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Effects of chronic application of propranolol on β-adrenergic signal transduction in heart ventricles from myopathic BIO TO2 and control hamsters

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1 In human congestive heart failure β-adrenoceptor antagonists improve exercise tolerance and cardiac contractility. These beneficial effects are thought to reflect an up-regulation of cardiac β-adrenoceptors, involving mainly the β_1-subtype. In the present study we evaluated the functional contribution of β-adrenoceptor subtypes to stimulation of adenylyl cyclase in an animal model of dilated cardiomyopathy, and compared the effects of treatment with propranolol on cardiac β-adrenergic signal transduction in myopathic and control hamsters.

2 Cardiomyopathic BIO TO2 hamsters and BIO F1B controls aged 270 days were used. In the treatment study, hamsters received drinking water with or without propranolol 40 mg kg^{-1} d^{-1} for 4 weeks prior to sacrifice. Density and subtype distribution of β-adrenoceptors were determined in radioligand binding studies. Functional contributions of β-adrenoceptors were evaluated by subtype-selective stimulation of adenylyl cyclase. Cardiac G-protein content was determined by immunoblotting.

3 Compared to BIO F1B controls, myopathic hamsters showed increases in cardiac total β- and β_2-adrenoceptor density, $G_s\alpha$ and $G_i\alpha$ content. In BIO TO2 ventricles, β_1-adrenoceptors were almost completely uncoupled from adenylyl cyclase stimulation despite an unchanged density. Treatment of hamsters with propranolol resulted in increased density of β_1-adrenoceptors in both strains, but had no effect on their functional efficacy. Moreover, β_2-adrenergic stimulation of adenylyl cyclase was even reduced in propranolol-treated animals, which could not be explained by changes in cardiac G-protein content.

4 Cardiomyopathic BIO TO2 hamsters showed functional uncoupling of cardiac β_1-adrenoceptors, which could not be normalized by propranolol and, therefore, is unlikely to be solely due to agonist-dependent desensitization. The paradoxical reduction in β_2-adrenergic efficiency in propranolol-treated myopathic and control hamsters deserves further investigation.

Keywords: Heart failure; myopathic hamster; adenylyl cyclase; β-adrenoceptor subtypes; coupling; G-proteins; treatment study

Introduction

Despite the progress in the treatment of congestive heart failure, the life expectancy of patients with advanced stages of the disease is still rather low. Addition of β-adrenoceptor antagonists to the standard therapy of heart failure was found to improve exercise tolerance and cardiac contractility in patients with dilated cardiomyopathy (Waagstein *et al*., 1993; Eichhorn *et al*., 1994; CIBIS Investigators, 1994; Bristow *et al*., 1994). Beneficial effects of β-adrenoceptor antagonists in heart failure could result from a reduction in heart rate (Steeds & Channer, 1998), which is often elevated in patients with heart failure as a consequence of the increase in sympathetic drive. In addition, prolonged treatment with β-adrenoceptor antagonists could prevent the down-regulation and uncoupling of β-adrenoceptors from their signaling pathway in the diseased myocardium. Discrepant data on receptor changes have been reported in treated patients: significantly higher densities of β-adrenoceptors were found in some studies (Michel *et al*., 1988; Heilbrunn *et al*., 1989), while in other studies unchanged β-adrenoceptor densities were observed (Kaumann *et al*., 1995; Molenaar *et al*., 1997). Prospective intervention studies on β-adrenergic signal transduction would require repeated sampling of sufficient amounts of cardiac tissue and, therefore, cannot easily be performed in these severely ill patients. Studies in animal models of congestive heart failure may help to elucidate the mechanisms contributing to the beneficial effects of β-adrenoceptor antagonists. Myopathic hamsters are often used as animal models of genetically induced heart failure. Disturbances in the β-adrenergic signal transduction cascade have been reported by several groups involving reductions in the activity of $G_s\alpha$ (Kessler *et al*., 1989; Feldman *et al*., 1990) and the amount of mRNA for both $G_s\alpha$ and $G_i\alpha$ (Katoh *et al*., 1990), increases in the number of cardiac β-adrenoceptors (Karliner *et al*., 1981), and reductions in basal (Kessler *et al*., 1989) and stimulated adenylyl cyclase activity (Kessler *et al*., 1989; Feldman *et al*., 1990). Kaura *et al*. (1996) observed a selective down-regulation of β_1-adrenoceptors and an increase in $G_i\alpha$ in hypertrophied myocardium from UM-X7 hamsters resembling the changes in human cardiomyopathy. In the failing human heart, β-adrenergic stimulation of contractile force and adenylyl cyclase activity is known to be mediated predominantly by β_2-adrenoceptors, although the β_1-subtype is more abundant in human cardiac tissue (Kaumann *et al*., 1989). Treatment with β-adrenoceptor antagonists might restore the functional coupling efficiency of β_1-adrenoceptors in the diseased myocardium and, thus, improve cardiac contractility. In an earlier study, we were able to show that an even more pronounced functional uncoupling of β_1-adrenoceptors occurred in myocardium from failing BIO 8262 hamster hearts (Witte *et al*., 1995). Therefore, cardiomyopathic hamsters seemed to be an appropriate model for investigating the

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functional consequences of treatment with β-adrenoceptor antagonists. Since BIO 8262 hamsters, which were used in our previous studies (Witte *et al*., 1995), develop myocardial hypertrophy prior to the final dilation of the heart, they differ in pathophysiology from human dilated cardiomyopathy. In contrast, BIO TO2 hamsters show cardiac dilation without preceding hypertrophy resembling the pattern in human idiopathic dilated cardiomyopathy. Moreover, at an age of 270 days, the myopathic animals show characteristic hemodynamic changes as observed in human heart failure: left ventricular systolic pressure, cardiac output and mean arterial pressure are reduced, peripheral resistance and left ventricular end-diastolic pressure are increased in myopathic compared to control hamsters (Panchal & Trippodo, 1993).

In the present study, we investigated disease-related changes in β-adrenergic signal transduction in dilated cardiomyopathy of BIO TO2 hamsters (study 1) and the effects of prolonged treatment with the β-adrenoceptor antagonist propranolol in control and BIO TO2 hamsters (study 2).

Methods

Animals

Male control (BIO F1B, $n=20$) and cardiomyopathic Syrian hamsters (BIO TO2, $n=20$) were obtained from Bio Breeders Inc., U.S.A. Hamsters were kept under constant environmental conditions with free access to food and water and a light-dark cycle of 12 : 12 h with lights on from 07.00–19.00 h. Animal experiments were approved by German federal regulations, which fully meet the requirements by the laws governing animal experimentation in the U.K.

Treatment protocol and preparation of cardiac tissue

In order to evaluate disease-related changes in β-adrenergic signaling (study 1) untreated cardiomyopathic and control hamsters ($n=8$ per strain, aged 270 days) were sacrificed by decapitation, heart ventricles were dissected quickly (within 1 min after decapitation), freed from fat and connective tissue, dried on filter paper, immediately frozen in liquid nitrogen and stored at −60°C until the biochemical assays were performed. Additional BIO F1B and BIO TO2 hamsters ($n=12$ per strain) were divided into two subgroups, receiving either drinking water or propranolol 40 mg kg^{-1} d^{-1} (PROP) in drinking water from 240–270 days of age (study 2). Daily fluid intake was monitored for 2 weeks before initiation of therapy and throughout the treatment period. At the end of the treatment period hamsters were sacrificed, and heart ventricles were prepared as described above.

Membrane preparation

Single ventricles were weighed and immediately homogenized in ice-cold assay buffer (Tris 50 mM, $MgCl_2$ 10 mM, pH 7.4 at 37°C) using an Ultra-Turrax-homogenizer (IKA, Staufen, Germany) at 20,000 r.p.m. The resulting suspension was divided into subsets for determination of β-adrenoceptor density (suspension A) and adenylyl cyclase activity (suspension B). Suspension A was homogenized a second time using a Potter S glass-homogenizer (Braun, Melsungen, Germany). After 10 min centrifugation at $25{,}000 \times g$ the supernatants of suspensions A and B were discarded. The pellet of suspension A was resuspended in 3 ml assay buffer, pellet B in 0.10 ml assay buffer per mg of tissue, resulting in protein concentrations of approximately 0.5 mg ml^{-1}. For determination of G-proteins, aliquots of resuspended pellet B were immediately frozen and stored at −20°C.

Radioligand binding studies

Cardiac β-adrenoceptor densities (B_{max}) were determined in saturation experiments using the non-selective β-adrenoceptor antagonist (−)-[^{3}H]CGP-12177 as described (Lemmer *et al*., 1993). Briefly, membranes were incubated with increasing concentrations of the radioligand (0.125–4 nM) in a total volume of 200 μl. Carteolol 0.1 mM was added for determination of non-specific binding. After a 2 h incubation at room temperature samples were filtered over Whatman GF/C filters, washed with 10 ml ice-cold assay buffer, and radioactivity bound was counted in a scintillation counter. For determination of β-adrenoceptor-subtypes displacement experiments were performed using increasing concentrations of the β_1-adrenoceptor antagonist CGP-20712A in the presence of (−)-[^{3}H]CGP-12177 (2 nM) under the same experimental conditions.

Adenylyl cyclase assay

The basal rate of cyclic AMP formation was determined in the presence of 3-isobutyl-1-methyl-xanthine (IBMX) and an ATP-regenerating system as described (Lemmer & Witte, 1989). Briefly, 0.3 ml of membrane suspension was added to 1.2 ml of prewarmed assay buffer (Tris 50 mM, $MgCl_2$ 10 mM, 37°C, pH 7.4) containing IBMX 1 mM, ATP 0.5 mM, phosphocreatine 10 mM and creatine phosphokinase 0.1 mg ml^{-1}. The reaction was stopped after 8 min by heating the tubes at 120°C, cyclic AMP formed was measured in the supernatant by radioassay (TRK 432, Amersham Buchler, Braunschweig, Germany).

G-protein activated adenylyl cyclase was assessed by addition of the non-hydrolyzable GTP analogue guanylyl-imidodiphosphate (GppNHp) 100 μM.

All assays for determination of β-adrenergic stimulation contained GTP 10 μM. For subtype-selective stimulation, concentration-response curves were performed using the non-selective β-adrenoceptor agonist isoprenaline and the rather β_1-selective β-adrenoceptor agonist noradrenaline in the presence of ICI-118.551 (1 μM), a highly selective β_2-adrenoceptor antagonist. The comparatively high concentration of ICI-118.551 was used in order to ensure that, even in the presence of noradrenaline 100 μM, increases in cyclic AMP were mediated exclusively *via* the β_1-adrenoceptor.

In the treatment study, an additional approach to subtype-selective stimulation of adenylyl cyclase was used: cyclic AMP formation was stimulated by the non-selective β-adrenoceptor agonist isoprenaline in the absence or presence of either CGP-20712A 1 μM or ICI-118.551 50 nM, in order to block β_1- and β_2-adrenoceptors, respectively.

Determination of cardiac G-protein content

The amount of $G_s\alpha$ and $G_i\alpha$ was determined by Western Blot analysis. Gel electrophoresis and immunoblotting were carried out according to the methods of Laemmli (1970) and Towbin *et al*. (1979), respectively, with minor modifications. Membranes were sonicated in sample buffer (Tris 125 mM, pH 6.8, sodium dodecyl sulfate (SDS) 1%, glycerol 10%, dithiotreitol 0.5%, bromphenol blue 0.01%), boiled for 5 min, and aliquots (20–30 μg protein) were subjected to SDS–polyacrylamide gel electrophoresis with 4% and 10% acrylamide in the

stacking and running gel, respectively. After transfer to nitrocellulose membranes (Hybond ECL, Amersham, Braunschweig, Germany) in blotting buffer (Tris 16 mM, glycine 120 mM, SDS 0.025%, methanol 20%), blots were incubated in TBS-T buffer (Tris 10 mM, pH 7.4, NaCl 0.9%, Tween-20 0.1%) with 3% bovine serum albumin for blocking of nonspecific binding sites. Subsequently blots were washed three times with TBS-T, and immunodetection was carried out with highly specific antibodies for $G_s\alpha$ and $G_i\alpha$ (RM/1 and AS/7, NEN, Bad Homburg, Germany). The membranes were then thoroughly washed three times for 10 min each with TBS-T and incubated with a horseradish peroxidase labeled anti-rabbit IgG (NA 934, Amersham Buchler, Braunschweig, Germany). Immunoreactivity was detected with the ECL-Western blotting analysis system (RPN 2106, Amersham Buchler, Braunschweig, Germany) according to the manufacturer's instructions. Intensities of the respective bands were quantified by transmission densitometry using G-protein standard from bovine brain (Calbiochem, Bad Soden, Germany) as reference. Representative immunoblots for $G_s\alpha$ and $G_i\alpha$ are shown in Figures 1 and 2, respectively.

Protein determination

The protein concentrations of membrane fractions A and B were determined by the method of Lowry *et al*. (1951) with minor modifications. Protein content in samples for determination of G-proteins was measured by using a commercially available Coomassie reagent (Pierce, oud-Beijerland, The Netherlands). Bovine serum albumine was used as standard and dissolved in the respective buffer.

Statistical analyses

Saturation curves for determination of B_{max} competition curves for calculation of β-adrenoceptor subtypes, and concentration-response curves for analysis of subtype-selective stimulation were fitted to the experimental data using PHARMFIT (Mattes *et al*., 1991). Differences between strains were tested by Student's *t*-test. In the second study, bivariate analysis of variance (ANOVA) was used to test for influence of strain versus treatment. The program package BiAS (Ackermann, 1996) was used. Data are expressed as means ±s.d. unless otherwise indicated.

Chemicals

(−)-Isoprenaline, (−)-noradrenaline, (±)-propranolol and IBMX were obtained from Sigma. GTP, GppNHp, and the components of the ATP-regenerating system were purchased from Boehringer Mannheim. (−)-[^{3}H]CGP-12177 (4-(3-t-butylamino-2-hydroxypropoxy)-[5,7-^{3}H]benzimidazol-2-one) was obtained from Amersham, ICI-118.551 (erythro[±]-1-[7-methylindan-4-yloxyl]-3-isopropylamino-butan-2-ol) was from Tocris Cookson. CGP-20712A (1-[2-(3-carbamoyl-4-hydroxyphenoxy)-ethylamino]-3-[4-(1-methyl-4-trifluormethyl-2-imidazolyl)-phenoxy]-propanol-methasulfonat) was donated by Ciba-Geigy, Basel.

Results

Study 1: cardiomyopathic versus control hamsters

β-Adrenoceptors In ventricular tissue from BIO TO2 hamsters, the density of total β-adrenoceptors was significantly higher than in BIO F1B controls, due to an increase in the β_2-subtype (Table 1). Relative proportions of β_1- and β_2-adrenoceptors were 74.3 and 25.7% in BIO F1B versus 65.9 and 34.1% in BIO TO2 myocardium, respectively. Due to marked interindividual variation in the myopathic group, strain-dependent differences in the relative proportions of β-

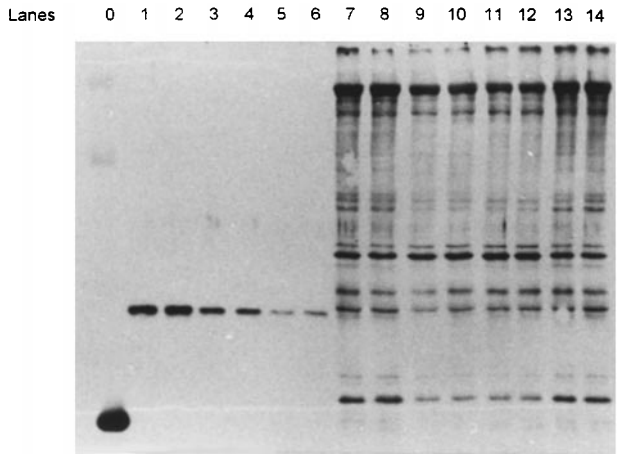

Figure 1 Immunoblot used for determination of $G_s\alpha$ in heart ventricles from BIO F1B and BIO TO2 hamsters. Lanes 1 – 6 represent three dilutions of G-protein standard, lanes 7, 8, 13 and 14 represent BIO F1B animals, lanes 9, 10, 11 and 12 are from BIO TO2 hamsters. Standards and tissue samples were assayed in duplicate. Please note that these blots are not corrected for protein concentration, which was higher in BIO F1B (2.8 and 3.1 mg ml^{-1}) than in BIO TO2 samples (1.4 and 1.6 mg ml^{-1}). Calculation of G-protein content per μg of membrane protein resulted in higher values in BIO TO2 than BIO F1B ventricles as shown in the manuscript. Lane 0 represents the molecular weight marker not specifically detected by the antibody.

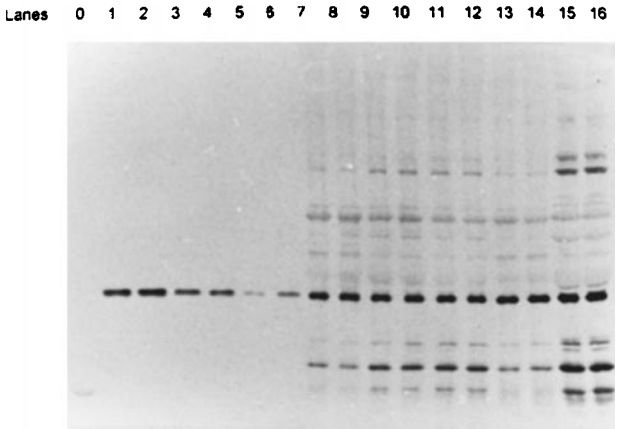

Figure 2 Immunoblot used for determination of $G_i\alpha$ in heart ventricles from BIO F1B and BIO TO2 hamsters. Lanes 1 – 6 represent three dilutions of G-protein standard, lanes 7, 8, 13 and 14 represent BIO TO2 animals, lanes 9, 10, 11 and 12 are from BIO F1B hamsters. Standards and tissue samples were assayed in duplicate. Please note that these blots are not corrected for protein concentration, which was higher in BIO F1B (2.1 and 1.3 mg ml^{-1}) than in BIO TO2 samples (0.5 and 1.2 mg ml^{-1}). Calculation of G-protein content per μg of membrane protein resulted in higher values in BIO TO2 than BIO F1B ventricles as shown in the manuscript. In lanes 15 and 16 an additional internal standard is shown, i.e. a membrane preparation from pooled cardiac tissue. Lane 0 represents the molecular weight marker not specifically detected by the antibody.

adrenoceptor subtypes did not achieve statistical significance (Table 1).

Adenylyl cyclase Under basal conditions, i.e. in the absence of any agonist, adenylyl cyclase activity was 46.8 ± 4.6 pmol mg^{-1} min^{-1} in BIO F1B and 35.7 ± 7.3 pmol mg^{-1} min^{-1} in BIO TO2 ventricles (*t*-test, $P<0.01$). GppNHp led to an increase in cyclic AMP formation (stimulated minus basal) by 217.4 ± 43.9 (BIO F1B) and 156.5 ± 62.8 pmol mg^{-1} min^{-1} (BIO TO2) being significantly higher (*t*-test, $P<0.05$) in the control myocardium. Activation of adenylyl cyclase by isoprenaline, representing total β-adrenergic stimulation, or noradrenaline in the presence of ICI-118.551, representing β_1-adrenergic stimulation, resulted in significantly lower rates of cyclic AMP formation in ventricular tissue from cardiomyopathic hamsters (Table 2). The efficacy of β_2-adrenoceptors, calculated as the difference between total and β_1-adrenergic stimulation, was unaffected in the diseased myocardium. Thus, the reduced β-adrenergic stimulation in BIO TO2 hamsters was due to a reduction in β_1-adrenoceptor function (Table 2). Consequently, the relative contribution of β_1-adrenoceptors to stimulation of adenylyl cyclase was $40.8 \pm 5.0\%$ in BIO F1B ventricles and only $17.6 \pm 9.2\%$ in cardiac tissue from BIO TO2, being significantly lower (*t*-test, $P<0.0001$) in the cardiomyopathic strain.

Cardiac G-protein content In BIO F1B-ventricles ($n=6$), the 52 kDa and 45 kDa forms of $G_s\alpha$ amounted to 1.86 ± 0.25 and $2.95 \pm 0.46\%$ of standard per μg of membrane protein (% μg^{-1}). In myopathic hamster hearts ($n=5$), the tissue content of 52 kDa $G_s\alpha$ was $3.16 \pm 0.39\%$ μg^{-1}, being significantly higher (*t*-test, $P<0.0001$) than in BIO F1B-controls, while the 45 kDa form amounting to $3.24 \pm 0.71\%$ μg^{-1} was slightly but not significantly higher in cardiomyopathic tissue. Membrane content of $G_i\alpha$ was markedly and significantly (*t*-test, $P<0.01$) higher in BIO TO2 ($3.75 \pm 0.62\%$ μg^{-1}) than in BIO F1B myocardium ($2.51 \pm 0.31\%$ μg^{-1}).

Study 2: effects of propranolol treatment

During the control period prior to treatment, the average daily water intake was 48.6 ± 1.9 ml kg^{-1} d^{-1} in BIO TO2 hamsters and 56.9 ± 1.9 ml kg^{-1} d^{-1} in BIO F1B controls. In the treatment period, myopathic hamsters ingested 31.9 mg kg^{-1} d^{-1} of propranolol, while BIO F1B hamsters received 38.4 ± 2.7 mg kg^{-1} d^{-1} due to the greater water intake. BIO TO2 hamsters showed lower absolute and relative (divided by body weight) ventricular weight of 349 ± 11 mg and 2.78 ± 0.10 mg g^{-1}, respectively, than control animals with 456 ± 40 mg and 2.91 ± 0.15 mg g^{-1}. Treatment of hamsters resulted in slightly reduced ventricular as well as body weight, leaving the ventricular:body weight ratio unaffected (2.76 ± 0.10 in treated BIO TO2 and 2.99 ± 0.16 in treated BIO F1B hamsters).

β-Adrenoceptors Strain-dependent differences in β-adrenoceptor subtype density could be reproduced in the second

Table 1 Density and subtype distribution of myocardial β-adrenoceptors in untreated and propranolol-treated (PROP) healthy BIO F1B- and myopathic BIO TO2 hamsters aged 270 days

Strain	*PROP* (mg kg^{-1} d^{-1})	(n)	*Total β-AR* (fmol mg^{-1})	*β_1-AR* (fmol mg^{-1})	*β_2-AR* (fmol mg^{-1})	*β_1-AR* (%)	*β_2-AR* (%)
BIO F1B	—	(7)	23.3 ± 6.7	17.3 ± 5.1	6.0 ± 2.0	74.3 ± 4.7	25.7 ± 4.7
BIO TO2	—	(8)	30.7 ± 3.2	20.0 ± 2.3	10.7 ± 4.3	65.9 ± 10.9	34.1 ± 10.9
t-test			$P<0.05$	n.s.	$P<0.05$	n.s.	n.s.
BIO F1B	—	(6)	24.4 ± 3.7	18.4 ± 3.4	6.0 ± 0.7	74.9 ± 4.0	25.1 ± 4.0
BIO F1B	40	(6)	33.8 ± 4.6*	28.2 ± 4.6*	5.6 ± 0.8	83.2 ± 3.3*	16.8 ± 3.3*
BIO TO2	—	(6)	29.0 ± 2.5	19.5 ± 2.4	9.5 ± 0.8	66.9 ± 3.8	33.1 ± 3.8
BIO TO2	40	(6)	35.9 ± 4.4*	25.6 ± 4.3*	10.2 ± 2.0	71.2 ± 6.2	28.8 ± 6.2
ANOVA (strain)			$P<0.05$	n.s.	$P<0.001$	$P<0.001$	$P<0.001$
ANOVA (treatment)			$P<0.001$	$P<0.001$	n.s.	$P<0.01$	$P<0.01$

Means ± s.d., ANOVA = bivariate analysis of variance, *$P<0.05$ (Student's *t*-test, untreated versus PROP), AR = adrenoceptor. Data are taken from two independent experimental series: in the second study, groups of hamsters received PROP in drinking water from 240–270 days of age.

Table 2 Analysis of concentration-response curves for isoprenaline and noradrenaline in untreated and propranolol-treated healthy BIO F1B- and myopathic BIO TO2 hamsters aged 270 days

			Stimulation of adenylyl cyclase				
Strain	*PROP* (mg kg^{-1} d^{-1})	(n)	Via *total β-AR* (pmol mg^{-1} min^{-1})	Via *β_1-AR* (pmol mg^{-1} min^{-1})	Via *β_2-AR* (pmol mg^{-1} min^{-1})	Via *β_1-AR* (%)	Via *β_2-AR* (%)
BIO F1B	—	(8)	152.7 ± 22.8	61.7 ± 7.9	91.1 ± 20.1	40.8 ± 5.0	59.2 ± 5.0
BIO TO2	—	(8)	92.4 ± 26.8	15.8 ± 7.7	76.6 ± 24.7	17.6 ± 9.2	82.4 ± 5.0
t-test			$P<0.001$	$P<0.0001$	n.s.	$P<0.0001$	$P<0.0001$
BIO F1B	—	(6)	133.4 ± 19.0	53.8 ± 12.3	79.6 ± 14.8	40.3 ± 7.1	59.7 ± 7.1
BIO F1B	40	(6)	112.6 ± 27.5	63.4 ± 12.0	49.2 ± 16.5*	57.0 ± 4.4*	43.0 ± 4.4*
BIO TO2	—	(6)	103.0 ± 12.7	15.4 ± 4.6	87.6 ± 14.2	15.3 ± 5.4	84.7 ± 5.4
BIO TO2	40	(6)	72.6 ± 23.2*	21.7 ± 6.9	50.9 ± 22.3*	31.1 ± 11.4*	68.9 ± 11.4*
ANOVA (strain)			$P<0.01$	$P<0.01$	n.s.	$P<0.01$	$P<0.01$
ANOVA (treatment)			$P<0.01$	n.s.	$P<0.01$	$P<0.01$	$P<0.01$

Means ± s.d., ANOVA = bivariate analysis of variance, *$P<0.05$ (Student's *t*-test, untreated versus PROP), AR = adrenoceptor. Total β-adrenergic stimulation was evaluated by addition of isoprenaline, β_1-adrenergic effects by noradrenaline in the presence of the β_2-adrenoceptor antagonist ICI-118.551, and β_2-adrenergic stimulation was calculated as the difference between them. Data from two independent experimental series: in the second study,groups of hamsters received PROP in drinking water from 240–270 days of age. The corresponding concentration-response curves for untreated and propranolol-treated groups are shown in Figure 4.

study: total β-adrenoceptor density was greater in untreated BIO TO2 than in BIO F1B controls due to a significantly higher density of the β_2-subtype (Table 1). Consequently, relative proportions of β_1- and β_2-adrenoceptors confirmed a shift towards the β_2-subtype in tissue from myopathic hamsters which, in the second study, achieved statistical significance.

Treatment of control BIO F1B and myopathic BIO TO2 hamsters with propranolol resulted in a significant increase in myocardial β-adrenoceptor density. The effect of propranolol was restricted to the β_1-subtype, whereas β_2-adrenoceptors were not affected (Figure 3).

The affinity of β-adrenoceptors towards the antagonist ligand [^{3}H]CGP-12177, expressed as the pK_D-value, was significantly higher in BIO TO2 ventricles than in BIO F1B controls, and was slightly reduced in both propranolol-treated groups (Table 3). Displacement curves using CGP-20712A revealed no effects of propranolol on the affinities, i.e. pK_i-values, of β_1- and β_2-adrenoceptors, but confirmed a slightly greater affinity of β-adrenoceptors in myopathic hamsters towards an antagonist ligand (Table 3).

Adenylyl cyclase Basal adenylyl cyclase activity was significantly higher in BIO F1B controls (untreated: 31.2±8.4, PROP: 32.8±2.0 pmol mg^{-1} min^{-1}) than in myopathic BIO TO2 hamsters (untreated: 25.6±4.1, PROP: 24.3±5.9 pmol mg^{-1} min^{-1}), but was not affected by propranolol treatment (bivariate ANOVA, influence of strain $P<0.01$, treatment n.s.). Stimulation of β-adrenoceptors by isoprenaline was significantly less effective in BIO TO2 than in BIO F1B ventricles, which was due to a smaller β_1-adrenergic effect in myopathic tissue (Table 2). Treatment with propranolol did not affect the functional efficacy of the β_1-subtype, either in BIO F1B or in BIO TO2 hamsters (Figure 4). In contrast, β_2-adrenergic stimulation of adenylyl cyclase did not differ between untreated BIO F1B and BIO TO2 ventricles, but was significantly decreased in both propranolol-treated groups (Table 2).

Desensitized β_1-adrenoceptors in BIO TO2 ventricles as well as the paradoxical reduction in β_2-adrenergic efficacy in propranolol-treated hamsters of both strains could be confirmed in an additional experimental approach, in which

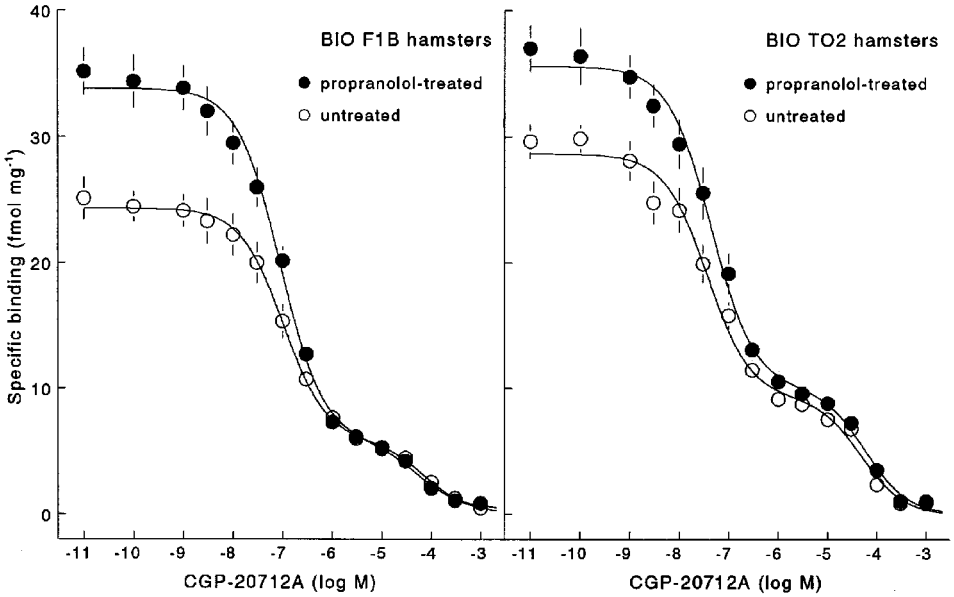

Figure 3 Subtype distribution of cardiac β-adrenoceptors in untreated and propranolol-treated control BIO F1B and myopathic BIO TO2 hamsters. Specific binding to β-adrenoceptor subtypes was determined by competition of the β_1-selective β-adrenoceptor antagonist CGP-20712A with the non-selective β-adrenoceptor antagonist [^{3}H]CGP-12177 (2 nM). Density of the β_2-subtype was significantly greater in BIO TO2 ventricles. Four weeks treatment with propranolol resulted in a significant increase in cardiac β_1-adrenoceptors in both strains. Means±s.e.mean of six ventricles per group.

Table 3 Receptor affinities and agonist potencies at β-adrenoceptor subtypes in untreated and propranolol-treated healthy BIO F1B- and myopathic BIO TO2 hamsters aged 270 days

			Receptor binding studies			*Stimulation of adenylyl cyclase*	
Strain	*PROP* (mg kg^{-1} d^{-1})	(n)	*pK_D [^{3}H]CGP-12177*	*pK_i-β_1 CGP-20712A*	*pK_i-β_2 CGP-20712A*	*pEC_{50} isoprenaline*	*pEC_{50} noradrenaline*
BIO F1B	—	(6)	9.08±0.12	7.61±0.22	4.74±0.34	6.52±0.19	5.58±0.29
BIO F1B	40	(6)	8.91±0.09	7.48±0.12	4.62±0.41	6.65±0.17	5.67±0.19
BIO TO2	—	(6)	9.42±0.17	8.38±0.42	5.20±0.10	6.36±0.27	5.46±0.16
BIO TO2	40	(6)	9.19±0.12	8.06±0.27	4.90±0.14	6.16±0.26	5.76±0.29
ANOVA (strain)			$P<0.01$	$P<0.01$	$P<0.01$	$P<0.01$	n.s.
ANOVA (treatment)			$P<0.01$	n.s.	n.s.	n.s.	n.s.

Means±s.d., ANOVA=bivariate analysis of variance, *$P<0.05$ (Student's *t*-test, untreated versus PROP), AR=adrenoceptor, pK_D, pK_i and pEC_{50} values are negative decadic logarithms of the respective parameters. Data from the second study: groups of hamsters received PROP in drinking water from 240–270 days of age.

isoprenaline was applied with or without addition of subtype-selective β-adrenoceptor antagonists (Figure 5).

Receptor-independent stimulation of cyclic AMP formation by GppNHp was not affected by treatment with propranolol, but differed between control and myopathic hamsters (bivariate ANOVA, strain $P<0.05$, treatment n.s.). Net increases in adenylyl cyclase activity by GppNHp 100 μM amounted to 176.7±35.3 (untreated) and

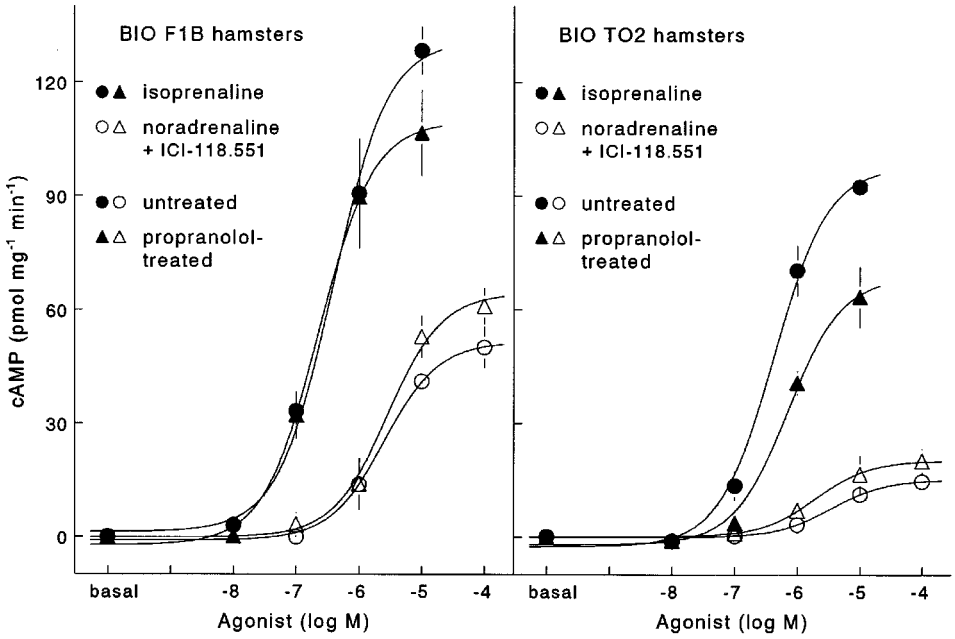

Figure 4 Stimulation of adenylyl cyclase by isoprenaline and noradrenaline in the presence of ICI-118.551 in ventricular tissue from control BIO F1B and myopathic BIO TO2 hamsters. From 240 – 270 days of age, groups of hamsters received either drinking water or propranolol in drinking water. The efficacy of both isoprenaline and noradrenaline was significantly reduced in BIO TO2 myocardium. Propranolol treated hamsters showed a reduced response towards isoprenaline, whereas the effects of β_1-adrenergic stimulation by noradrenaline were not affected. Means±s.e.mean of six ventricles per group. Data derived from individual concentration-response curves are summarized in Table 2.

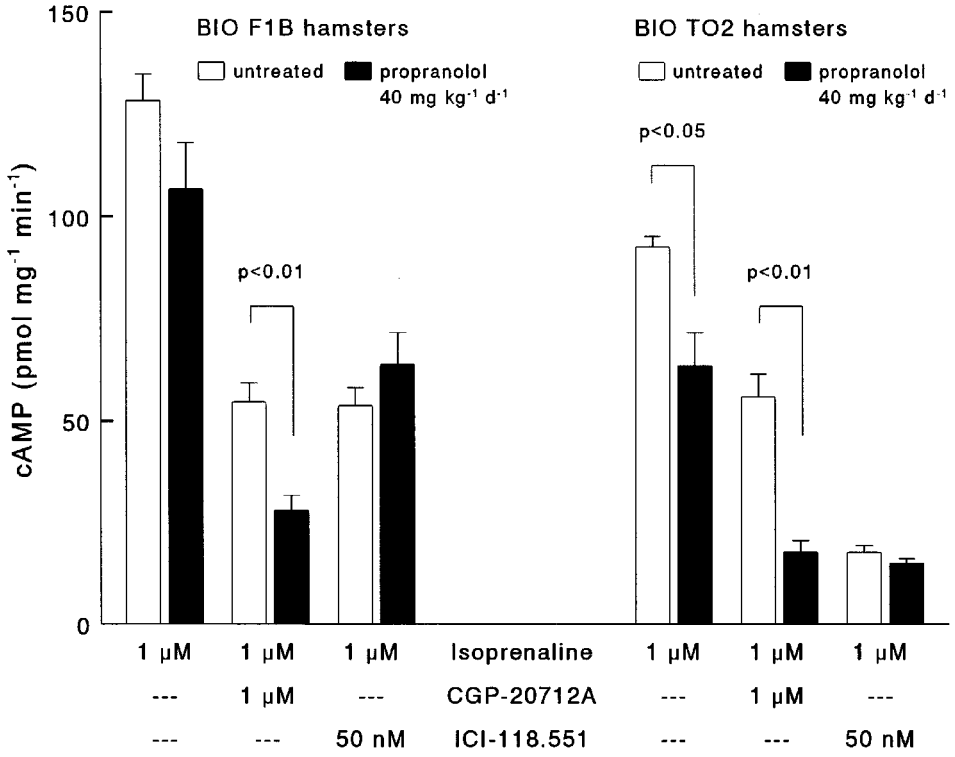

Figure 5 Net increase in cardiac cyclic AMP formation by isoprenaline in the absence or presence of the β_1- and β_2-selective β-adrenoceptor antagonists CGP-20712A and ICI-118.551, respectively. From 240 – 270 days of age, groups of control BIO F1B and myopathic BIO TO2 hamsters received either drinking water or propranolol in drinking water. In both strains, propranolol treatment induced a significant reduction in β_2-adrenergic stimulation (isoprenaline + CGP-20712A), whereas β_1-adrenergic effects (isoprenaline + ICI-118.551) were unchanged. Means±s.e.mean of six ventricles per group.

161.2±47.9 pmol mg^{-1} min^{-1} (PROP) in BIO F1B ventricles, and to 138.4±23.3 (untreated) and 134.0±39.0 pmol mg^{-1} min^{-1} (PROP) in tissue from BIO TO2 hamsters.

Cardiac G-protein content As observed in the first study, the membrane content of both $G_s\alpha$ and $G_i\alpha$ was significantly greater in myopathic than in control ventricles (Figure 6). Treatment of BIO F1B and BIO TO2 hamsters with propranolol had no significant effect on the amount of G-proteins (bivariate ANOVA, strain $P<0.05$, treatment n.s.).

Discussion

In two independent experimental series we observed that, in cardiac tissue from control BIO F1B hamsters, β_1-adrenoceptors were less efficiently coupled to adenylyl cyclase stimulation than the β_2-subtype. The lower coupling efficiency of the β_1-adrenoceptors in BIO F1B hamster ventricles is in agreement with our previous observation in another strain of Syrian hamsters without cardiac disease (Witte *et al*., 1995) and gives further support to the hypothesis of inherent differences in receptor-effector coupling of β-adrenoceptor subtypes (Kaumann *et al*., 1989; Green *et al*., 1992; Levy *et al*., 1993; Rousseau *et al*., 1996). In cardiomyopathic BIO TO2 hamsters, coupling of cardiac β_1-adrenoceptors to adenylyl cyclase was further reduced resulting in a functional contribution to total β-adrenergic stimulation of less than 20%. In contrast, β_1-adrenoceptor density did not differ between control and myopathic hamsters, while β_2-adrenoceptors and G-proteins were increased in cardiomyopathic tissue.

In 100-day-old BIO TO2 hamsters Feldman *et al*. (1990) observed a decrease in isoprenaline-mediated cardiac cyclic AMP formation, resulting from a disturbed function of the G_s-protein. In our 270-day-old animals, a reduced stimulation of adenylyl cyclase by GppNHp in cardiomyopathic hamster hearts could be confirmed despite increased levels in $G_s\alpha$, giving further support to a functional abnormality of the G_s-protein in BIO TO2 hamsters. However, subtype-specific disturbancies in β-adrenergic function cannot exclusively be due to a defective G_s-protein, because both β-adrenoceptor subtypes are coupled to the same signal transduction pathway. Phosphorylation of β-adrenoceptors by β-adrenergic receptor kinase (βARK) is known to functionally uncouple the receptor from G-proteins (Lohse *et al*., 1996) and may, therefore, be involved in the disturbed β_1-adrenergic function in BIO TO2 ventricles. Urasawa *et al*. (1996) were able to demonstrate the expression of βARK1 mRNA in cardiac tissue from BIO F1B and BIO TO2 hamsters, being 2 fold higher in the cardiomyopathic strain. A comparable increase in βARK mRNA has also been found in human myocardium from patients with severe heart failure (Ungerer *et al*., 1993). Our observation of desensitized β_1-adrenoceptors in BIO TO2 ventricles could, therefore, result from an increased receptor phosphorylation by βARK. Since β_2-adrenoceptors are equally or even more susceptible to βARK mediated desensitization than the β_1-subtype, the exclusive uncoupling of β_1-adrenoceptors in BIO TO2 myocardium may reflect the increased level and turnover of the rather β_1-selective agonist noradrenaline described to occur in diseased hamsters (Sole *et al*., 1975; Yamada *et al*., 1997).

The increase in cardiac $G_i\alpha$ in cardiomyopathic animals is in agreement with data observed in human heart failure (Bristow, 1997), and could contribute to the reduced basal and stimulated cyclic AMP formation. However, the greater membrane content of $G_s\alpha$ in BIO TO2 ventricles has no

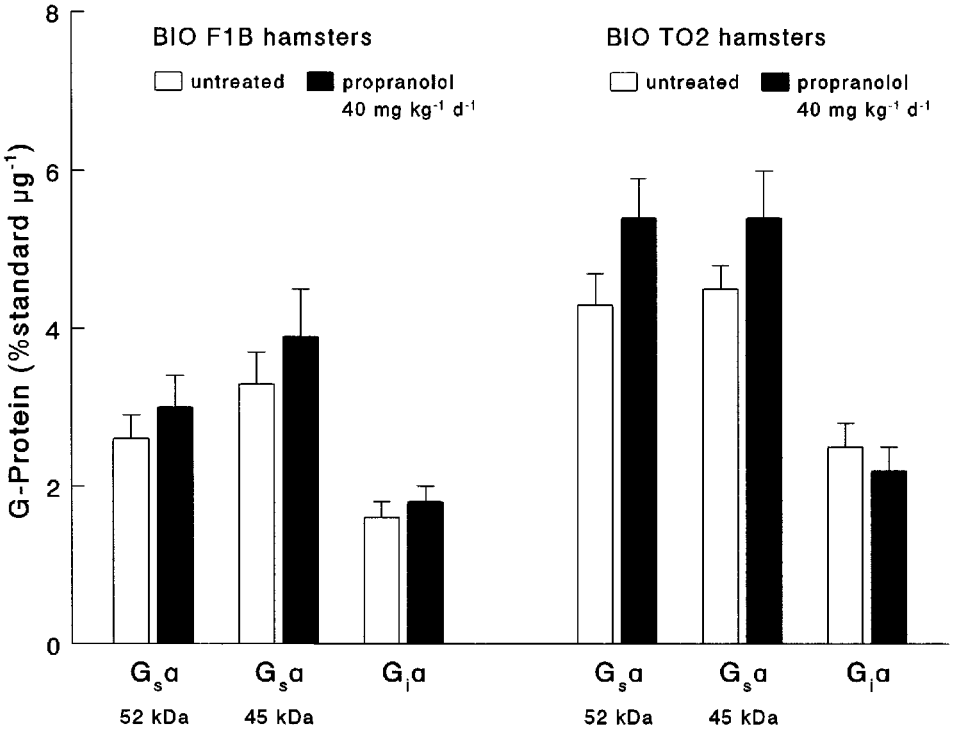

Figure 6 Content of $G_s\alpha$ and $G_i\alpha$ in myocardial membranes from untreated and propranolol-treated control BIO F1B and myopathic BIO TO2 hamsters. G-proteins were quantified by immunoblotting and transmission densitometry and expressed as percent of G-protein standard per µg of membrane protein. BIO TO2 ventricles showed higher concentrations of stimulatory and inhibitory G-proteins. Treatment with propranolol had no significant effects on membrane G-protein content. (bivariate ANOVA, strain $P<0.05$, treatment n.s.). Means±s.e.mean of five to six ventricles per group.

human equivalent and needs to be discussed. It seems reasonable to speculate that the increased $G_s\alpha$ could represent an attempt of the diseased heart to restore β-adrenergic regulation of cyclic AMP formation. Unfortunately, no data are available in BIO TO2 hamsters concerning myocardial $G_s\alpha$-mRNA levels. However, in myocardium from 240-day-old UM-X7.1 hamsters, Kaura *et al*. (1996) observed reduced β-adrenergic and G-protein mediated stimulation of adenylyl cyclase accompanied by elevated $G_s\alpha$ mRNA and protein levels. These findings in hypertrophic cardiomyopathy are in line with the hypothesis of a compensatory up-regulation of $G_s\alpha$-protein in the failing hamster heart. It should, however, be noted that in another hamster model of hypertrophic cardiomyopathy, BIO 14.6, cardiac $G_s\alpha$ was found to be unchanged (Cai *et al*., 1993) or even decreased (Nakamura *et al*., 1996). In the light of these discrepant findings it is of interest that all myopathic hamster strains are derived from a single original line, i.e. the BIO 1.5 hamster, and share the same causative gene defect (Sakamoto *et al*., 1997). However, in BIO TO2 hamsters the compensatory cardiac hypertrophy of the other strains does not occur, leading to primary dilation of the ventricles (Sakamoto *et al*., 1997). This may result from an additional genetic defect in the BIO TO2 strain and lead to different hemodynamic alterations than in BIO 14.6, CHF 146 and UM-X7.1 hamsters. Which of these factors, an additional gene defect versus different hemodynamics, is responsible for the differences in cardiac β-adrenergic signaling cannot be answered conclusively.

Four weeks treatment of BIO F1B and BIO TO2 hamsters with propranolol resulted in an up-regulation of β_1-adrenoceptors but could not restore their disturbed function in BIO TO2 ventricles. The increase in β-adrenoceptor density by propranolol treatment confirms previous observations by Yamada *et al*. (1997), who observed a 30% increase in binding of [^{125}I]cyanopindolol in myocardial homogenates from BIO 53.58 (=TO2) hamsters treated with metoprolol 1, 10 or 50 mg kg^{-1} d^{-1} for 7 weeks. Unfortunately, the authors did not include functional parameters in their study and did not differentiate between β_1- and β_2-subtypes. Thus, the opposite effects of *in vivo* treatment with propranolol in the present study, i.e. up-regulation of cardiac β_1-adrenoceptors and functional uncoupling of β_2-adrenoceptors from adenylyl cyclase, cannot easily be explained. A contamination of the membrane preparation with propranolol is unlikely to play a role, because EC_{50}-values of isoprenaline and noradrenaline did not differ between untreated and propranolol-treated groups. It has been shown that stimulation of receptors coupled to activation of proteinkinase C results in heterologous desensitization of cardiac β-adrenoceptors (Schwartz & Naff, 1997), presumably by promoting the translocation of β ARK (Lohse *et al*., 1996). Since myopathic hamsters show an increased basal activity of cardiac proteinkinase C (Cai *et al*., 1993), increased the density of α_1-adrenergic (Karliner *et al*., 1981; Kagiya *et al*., 1991) and angiotensin AT_1-receptors (Lambert *et al*., 1995), such cross-regulation could contribute to functional uncoupling of β_2-adrenoceptors in propranolol treated hamsters. Treatment-induced changes in $G_i\alpha$ could not be found in the present study, but may have been obscured by the fact that the AS/7 antibody detects α-subunits of both G_{i1} and G_{i2}, but not of G_{i3} which is expressed at low level in hamster heart (Katoh *et al*., 1990). Thus, alterations restricted to G_{i3} would have been undetected by the antibody used.

In conclusion, the present study demonstrates that cardiomyopathic BIO TO2 hamsters show functional uncoupling of cardiac β_1-adrenoceptors, which could not be reversed by treatment with propranolol despite an increased myocardial density of the β_1-subtype. Moreover, propranolol treatment resulted in a decrease in β_2-adrenergic stimulation of adenylyl cyclase, which was neither due to a reduction in β_2-adrenoceptor density nor to changes in membrane content of G_s- and G_i-proteins. The final molecular mechanisms involved in these effects should be addressed in future studies.

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