weight) was not influenced at any time of treatment, indicating that cardiac enlargement was accompanied by similar increases in total ventricular protein. Consistent with these data, standard histological examination of formaldehyde-fixed tissue sections from hearts treated for 1, 4, or 8 days revealed an increase in diameter of the single myocyte. The increase started at day 1 (15.0 ± 0.5 μm versus 14.0 ± 0.5 μm, $n = 32$–41) and reached significance on day 4 (17.9 ± 0.5 μm, $n = 30$) and 8 (17.8 ± 0.5, $n = 31$). Isoprenaline infusion at a dose of 2.4 mg/kg/day induced disseminated focal necrosis throughout the myocardium, which showed signs of fibrotic rebuilding from day 4 on. Propranolol antagonized or attenuated the effects of isoprenaline on heart rate (Fig. 1), heart weight, and total RNA when given in combination with isoprenaline, indicating a sufficient dose of the β-adrenoceptor antagonist. Propranolol, given alone, lowered heart rate constantly over the treatment period by 72–97 beats/min (Fig. 1) and had no effect on heart weight or total RNA yield (not shown).

Serum concentrations of norepinephrine, epinephrine, dopamine, and isoprenaline were determined in rats treated for 1 or 8 days with NaCl or isoprenaline, to characterize the impact of chronic isoprenaline infusions on the endogenous sympathetic system and to control drug delivery by the minipumps (Table 1). Three main results were obtained. Norepinephrine concentrations doubled at day 1 of treatment and returned to control values at day 8. The increase is most likely due to the β_2-adrenoceptor-mediated presynaptical norepinephrine release, whereas the decrease to control values probably reflects either the depletion of norepinephrine stores known to occur under isoprenaline infusion (19) or the effects of desensitization. Dopamine concentrations increased with time of isoprenaline infusion, with an increase of 123% at day 8. The increase in dopamine is in agreement with studies in cardiomyopathic Syrian hamsters indicating that augmented sympathetic nerve traffic is in general accompanied by a shift in the rate-limiting step for norepinephrine synthesis from the hydroxylation of tryrosine to the hydroxylation of dopamine, leading to accumulation of dopamine (20). Isoprenaline concentrations decreased over time and ranged from 3×10^{-8} M to 1×10^{-8} M, which is in the range of the half-maximal positive inotropic effect of isoprenaline under *in vitro* conditions. The decrease in isoprenaline serum concentration cannot be attributed to a decreased pumping rate or other technical artifacts, because reimplantation of pumps into another rat always induced changes in all investigated parameters that were indistinguishable from those induced by freshly filled minipumps. Therefore, the decrease in isoprenaline levels most likely reflects an increased clearance of the drug after prolonged infusion, perhaps by induction of catechol-*O*-methyltransferase or monoaminooxidase.

Northern analysis. A prerequisite of measuring specific mRNA levels by slot blotting is to verify specificity of the radiolabeled probes under the conditions of hybridization and washing used. This was performed by Northern blotting (Fig. 3). Northern blots of 20 μg of total RNA extracted from ventricular myocardium of rats treated by 8-day subcutaneous infusions of 0.9% NaCl, isoprenaline, propranolol, or isoprenaline plus propranolol confirmed and extended other reports (13) and our own former results on rat heart (5). The nick-translated ^{32}P-labeled cDNA probe encoding $G_{i\alpha\text{-}2}$ hybridized to a single band at 2.4 kb, $G_{i\alpha\text{-}3}$ cDNA detected a single band at about 3.5 kb, and $G_{s\alpha}$ cDNA detected one prominent band at about 1.9 kb. The ^{32}P-$G_{o\alpha\text{-}1}$ cRNA, which was derived from the 3′ coding and noncoding region of a $G_{o\alpha\text{-}1A}$ cDNA (12) common to both $G_{o\alpha\text{-}1A}$ and $G_{o\alpha\text{-}1B}$ cDNAs, detected two main mRNA species of about 4.7 ($G_{o\alpha\text{-}1B}$) and 3.8 kb ($G_{o\alpha\text{-}1A}$) and two faint bands of about 2.8 and 1.9 kb. The identity of the smaller mRNA species is not known. The G_β cDNA hybridized to two bands at about 3.5 and 1.9 kb (see Fig. 6). In accord with previous studies (5, 11), $G_{i\alpha\text{-}1}$ mRNA was only marginally detectable in the heart after very long exposure times and was, therefore, not measured by slot blotting.

Fig. 3 depicts a representative Northern blot hybridized subsequently with different G_α probes. In RNA from isoprenaline-treated rats (Fig. 3, *lane 2*) signal intensity of $G_{i\alpha\text{-}2}$, $G_{i\alpha\text{-}3}$,

TABLE 1

Influence of treatment with 0.9% NaCl or isoprenaline (2.4 mg/kg/day) on serum concentrations of catecholamines

Values from rats treated with NaCl for 1 or 8 days did not differ and were pooled. All concentrations are expressed as pg/ml of serum.

Treatment	n	Concentration			
		Norepinephrine	Epinephrine	Dopamine	Isoprenaline
		pg/ml			
NaCl	9	1588 ± 210	2453 ± 395	75 ± 9	
Isoprenaline (1 day)	6	3223 ± 511*	1558 ± 290	108 ± 15	7139 ± 1560
Isoprenaline (8 days)	6	1838 ± 222	2093 ± 251	167 ± 50*	2828 ± 383

* $p < 0.05$ versus NaCl.

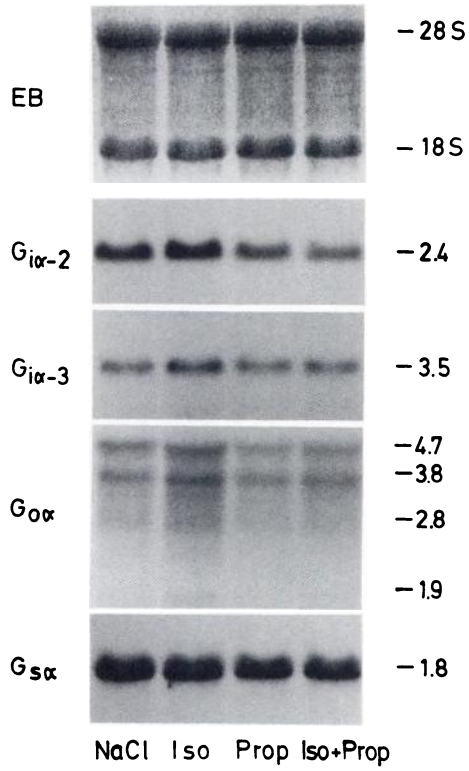


Fig. 3. Northern analysis of G_α mRNA expression in rat heart. RNA was isolated from hearts of rats treated by 8-day subcutaneous infusions of 0.9% NaCl, isoprenaline (*Iso*) (2.4 mg/kg/day), propranolol (*Prop*) (9.9 mg/kg/day), or a combination thereof (*Iso + Prop*) (same doses). Total RNA (20 μg) was subjected to agarose gel electrophoresis (ethidium bromide-stained gel) (*EB*) and to RNA blot analysis. The blot was probed subsequently with ^{32}P-labeled cDNA encoding rat $G_{i\alpha-2}$ (full length probe), $G_{i\alpha-3}$ (625-bp *Eco*RV/*Eco*RI fragment, 3′ noncoding region). Another blot with identical RNA samples was hybridized with a ^{32}P-labeled cRNA probe of rat $G_{o\alpha}$ (600-base 3′ coding and noncoding *Bam*HI/*Eco*RI fragment) under high stringency conditions, as outlined in Materials and Methods. Time of autoradiographic exposure was 2 days ($G_{i\alpha-2}$), 4 days ($G_{i\alpha-3}$), 18 hr ($G_{s\alpha}$), or 7 days ($G_{o\alpha}$). The mRNA size of the different G_α mRNAs is indicated in kb.

and $G_{o\alpha}$ was enhanced, whereas signal intensity of $G_{s\alpha}$ was unchanged, compared with RNA from the other treatment groups. There were no qualitative differences in hybridization pattern between the four groups.

G_α mRNA levels. mRNA levels of $G_{i\alpha-2}$, $G_{i\alpha-3}$, $G_{o\alpha}$, and $G_{s\alpha}$, expressed as pg/μg of total RNA, were measured on slot blots using six-point standard curves of *in vitro* transcribed cRNA standards. In the case of $G_{i\alpha-2}$, $G_{i\alpha-3}$, and $G_{s\alpha}$, full length cRNAs were used that were approximately similar in size to the respective mRNAs. Therefore, mRNA levels of $G_{i\alpha-2}$, $G_{i\alpha-3}$, and $G_{s\alpha}$ were directly deducible from the standard curve. In the case of $G_{o\alpha}$, the unlabeled cRNA standard amounted to 0.6 kb and the average length of the four $G_{o\alpha}$ mRNA bands was assumed to be 3 kb. Therefore, values from the standard curve were multiplied by a factor of 5. These measurements revealed a rank order of basal G_α mRNA levels (pg/μg of total RNA) in rat ventricular myocardium of $G_{s\alpha}$ (~30) > $G_{i\alpha-2}$ (~10) > $G_{i\alpha-3}$ (~3) > $G_{o\alpha}$ (~0.25).

Isoprenaline infusion induced a sustained increase in mRNA levels of all myocardial members of the G_i/G_o family, i.e., $G_{i\alpha-2}$, $G_{i\alpha-3}$, and $G_{o\alpha-1}$ (Fig. 4, A–C). $G_{o\alpha}$ mRNA level (Fig. 4C) was slightly increased from the first day on and reached significant values at the second day of treatment. $G_{i\alpha-2}$ and $G_{i\alpha-3}$ mRNA levels (Fig. 4B) increased significantly at the third day of treatment. Maximal increases in $G_{i\alpha}/G_{o\alpha}$ mRNA levels amounted to 77% ($G_{i\alpha-2}$), 58% ($G_{i\alpha-3}$), and 78% ($G_{o\alpha}$), compared with control. In general, the maximal increase was reached after 3 days of isoprenaline infusion and levels remained approximately similar over the entire treatment period of 4 weeks.

The effect of isoprenaline on $G_{i\alpha-2}$, $G_{i\alpha-3}$, and $G_{o\alpha}$ mRNA levels was antagonized by simultaneously administered propranolol, which had no effect on G_α mRNA levels when given alone (determined for days 1–8; Fig. 5). The effect of propranolol alone or the combination of isoprenaline plus propranolol on $G_{o\alpha}$ mRNA levels was only determined for day 4, i.e., the time point when the effect of isoprenaline was maximal. $G_{s\alpha}$ mRNA levels were not influenced by any treatment at any time (Fig. 4D).

Dose dependency. One might argue that the effects seen after isoprenaline infusion are, in spite of a relatively low concentration (EC_{50} serum concentration), a pathological response accompanying the myocardial necrosis and not a physiological regulation. We, therefore, performed an additional set of experiments using two different doses of isoprenaline (0.24 and 0.07 mg/kg/day). The lower doses of isoprenaline still had biological effects, although less pronounced than the effects of 2.4 mg/kg/day, i.e., increases in heart rate, heart/body weight ratio, or total RNA yield (Table 1). However, histological examination revealed a normal finding, i.e., both doses induced no necrosis, no lymphocyte infiltration, and no fibrosis. Nevertheless, treatment with 0.24 mg/kg/day induced an increase in $G_{i\alpha-2}$ mRNA level by 24% (Table 2), and 0.07 mg/kg/day isoprenaline still induced a trend towards higher $G_{i\alpha-2}$ mRNA levels (Table 2), indicating that the increased expression of $G_{i\alpha}/G_{o\alpha}$ proteins is a physiological response in the myocardium that is independent of the pathological reactions induced by isoprenaline.

G_β mRNA level. G_β mRNA levels were determined after 8 days of treatment, i.e., at the time when the effect of isoprenaline on other mRNA levels was maximal. Two-dimensional densitometry of Northern blots (three independent blots) re-

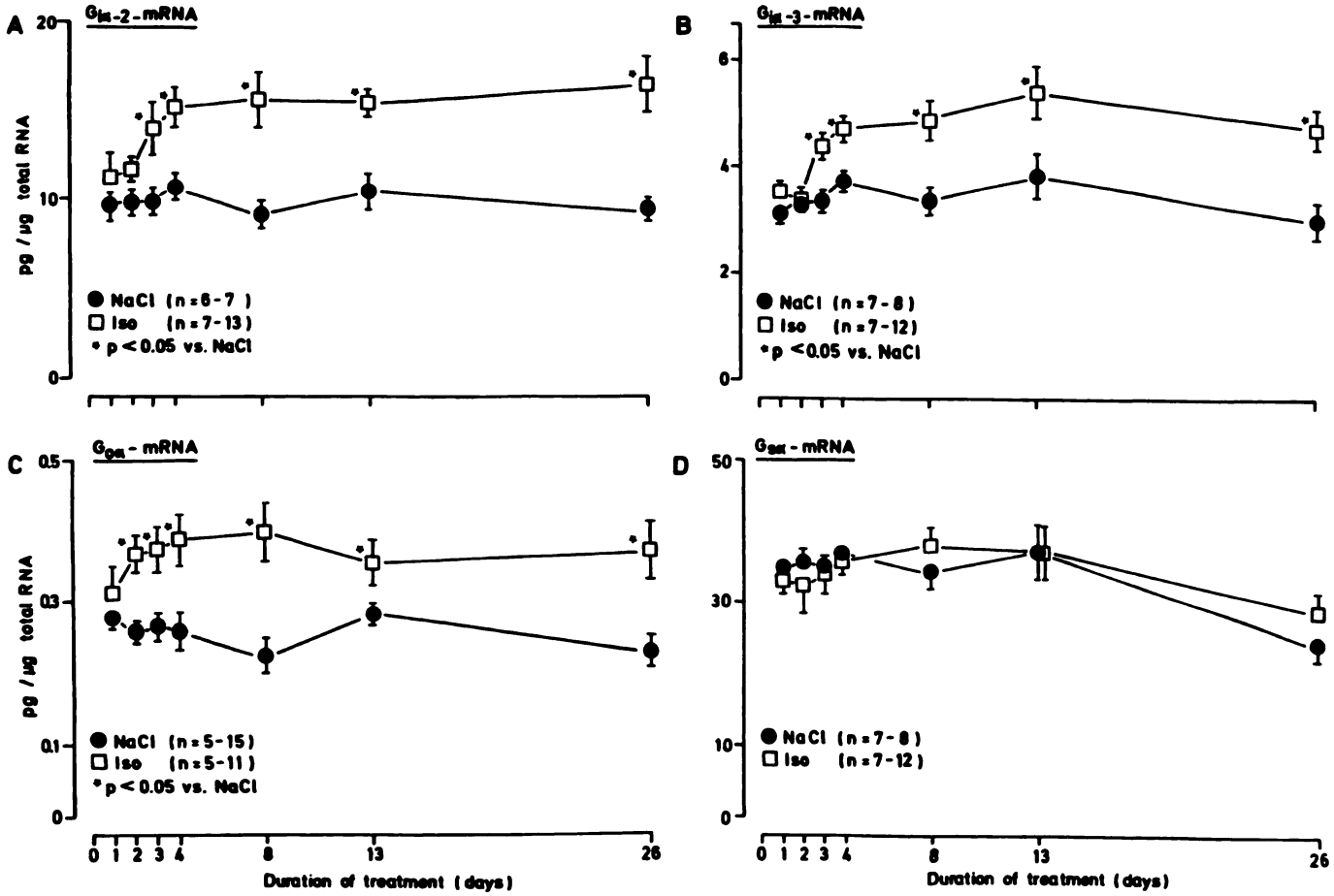


Fig. 4. Influence of isoprenaline on $G_{i\alpha-2}$ (A), $G_{i\alpha-3}$ (B), $G_{o\alpha}$ (C), and $G_{s\alpha}$ mRNA levels (D) in the heart. Total RNA was extracted from left and right ventricular myocardium of rats treated by 1–26-day subcutaneous infusions of 0.9% NaCl (*NaCl*) or isoprenaline (*Iso*) (2.4 mg/kg/day). Total RNA (10 µg) was subjected to RNA slot blot analysis and probed with ^{32}P-labeled cDNA encoding rat $G_{i\alpha-2}$ (full length), $G_{i\alpha-3}$ (625-bp *Eco*RV/*Eco*RI fragment, 3′ noncoding), and $G_{s\alpha}$ (1120-bp *Eco*RI fragment, 3′ coding and noncoding region) or a ^{32}P-labeled cRNA probe of rat $G_{o\alpha}$ (600-bp *Bam*HI/*Eco*RI fragment, 3′ coding and noncoding) under high stringency conditions, as outlined in Materials and Methods. mRNA concentrations of $G_{i\alpha-2}$, $G_{i\alpha-3}$, $G_{o\alpha}$, and $G_{s\alpha}$ were calculated by referring individual autoradiographic densities on slot blots to cRNA standard curves. *, $p < 0.05$ versus NaCl.

vealed that isoprenaline infusions had no effect on G_β mRNA levels after 4 days (data not shown) or 8 days of treatment and did not alter the ratio of the 3.5- and 1.9-kb bands (Fig. 6).

PTX-sensitive G proteins. The level of PTX-sensitive G proteins was measured in crude homogenates to investigate whether alterations in G_i/G_o mRNA levels were accompanied by parallel changes in the amount of G_i/G_o proteins. The apical half and the valvular half of both right and left ventricules were taken alternately for G_α mRNA or G_α protein determinations, to allow a general statement about G protein expression in ventricular myocardium and to exclude systematic errors that might derive from regional differences in G protein expression. PTX-catalyzed ADP-ribosylation resulted in specific labeling of proteins that migrated as a doublet at 40/41 kDa (Fig. 7A).

Isoprenaline infusion induced an increase in the amount of PTX-sensitive G proteins, which involved both the 40- and 41-kDa bands to approximately the same degree (Fig. 7A). The increase was biphasic, with an increase of 27% on day 2 and a decline to control values on day 3 (Fig. 7B). After 4 days of isoprenaline infusion, myocardial levels of PTX-sensitive G proteins increased again by maximally 54% and remained constantly elevated over the additional treatment of up to 4 weeks. During this time there was no obvious shift in the ratio between 40- and 41- kDa bands in SDS gels (data not shown). Propranolol antagonized the effect of isoprenaline and had no effect on the level of PTX-sensitive G proteins when given alone (determined for days 1–8; Fig. 7C).

Discussion

The major findings of the present report are (i) that mRNA levels of all members of the $G_{i\alpha}/G_{o\alpha}$ family, which are expressed in the heart, increased after β-adrenergic stimulation, whereas $G_{s\alpha}$ and G_β mRNA levels were unchanged, (ii) that increases in both mRNA and protein levels were slow in onset and persisted over the treatment period of 4 weeks, (iii) that up-regulation of $G_{i\alpha}$ and $G_{o\alpha}$ mRNA levels and that of PTX-sensitive G proteins were approximately similar in quantity and paralleled each other after 4 days of treatment, and (iv) that the increase in $G_{i\alpha-2}$ mRNA levels is a dose-dependent phenomenon not associated with histopathological effects of isoprenaline in the heart. These data provide evidence that β-adrenoceptor-mediated regulation of $G_{i\alpha}/G_{o\alpha}$ mRNA levels plays a crucial and physiological role in determination of cellular levels of $G_{i\alpha}/G_{o\alpha}$ in the heart. They also demonstrate that chronic β-adrenergic stimulation *in vivo* does not up-regulate the mRNA expression of all G proteins (including $G_{s\alpha}$ and the β subunits) but affects

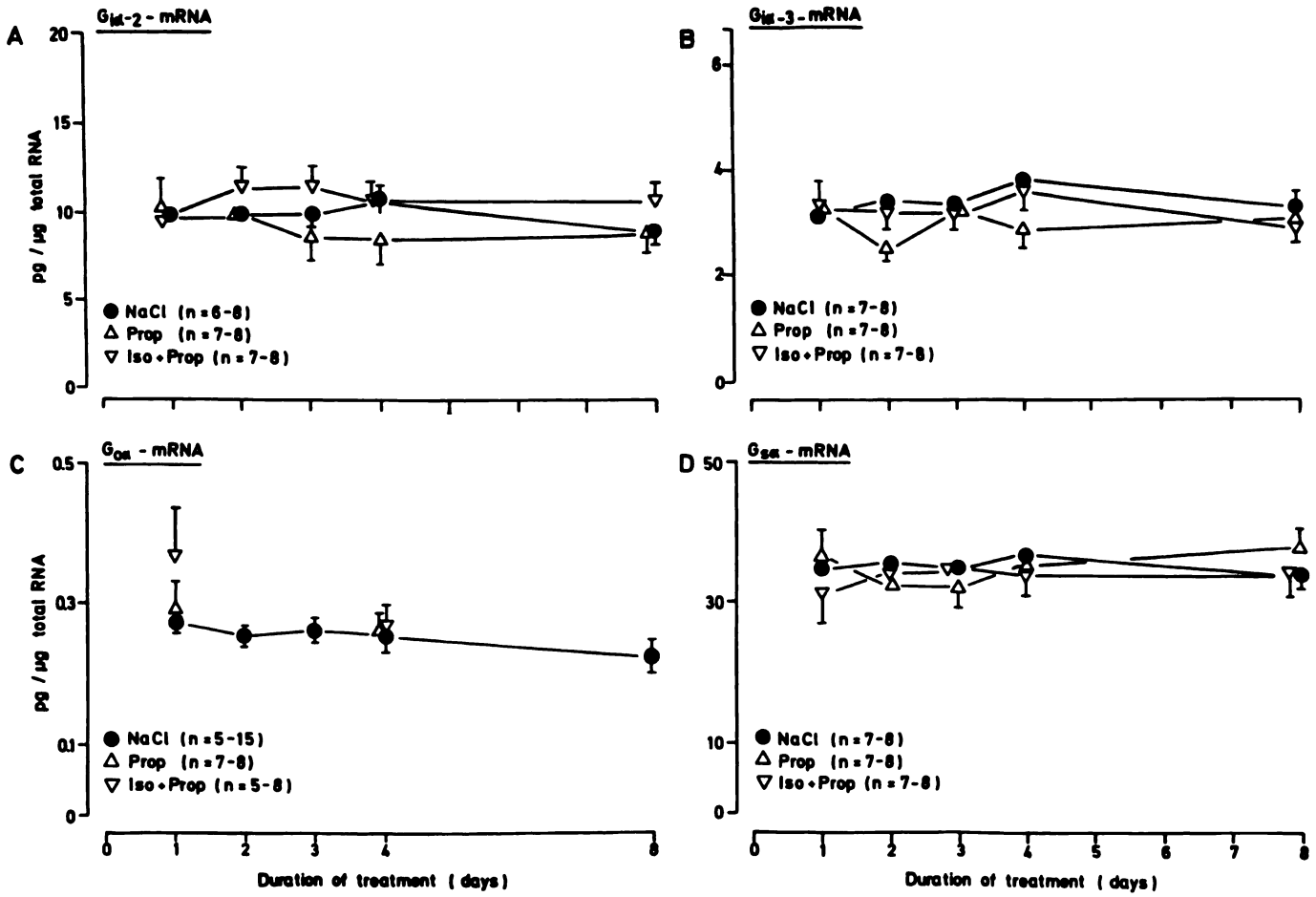


Fig. 5. Influence of the β-adrenoceptor antagonist propranolol on $G_{i\alpha 1-2}$ (A), $G_{i\alpha 1-3}$ (B), $G_{o\alpha}$ (C), and $G_{s\alpha}$ mRNA levels (D) in the heart. Total RNA was extracted from left and right ventricular myocardium of rats treated by 1–8-day subcutaneous infusions of 0.9% NaCl (*NaCl*), propranolol (*Prop*) (9.9 mg/kg/day), or isoprenaline plus propranolol (*Iso + Prop*) (same dose). Total RNA (10 µg) was subjected to RNA slot blot analysis and probed with ^{32}P-labeled cDNA encoding rat $G_{i\alpha 1-2}$ (full length), $G_{i\alpha 1-3}$ (625-bp *Eco*RV/*Eco*RI fragment, 3′ noncoding), and $G_{s\alpha}$ (1120-bp *Eco*RI fragment, 3′ coding and noncoding region) or a ^{32}P-labeled cRNA probe of rat $G_{o\alpha}$ (600-bp *Bam*HI/*Eco*RI fragment, 3′ coding and noncoding) under high stringency conditions, as outlined in Materials and Methods. mRNA concentrations of $G_{i\alpha 1-2}$, $G_{i\alpha 1-3}$, $G_{o\alpha}$, and $G_{s\alpha}$ were calculated by referring individual autoradiographic densities on slot blots to cRNA standard curves.

TABLE 2

Dose dependency of the effects of isoprenaline on physiological parameters and $G_{i\alpha 1-2}$ mRNA levels

Rats were treated for 4 days with NaCl or 2.4 mg/kg/day (ISO I), 0.24 mg/kg/day (ISO II), or 0.07 mg/kg/day (ISO III) isoprenaline. Heart rate was measured before and after 4 days of treatment. Heart weight is given as left and right ventricular wet weight relative to body weight and total RNA yield as µg of RNA/mg of ventricular wet weight. Note that heart rate and total RNA yield cannot be compared directly with data from the time course experiments (Figs. 1 and 2) because electrocardigram measurements on awake rats strongly depend on handling of the rats, which differs among investigators, and total RNA was extracted using a different method (12).

	n	Heart rate		Heart weight	Total RNA	$G_{i\alpha 1-2}$ mRNA
		Before	After			
		beats/min		*mg/g of wet weight*	*µg/mg of wet weight*	*pg/µg of RNA*
NaCl	4	362 ± 14	394 ± 12	2.6 ± 0.07	1.3 ± 0.09	9.5 ± 0.7
ISO III	4	385 ± 10	402 ± 14[a]	2.83 ± 0.05[b]	1.5 ± 0.21	9.9 ± 1.0
ISO II	4	397 ± 13	463 ± 23[a]	2.97 ± 0.06[b]	1.3 ± 0.13	11.7 ± 1.1
ISO I	2	398	513	3.39	1.8	14.2

[a] $p < 0.05$ versus pretreatment value.
[b] $p < 0.05$ versus NaCl.

specifically the $G_{i\alpha}/G_{o\alpha}$ subunits, which might serve as transducers of functionally "antiadrenergic" pathways.

The results of the present study have been obtained in an *in vivo* model that is frequently used as a model of β-adrenoceptor-induced cardiac hypertrophy. Therefore, changes in physiological parameters, β-adrenoceptors, adenylyl cyclase activity, and force of contraction have been well characterized (5, 6, 21, 22). The dose of isoprenaline used in our experiments (2.4 mg/kg/day) was relatively low, compared with other studies, which used 4.8–160 mg/kg/day in rats (21, 22). The low dose of the β-adrenoceptor agonist was chosen to prevent development of gross myocardial necrosis (21); this was largely achieved, as verified by histological examination. Furthermore, two lower doses (0.24 and 0.07 mg/kg/day) were used, which clearly did not induce any pathological reaction throughout the myocardium.

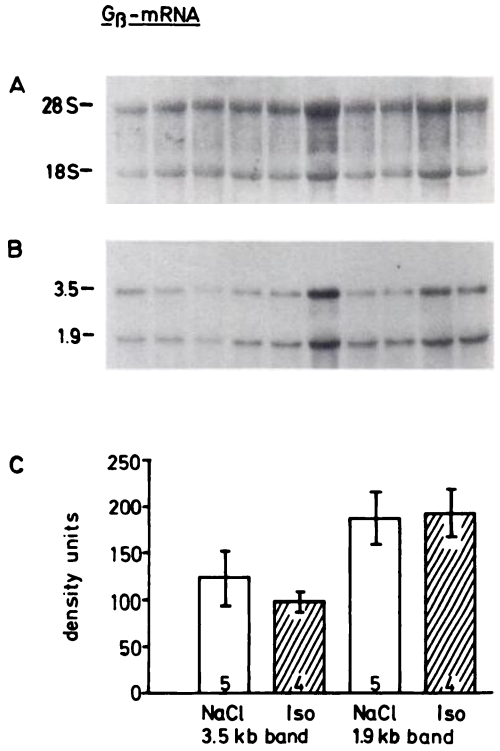


Fig. 6. Influence of isoprenaline on G_β mRNA levels in the heart: analysis by Northern blotting. RNA was isolated from hearts of rats treated by 8-day subcutaneous infusions of 0.9% NaCl or isoprenaline (*Iso*) (2.4 mg/kg/day). Total RNA (20 μg) was subjected to RNA blot analysis and probed with ^{32}P-labeled G_β cDNA (0.840-kb *BglII* fragment). The blot was hybridized and washed as outlined in Materials and Methods. Time of autoradiographic exposure was 5 days. The positions of 18 S and 28 S rRNA are indicated. Shown are the ethidium bromide-stained gel (A), the autoradiograph (B), and the signal intensities (mean ± standard error) of the 3.5-kb and 1.9-kb bands determined by two-dimensional densitometry (C). RNA samples of NaCl- and isoprenaline-treated rats were applied alternately, starting with NaCl. Note that one sample (isoprenaline, *lane 6* in A and B) was excluded from calculation because of overload of RNA. *Numbers in columns*, numbers of RNA samples.

Physiological parameters were determined to characterize the model and to ensure adequate drug delivery by the minipumps. Although they essentially confirm former results concerning an isoprenaline-induced increases in heart rate, heart weight, and total RNA (5), some aspects of the present results need further discussion. Unexpectedly, heart/body weight ratio increased very quickly and nearly reached its maximum on day 1. In spite of similar or even larger increases in protein content and total RNA (Fig. 2B), this rapid increase in heart weight is hardly explained exclusively by hypertrophy of the single myocyte and suggests concomitant initial cardiac edema. This is supported by a slower time course of development of histological enlargement of myocytes. The heart/body weight ratio declined after 8 days of isoprenaline infusion and nearly reached control values after 26 days of treatment. Because it was beyond the scope of this study, we did not investigate this question further. There are, however, recent data from cultured neonatal rat cardiomyocytes that demonstrated that β-adrenoceptor-mediated hypertrophy is probably an indirect result of increased force of contraction and stretch of the myocytes and does not occur if cultured cardiomyocytes are not beating (23). In this respect, the decline of cardiac hypertrophy under chronic isoprenaline infusion in our study may simply demonstrate the physiological significance of desensitization of the β-adrenoceptor/G protein/adenylyl cyclase system of the heart and the circulatory system. In a steady state condition, which might develop in days, these alterations are likely to result in normalization of contractile parameters and thereby of cardiac growth. The latter hypothesis is supported by complete normalization of heart rate in the isoprenaline-treated rats after 3 days (Fig. 1) and normalization of norepinephrine serum concentrations after 8 days (Table 1). Decreased isoprenaline serum concentrations after 8 days (Table 1) might also contribute to the decrease in cardiac growth. Isoprenaline infusion very quickly induced marked increases in total RNA yield, thus indicating a general stimulation of transcriptional and probably translational activity in the heart. It is important to consider this general increase in RNA when interpreting individual mRNA levels. The lower doses of isoprenaline resulted in smaller increases in heart rate, heart/body weight ratio, and total RNA yield, showing a dose dependency of the effects of isoprenaline. These results indicate that chronic β-adrenergic stimulation is a hypertrophic stimulus *in vivo* that is not linked to the induction of myocardial necrosis. Similarly and most importantly for interpretation of the present data, isoprenaline-induced increases in $G_{i\alpha\text{-}2}$ mRNA levels were shown to be clearly dose dependent and to occur independently from the induction of fibrotic necrosis (Table 2).

Up-regulation of the expression of PTX-sensitive G proteins reported in the present study involved mRNA levels of all members of the $G_{i\alpha}/G_{o\alpha}$ family, which are expressed at detectable levels in the heart. Their up-regulation reached the same levels and followed very similar time courses. This strongly suggests, although does not prove, that all $G_{i\alpha}/G_{o\alpha}$ family members contribute to the increases in the amounts of PTX-sensitive G proteins in the heart, which reached similar decrees of increment. Further support is provided by approximately similar increases in both 40- and 41- kDa PTX substrates in the SDS gel. On the other hand, the increase was restricted to $G_{i\alpha}/G_{o\alpha}$ mRNA and did not involve $G_{s\alpha}$ or G_β mRNA levels. This underlines the specificity and the possible functional impact of these alterations.

We have reported previously that the increased amount of PTX-sensitive G proteins is accompanied by an increased negative inotropic potency of carbachol and a decreased positive inotropic effect of isoprenaline and forskolin on isolated papillary muscles (6, 7). The results of the present time course study provide further evidence that up-regulation of G_i/G_o proteins might play a central role in the long term adaptation of the myocardium to chronic activation of the stimulatory adenylyl cyclase signaling pathway. Different G_i/G_o subtypes have been implicated in a complex variety of signal-transducing pathways, and alterations in cellular levels of all $G_{i\alpha}/G_{o\alpha}$ subtypes would be expected to influence multiple cellular responses. Although not unequivocally established, $G_{i\alpha\text{-}2}$ seems to be the principal subtype in the inhibition of adenylyl cyclase (24, 25), $G_{i\alpha\text{-}3}$ and $G_{o\alpha}$ might preferentially couple muscarinic receptors to cardiac pacemaker I_f channels (26), all three $G_{i\alpha}$ subtypes might be involved in the opening of atrial K^+ channels (27), and $G_{o\alpha}$, but not members of the G_i family, mediate the somatostatin- and carbachol-induced inhibition of neuronal voltage-sensitive Ca^{2+} channels (27, 28). Furthermore, $G_{o\alpha}$ has been implicated in the PTX-sensitive phospholipase C pathway (29). It should be emphasized that more than one $G_{o\alpha}$ isoform exists, which might possess different functions. It is not known

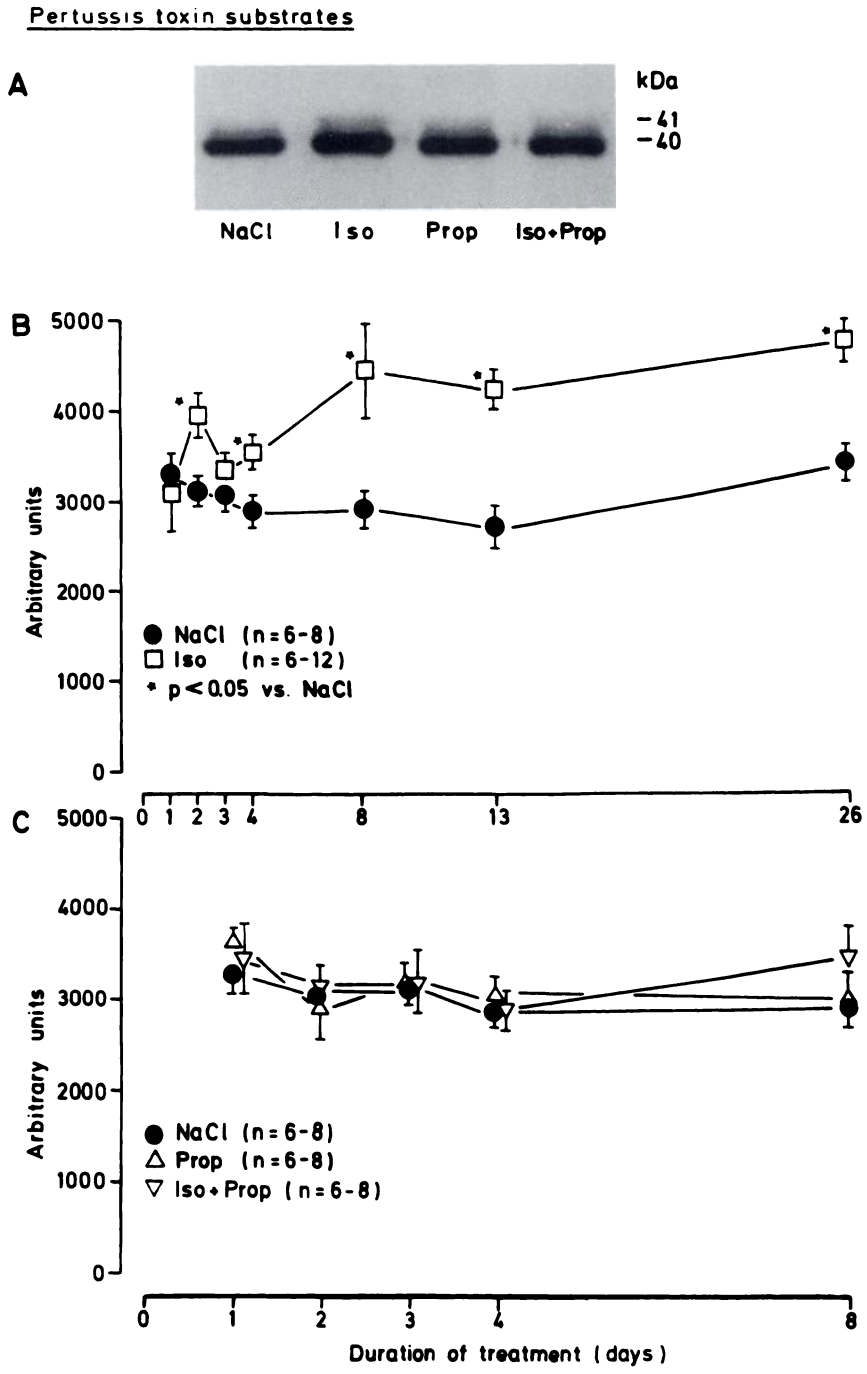


Fig. 7. Influence of isoprenaline and the β-adrenoceptor antagonist propranolol on PTX-catalyzed ADP-ribosylation in the heart. Crude homogenates were prepared from left and right ventricular myocardium of rats treated by 1–26-day subcutaneous infusions of 0.9% NaCl (*NaCl*) or isoprenaline (*Iso*) (2.4 mg/kg/day) (B) or by 1–8-day infusions of propranolol (*Prop*) (9.9 mg/kg/day) or isoprenaline plus propranolol (*Iso+Prop*) (same doses) (C). The homogenates (30 μg of protein) were subjected to PTX-catalyzed [^{32}P] ADP-ribosylation and resolved by SDS-polyacrylamide gel electrophoresis on urea (4 M)-containing gels. A, Representative autoradiograph of the 40/41-kDa region. Arbitrary units were obtained by two-dimensional light densitometry of autoradiograms, as outlined in Materials and Methods. *, $p < 0.05$ versus NaCl.

exactly which protein form of $G_{o\alpha}$ is associated with which cDNA (15). The $G_{o\alpha}$ cRNA used in the present study was derived from a $G_{o\alpha\text{-}1A}$ clone (14). It detected both $G_{o\alpha\text{-}1A}$ (~3.8 kb) and $G_{o\alpha\text{-}1B}$ (~4.7 kb) mRNA species and two more faint mRNA bands, at 2.8 and 1.9 kb, of unknown identity. It did not cross-react with $G_{o\alpha\text{-}2}$ mRNA (5.7 kb according to Ref. 30). Thus, all measurements refer to $G_{o\alpha\text{-}1A/B}$.

The stochiometry between receptors, different G protein α subunits, and G_β and the impact of alterations of one of these components on signal transduction are not well understood. Nevertheless, assuming that $G_{s\alpha}$ and G_β protein levels correlate with their mRNA levels, chronic β-adrenergic stimulation leads to an altered ratio between $G_{i\alpha}$ and $G_{o\alpha}$ on one hand and $G_{s\alpha}$ and the common scavenger for all G_α subunits, G_β, on the other hand. Theoretically, only such an altered ratio would be expected to sensitize inhibitory and desensitize stimulatory signal transduction pathways. Recent data on tumor necrosis factor-α-induced increases in the expression of both G_i and G_β in cultured cardiomyocytes, which did not lead to alterations in adenylyl cyclase activity (9), support this assumption.

The onset of increases in $G_{i\alpha}/G_{o\alpha}$ mRNA levels was delayed, compared with changes in heart rate, RNA yield, and heart weight, and maximal increases occurred after 3 days of isoprenaline infusion. The only other study measuring the time course of β-adrenoceptor-mediated up-regulation of $G_{i\alpha}$ protein levels (4) reported an increase in $G_{i\alpha\text{-}2}$ mRNA levels in S49 mouse lymphoma cells that started after 8 hr and was only transient, lasting for 18 hr. Differences from our data may be due to

differences in the cell type, the very high agonist concentrations in these experiments (1–10 mM), and differences in the experimental design, i.e., *in vitro* versus *in vivo*. It is well known that culture conditions, such as different amounts and sources of serum factors, as well as different phases of cell growth, do have a strong impact on several biological parameters. *In vitro* experiments also do not reflect physiological interactions between the target cell and local or systemic regulatory mechanisms. On the other hand, the *in vivo* system is limited by multiple physiological compensatory mechanisms, which are often not known in detail. The present results demonstrate that information from *in vitro* and *in vivo* experiments are complementary. Pathological states with increased expression of G_i, such as human heart failure, are chronic disorders. It is important, therefore, that the β-adrenoceptor-mediated increase in $G_{i\alpha}/G_{o\alpha}$ mRNA and PTX-sensitive G proteins in the heart seems to be a chronic regulatory event *in vivo*, lasting as long as stimulation persists. The observation of a relatively slow, moderate, and persistent regulation of G_i/G_o expression is also compatible with the characterization of the $G_{i\alpha\text{-}2}$ gene as a "housekeeping gene" (31). Recent studies on DDT-MF2 cells have shown that the isoprenaline- or forskolin-induced increase in $G_{i\alpha\text{-}2}$ protein involves increases in mRNA levels, protein synthesis, and protein decay (4, 32). Although the present study did not determine transcription rate and/or decay rate of the mRNAs or proteins, the largely parallel time courses of the changes in $G_{i\alpha}/G_{o\alpha}$ mRNA levels on one hand and in the PTX-sensitive G proteins on the other hand suggest that pretranslational changes in the expression of $G_{i\alpha}/G_{o\alpha}$ play the dominant role in long term up-regulation. It needs to be elucidated whether alterations in translational efficiency and/or in the decay rate of protein are also involved in the early time course.

How does persistent activation of the stimulatory adenylyl cyclase pathway induce changes in $G_{i\alpha}/G_{o\alpha}$ mRNA levels? Data on consensus sequences of a cAMP-response element in the $G_{i\alpha\text{-}2}$ gene (31), characterization of transcriptional factors, like AP-2, that are under cAMP-dependent control in eucaryotic cells (33), and the inability of persistent activation of adenylyl cyclase to increase the $G_{i\alpha\text{-}2}$ mRNA in S49-*kin*$^-$ cells lacking functional active protein kinase A (34) strongly suggest an important role of cAMP and protein kinase A in the elevation of $G_{i\alpha}$ mRNA levels. From these data, it seems reasonable to assume that isoprenaline causes increased transcription of the $G_{i\alpha}$ gene(s) via cAMP-dependent activation of transcriptional factors. Although still speculative, this is further supported by the present finding that the expression of the $G_{s\alpha}$ gene, lacking a cAMP-response element (35), was not influenced by isoprenaline infusion at any time. In accord with this hypothesis, recent run-on assays have shown that transcription of the $G_{i\alpha\text{-}2}$ gene, but not of the $g_{s\alpha}$ gene, indeed increases after a 4-day infusion of isoprenaline (36).

In summary, β-adrenergic stimulation led to a gradual and persistent increase in the mRNA expression of all members of the G_i/G_o family and of PTX substrates in the heart under *in vivo* conditions. In contrast, $G_{s\alpha}$ and G_β mRNA levels remained unchanged. It is conceivable that the increase in $G_{i\alpha}/G_{o\alpha}$ in the heart modulates signal transduction to several intracellular pathways, leading to an overall subsensitivity to adrenergic stimulation that is maintained at new steady states as long as β-adrenergic stimulation persists. Although one should always be cautious in extrapolating data from animal models to the human, the results of the present study support the hypothesis that similar increases in $G_{i\alpha\text{-}2}$ mRNA (37) and in PTX-sensitive G proteins (38–40) in human end-stage heart failure probably result from the adrenergic overdrive that generally accompanies end-stage heart failure.

Acknowledgments

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