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European Journal of Pharmacology 334 (1997) 209–216

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Effects of β-adrenoceptor blockade on β-adrenergic signal transduction in cardiomyopathic hamster (BIO 8262) hearts

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Received 1 May 1997; revised 1 July 1997; accepted 25 July 1997

Abstract

In myopathic BIO 8262-hamsters β_1-adrenergic stimulation of cardiac adenylyl cyclase has been found to be markedly reduced compared to that of healthy controls. In order to test the hypothesis that the functional uncoupling of β_1-adrenoceptors in diseased hamster hearts is due to agonist-dependent desensitization, we investigated the effects of prolonged treatment with β-adrenoceptor antagonists on cardiac β-adrenergic signaling. Groups of hamsters aged 240 days received either drinking water, or drinking water containing metoprolol (10 or 100 mg/kg/day) or propranolol (4 or 40 mg/kg/day). After 4 weeks' treatment animals were killed and heart ventricles were prepared for determination of β_1- and β_2-adrenoceptor densities and their functional contribution to stimulation of adenylyl cyclase. Markers of myocardial hypertrophy, i.e. absolute and relative ventricular weight and 5-nucleotidase activity, were not affected by the different treatment regimens. Neither absolute densities nor relative proportions of β-adrenoceptor subtypes differed between untreated and treated hamster groups. Metoprolol had no effects on the functional efficacy of β_1- and β_2-adrenoceptors. Hamsters treated with high dose propranolol showed unchanged β_1-adrenoceptor function but reduced β_2-adrenergic stimulation of adenylyl cyclase. The findings of the present study demonstrate that the disturbed coupling of cardiac β_1-adrenoceptors to adenylyl cyclase cannot be reversed by in vivo treatment with β-adrenoceptor antagonists and, therefore, is unlikely to be due to agonist-dependent desensitization.

Keywords: Cardiomyopathy; β-Adrenoceptor subtypes; Coupling efficacy; Adenylyl cyclase; (Hamster); Propranolol; Metoprolol

1. Introduction

In previous studies we were able to demonstrate that, in healthy Syrian hamsters, cardiac β_1-adrenoceptors are less efficiently coupled to adenylyl cyclase than the β_2-subtype (Witte et al., 1993; Witte et al., 1995), which is comparable to findings in human heart tissue (Kaumann et al., 1989) and, probably, reflects inherent differences in subtype-specific coupling to the stimulatory G-protein. Therefore, the hamster could be a suitable animal model for studies of cardiac β-adrenergic signal transduction. In cardiomyopathic BIO 8262-hamsters aged 30 to 300 days, we observed a further uncoupling of β_1-adrenoceptors from adenylyl cyclase compared to healthy control animals, while the β_2-subtype was not affected by the myopathic disease. Similar changes involving β_1-adrenoceptor desensitization and down-regulation have been observed in ventricular tissue from patients with idiopathic dilated cardiomyopathy. The subtype-selective down-regulation of β_1-adrenoceptors in human heart failure is thought to result from an increased concentration and turnover of the endogenous neurotransmitter noradrenaline that affects predominantly the β_1-adrenoceptor subtype (for review see Brodde, 1993; Harding et al., 1994). In cardiomyopathic hamsters of different ages Sole et al. (1975) observed increases in the concentration, content, and turnover rate of noradrenaline compared to those of age-matched controls. However, in animals at the prenecrotic stage, aged 20 days, ventricular noradrenaline content and turnover in myopathic hearts did not differ from those of controls (Sole et al., 1975), indicating that the increase in sympathetic tone does not precede focal myocardial necrosis. In our previous study the functional coupling efficacy of β_1-adrenoceptors was reduced already in 30 day-old BIO

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8262-hamsters (Witte et al., 1995). It is, therefore, still unclear whether the disturbed β_1-adrenoceptor function in BIO 8262-hamster hearts is due to agonist-dependent desensitization or represents a specific defect in receptor–effector coupling in this animal model of heart failure. In order to address this question, we investigated the effects of treatment with the β_1-selective β-adrenoceptor antagonist metoprolol or the non-selective β-adrenoceptor antagonist propranolol on β-adrenergic signaling in diseased hamsters. If the disturbed function of β_1-adrenoceptors in BIO 8262-hamster hearts is due to increased noradrenaline turnover, it should be normalized by prolonged antagonist treatment.

2. Methods

2.1. Animals

Cardiomyopathic Syrian hamsters (BIO 8262, $n = 45$) were obtained from the ''Pharmakologisches Institut für Naturwissenschaftler'', J.W. Goethe-University, Frankfurt/Main, Germany. Hamsters were kept under constant environmental conditions with free access to food and tap water and a light–dark cycle of 12:12 h with lights on from 07.00–19.00 h. At the age of 240 days, hamsters were divided into treatment groups receiving either drinking water, metoprolol 10 or 100 mg/kg/day, or propranolol 4 or 40 mg/kg/day. Water intake was monitored by weighing the bottles 3 times per week prior to and during the whole treatment period. After 4 weeks treatment, groups of animals were killed by decapitation and heart ventricles were dissected quickly (within 1 min after decapitation), freed from fat and connective tissue, rinsed in ice-cold saline solution, dried on filter paper, immediately frozen in liquid nitrogen and stored at -60°C.

2.2. 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) at 20000 rpm. The resulting suspension was divided into 3 portions for determination of β-adrenoceptor density (suspension A), adenylyl cyclase activity (suspension B), and 5-nucleotidase activity (suspension C). Suspension A was homogenized a second time by sonication. After 10 min centrifugation at $25000 \times g$ the supernatants of suspensions A, B, and C were discarded. The pellet of suspension A was resuspended in 3 ml assay buffer and pellet B in 0.10 ml assay buffer per mg of tissue. Pellet C was resuspended in assay buffer (0.10 ml per mg of tissue) containing 0.3% Triton-X100 for solubilization of membrane-bound 5-nucleotidase; after a second centrifugation (10 min, $25000 \times g$) the resulting supernatant was used for photometric determination of 5-nucleotidase activity.

2.3. Determination of β-adrenoceptor densities

Densities of cardiac β-adrenoceptor subtypes were determined in radioligand saturation and displacement studies (Fig. 1), using the non-selective β-adrenoceptor antagonist [^{3}H](−)-CGP-12177 (saturation) and the β_1-selective β-adrenoceptor antagonist CGP-20712A (competition) as described in detail (Witte et al., 1995).

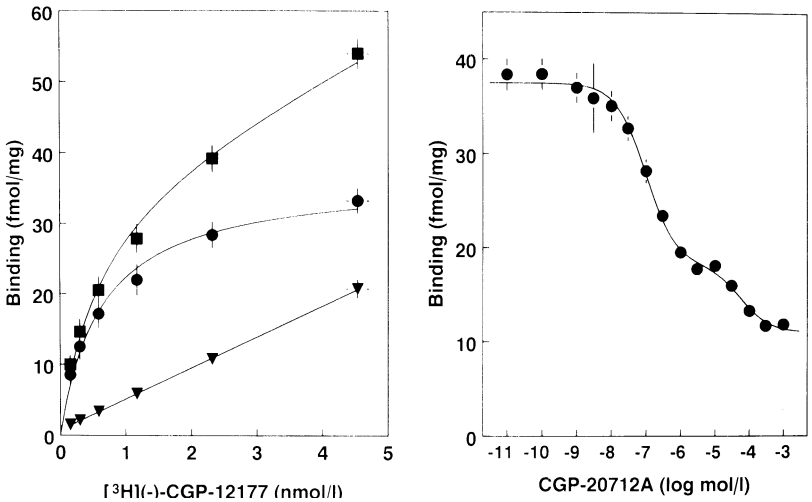

Fig. 1. Radioligand saturation (left graph) and competition experiments (right graph) in ventricular tissue from untreated 270 day-old BIO 8262-hamsters ($n = 14$, means ± S.E.M.). The non-selective β-adrenoceptor antagonist [^{3}H](−)-CGP-12177 was used as radioligand, the highly β_1-selective β-adrenoceptor antagonist CGP-20712A as competitor. In the left graph, total (squares), non-specific (triangles) and specific binding (circles) are shown together with the respective functions fitted to the experimental data. In the right graph, the biphasic displacement of the radioligand (2 nM) by increasing concentrations of CGP-20712A demonstrates the presence of β_1- and β_2-adrenoceptors in a ratio of 70% β_1:30% β_2.

2.4. Adenylyl cyclase assay

The basal and stimulated formation of cAMP was determined in the presence of 3-isobutyl-1-methyl-xanthine (IBMX) and an ATP-regenerating system as described (Witte et al., 1995). The non-hydrolyzable GTP-analogue guanylyl–imidodiphosphate (GppNHp 100 μM) was used for stimulation via G-proteins and a water-soluble forskolin-derivative (forskolin-7-deacetyl-7-butyryl 100 μM) for direct activation of the adenylyl cyclase. Stimulation of adenylyl cyclase via β-adrenoceptor subtypes was determined by two different approaches, functional competition experiments and subtype-selective stimulation (Fig. 2). For functional competition experiments adenylyl cyclase activity was determined in the presence of a fixed concentration of isoprenaline (1 μM) with increasing concentrations of the β_1-adrenoceptor antagonist CGP-20712A (100 pM–100 μM). For subtype-selective stimulation concentration–response curves were made by using the non-selective β-adrenoceptor agonist isoprenaline (10 nM–10 μM) and the rather β_1-selective β-adrenoceptor agonist noradrenaline (10 nM–10 μM) in the presence of ICI-118.551 (1 μM), a highly selective β_2-adrenoceptor antagonist. Both experimental approaches gave very similar results concerning the functional contribution of β-adrenoceptor subtypes. For reasons of clarity, only results from subtype-selective stimulation experiments are shown.

2.5. 5-Nucleotidase assay

The activity of solubilized 5-nucleotidase was measured photometrically, using a commercially available kit (No. 265-UV, Sigma, Deisenhofen).

2.6. Protein measurement

The protein content of the membrane fractions A and B was determined by the method of Lowry et al. (1951) with minor modifications and that of solubilized fraction C was measured using the Coomassie® Plus assay (Pierce, Oud-Beijerland). Bovine serum albumin was used as standard and was dissolved in the respective assay buffers.

2.7. Statistical analyses

Saturation curves for determination of maximal specific binding (B_{max}), competition curves for calculation of the β-adrenoceptor subtype distribution, and concentration–response curves for analysis of subtype-selective stimulation were fitted to the experimental data by using PHARMFIT (Mattes et al., 1991). Differences between treatment groups were tested by analysis of variance (ANOVA) followed by Scheffé's test for multiple pairwise comparisons. The program package BiAS (Ackermann, 1997) was used. Data are expressed as means ± S.D. unless otherwise indicated.

2.8. Chemicals

Isoprenaline, noradrenaline, IBMX, and the 5-nucleotidase kit were obtained from Sigma. Forskolin-7-deacetyl-7-butyryl was from Calbiochem. 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. CGP-20712A (1-[2-(3-carbamoyl-4-hydroxy-phenoxy)-ethylamino]-3-[4-(1-methyl-4-trifluormethyl-2-imidazo-lyl)-

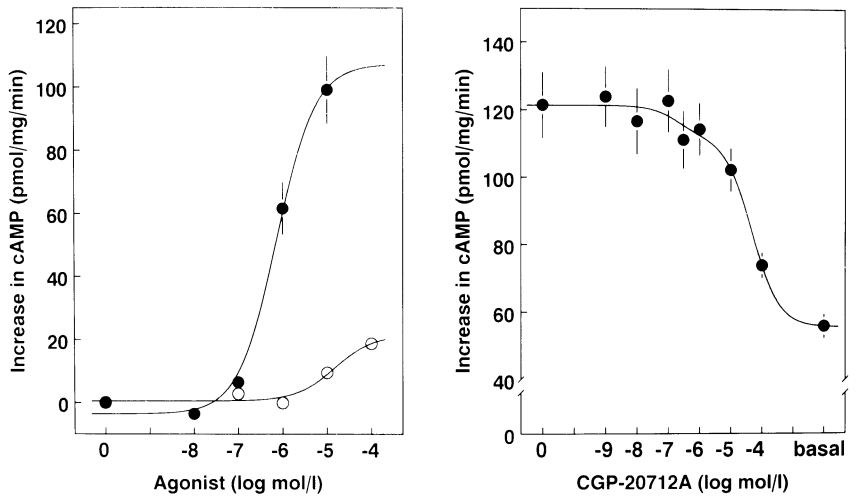

Fig. 2. Subtype-selective stimulation (left graph) and functional competition experiments (right graph) in ventricular tissue from untreated 270 day-old BIO 8262-hamsters (n = 11, means ± S.E.M.). In the left graph, concentration–response curves are shown for stimulation of adenylyl cyclase by isoprenaline (closed circles), representing β_1- and β_2-adrenergic stimulation, and noradrenaline in the presence of 1 μM ICI-118.551 (open circles), representing the exclusive β_1-adrenergic effect. In the right graph, the increase in cAMP by 1 μM isoprenaline is inhibited biphasically by the β_1-selective β-adrenoceptor antagonist CGP-20712A, demonstrating that 80% of the β-adrenergic stimulation depends on the β_2-subtype.

phenoxy]-propanol-methansulfonat) and ICI-118.551 (erythro[±]-1-[7-methylindan-4-yloxyl]-3-isopropylamino-butan-2-ol) were from Ciba-Geigy, Basel, and Tocris Cookson, Langford, respectively.

3. Results

Mean drug intake was 9.1 and 96.7 mg/kg/day in the metoprolol-treated hamsters and 3.6 and 34.4 mg/kg/day in hamsters receiving propranolol. During the treatment period, 9 hamsters died, none in the control group, 2 and 5 hamsters on low and high dose metoprolol and one hamster in each of the propranolol groups. Causes of death could not be studied, because dead hamsters were immediately eaten by their cage mates.

3.1. Markers of myocardial hypertrophy

Untreated BIO 8262-hamsters had a ventricular weight of 380 ± 48 mg, a relative ventricular weight (divided by body weight) of 3.6 ± 0.5 mg/g, and a 5-nucleotidase

Table 1
Effects of 4 weeks' treatment with β-adrenoceptor antagonists on parameters of myocardial hypertrophy

Drug	Dose	*n*	Body weight (g)	Ventricular weight (mg)	Ratio (mg/g)	5-ND (nmol/mg/min)	5-ND (μmol/min)
Untreated		14	106.1 ± 14.2	379.8 ± 47.8	3.6 ± 0.5	504.1 ± 115.4	5.3 ± 1.3
Metoprolol	10 mg/kg/day	5	102.4 ± 17.7	359.8 ± 14.3	3.6 ± 0.6	534.6 ± 136.5	4.9 ± 0.6
Metoprolol	100 mg/kg/day	4	116.0 ± 4.2	374.8 ± 15.6	3.2 ± 0.2	511.2 ± 16.5	5.1 ± 0.6
Propranolol	4 mg/kg/day	5	107.3 ± 10.5	364.0 ± 27.2	3.4 ± 0.3	604.2 ± 98.6	5.2 ± 0.3
Propranolol	40 mg/kg/day	8	93.4 ± 16.4	372.1 ± 40.2	4.1 ± 0.9	429.7 ± 81.5	4.6 ± 0.7
ANOVA			n.s.	n.s.	n.s.	($P < 0.10$)	n.s.

The activity of 5-nucleotidase (5-ND) was calculated per mg of protein (nmol/mg/min) and for the whole ventricle (μmol/min).
Means ± S.D.; Ratio = ventricular weight/body weight; ANOVA = analysis of variance.

Table 2
Effects of 4 weeks' treatment with β-adrenoceptor antagonists on β-adrenoceptor density in ventricular tissue from myopathic BIO 8262-hamsters

Drug	Dose	*n*	β-AR (fmol/mg)	β_1-AR (fmol/mg)	β_2-AR (fmol/mg)	β_1-AR (%)	β_2-AR (%)
Untreated		14	38.6 ± 8.0	27.2 ± 7.7	11.4 ± 3.3	69.6 ± 10.5	30.4 ± 10.5
Metoprolol	10 mg/kg/day	5	35.7 ± 5.2	22.7 ± 3.9	13.0 ± 5.1	64.1 ± 10.5	35.9 ± 10.5
Metoprolol	100 mg/kg/day	4	42.0 ± 6.3	32.8 ± 6.6	9.1 ± 1.0	77.8 ± 4.8	22.2 ± 4.8
Propranolol	4 mg/kg/day	5	39.1 ± 13.7	28.4 ± 10.6	0.7 ± 3.3	72.1 ± 3.2	27.9 ± 3.2
Propranolol	40 mg/kg/day	7	41.6 ± 5.7	31.6 ± 4.6	9.9 ± 5.1	76.7 ± 10.6	23.3 ± 10.6
ANOVA			n.s.	n.s.	n.s.	n.s.	n.s.

The density of β-AR was determined by radioligand binding studies. Proportions and densities of subtypes were calculated from competition curves by using the β_1-selective β-adrenoceptor antagonist CGP-20712A.
Means ± S.D., AR = adrenoceptors, ANOVA = analysis of variance.

Table 3
Effects of 4 weeks' treatment with β-adrenoceptor antagonists on β-adrenergic stimulation of adenylyl cyclase in ventricular tissue from myopathic BIO 8262-hamsters

Drug	Dose	*n*	Effect of β-adrenergic stimulation on cAMP formation				
			via all β-AR (pmol/mg/min)	via β_1-AR (pmol/mg/min)	via β_2-AR (pmol/mg/min)	via β_1-AR (%)	via β_2-AR (%)
Untreated		11	113.5 ± 37.5	21.3 ± 11.8	92.2 ± 37.4	20.5 ± 11.6	79.5 ± 11.6
Metoprolol	10 mg/kg/day	4	117.3 ± 30.1	16.6 ± 11.7	100.7 ± 20.1	12.9 ± 8.7	87.1 ± 8.7
Metoprolol	100 mg/kg/day	3	99.5 ± 10.6	19.5 ± 3.5	80.0 ± 14.0	20.0 ± 5.4	80.0 ± 5.4
Propranolol	4 mg/kg/day	5	92.5 ± 19.1	18.6 ± 6.4	73.9 ± 18.9	20.8 ± 9.6	79.2 ± 9.6
Propranolol	40 mg/kg/day	8	56.2 ± 19.4[b]	18.2 ± 9.8	38.0 ± 11.0[b]	30.8 ± 8.3	69.2 ± 8.3
ANOVA			$P < 0.01$	n.s.	$P < 0.01$	($P < 0.10$)	($P < 0.10$)

The adenylyl cyclase was stimulated by isoprenaline (via β_1- and β_2-AR) and by noradrenaline + ICI-118.551 (via β_1-AR); β_2-adrenergic stimulation was calculated as the difference between the effects of isoprenaline and noradrenaline.
Means ± S.D., AR = adrenoceptors, ANOVA = analysis of variance.
[a] $P < 0.05$, [b] $P < 0.01$, treated versus untreated.

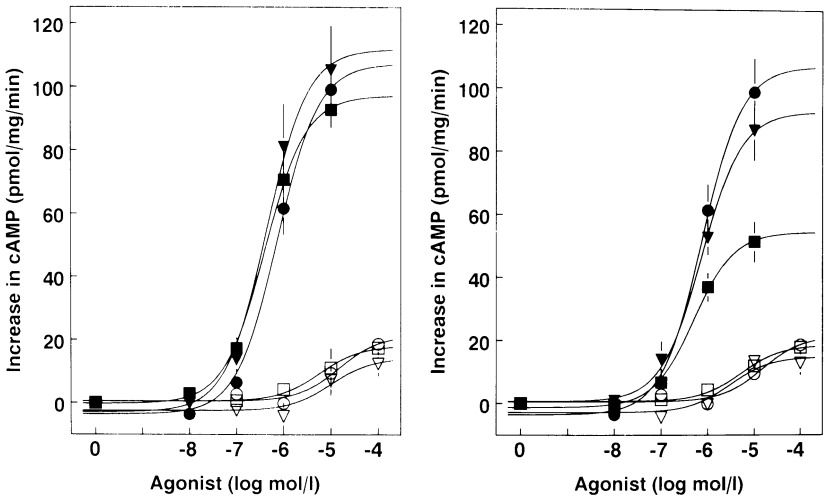

Fig. 3. β-Adrenergic stimulation of cAMP formation in ventricular tissue from untreated (both graphs: circles), metoprolol- (left graph: 10 mg/kg/day, triangles; 100 mg/kg/day, squares) and propranolol-treated (right graph: 4 mg/kg/day, triangles; 40 mg/kg/day, squares) myopathic BIO 8262-hamsters. Adenylyl cyclase was stimulated via β_1- and β_2-adrenoceptors by isoprenaline (closed symbols) and exclusively via the β_1-subtype by noradrenaline in the presence of 1 μM ICI-118.551 (open symbols). Hamsters treated with propranolol 40 mg/kg/day showed a significant reduction in the isoprenaline mediated effect, which was due to a decrease in β_2-adrenergic efficacy, because the response to noradrenaline was not affected by propranolol treatment.

activity of 504 ± 115 nmol/mg/min. None of these parameters differed significantly between treated and untreated animals (Table 1).

3.2. Receptor binding

In untreated myopathic hamsters cardiac β-adrenoceptor density was 38.6 ± 8.0 fmol/mg, with a ratio β_1-:β_2-adrenoceptors of 70%:30% (Fig. 1). Absolute and relative densities of β-adrenoceptor subtypes were not affected by treatment with metoprolol and propranolol (Table 2).

3.3. Adenylyl cyclase

In untreated BIO 8262-hamsters, stimulation of adenylyl cyclase by isoprenaline resulted in an increase in ventricular cAMP formation by 113.5 ± 37.5 pmol/mg/min. Only 20% of this β-adrenergic stimulation could be achieved by selective stimulation of β_1-adrenoceptors (Table 3, Fig. 2). Treatment of hamsters with metoprolol and with low dose propranolol had no effect on β-adrenergic stimulation of adenylyl cyclase. In contrast, prolonged application of propranolol 40 mg/kg/day resulted in a significantly lower functional efficacy of β_2-adrenoceptors, while β_1-adrenergic function was unchanged (Fig. 3). As a consequence, there was a trend towards greater contributions of the β_1-subtype to total β-adrenergic stimulation of adenylyl cyclase in propranolol-treated hamsters (Table 3).

The potencies of isoprenaline and noradrenaline (in the presence of ICI-118.551, 1 μM) did not differ between treatment groups. The pEC_{50}-values (negative decadic logarithm of the EC_{50}-value) of isoprenaline and noradrenaline were 6.1 ± 0.3 and 4.9 ± 0.4 in untreated hamsters. In metoprolol-treated groups, pEC_{50}-values of both agonists amounted to 6.3 ± 0.3 and 5.2 ± 0.7 (metoprolol 10 mg/kg/day), and 6.3 ± 0.3 and 5.4 ± 0.4 (metoprolol 100 mg/kg/day), respectively. In hamsters treated with pro-

Table 4
Effects of 4 weeks' treatment with β-adrenoceptor antagonists on receptor-independent stimulation of adenylyl cyclase in ventricular tissue from myopathic BIO 8262-hamsters

Drug	Dose	n	GppNHp 100 μM (pmol/mg/min)	Forskolin 100 μM (pmol/mg/min)	GppNHp 100 μM (-fold basal)	Forskolin 100 μM (-fold basal)
Untreated		12	157.7 ± 40.2	591.3 ± 160.3	5.5 ± 1.7	17.5 ± 5.4
Metoprolol	10 mg/kg/day	5	163.3 ± 35.4	432.2 ± 101.8	5.7 ± 1.3	13.3 ± 3.9
Metoprolol	100 mg/kg/day	3	137.7 ± 12.0	518.4 ± 56.4	6.3 ± 1.2	20.4 ± 1.6
Propranolol	4 mg/kg/day	5	171.2 ± 16.5	528.3 ± 125.1	5.6 ± 1.0	14.5 ± 1.4
Propranolol	40 mg/kg/day	8	150.1 ± 52.8	510.5 ± 193.4	6.1 ± 1.7	18.4 ± 6.5
ANOVA			n.s.	n.s.	n.s.	n.s.

Adenylyl cyclase activity was determined in the absence and presence of the different activators. Stimulation was calculated as net increase (pmol/mg/min) and as relative stimulation over basal values (-fold basal).
Means $\pm$ S.D., ANOVA = analysis of variance, n.s. = not significant.

pranolol, the following pEC_{50}-values of isoprenaline and noradrenaline were observed: 6.1 ± 0.1 and 5.0 ± 0.5 (propranolol 4 mg/kg/day), and 6.3 ± 0.2 and 5.4 ± 0.4 (propranolol 40 mg/kg/day).

3.4. Receptor-independent stimulation of adenylyl cyclase

In untreated myopathic hamsters, stimulation of cardiac adenylyl cyclase by GppNHp 100 μM (via G-proteins) and forskolin 100 μM (direct activation of adenylyl cyclase) increased cAMP formation by 157.7 ± 40.2 and 591.3 ± 160.3 pmol/mg/min, respectively. The magnitude of stimulation (divided by basal adenylyl cyclase activity) amounted to 5.5 ± 1.7-fold (GppNHp) and 17.5 ± 5.4-fold (forskolin). Neither absolute increases in cAMP formation nor relative magnitude of stimulation differed between untreated and treated hamsters (Table 4).

4. Discussion

The present study confirms earlier findings (Witte et al., 1993, 1995) that in myopathic BIO 8262-hamsters myocardial β_1-adrenoceptors are of minor functional importance with regard to stimulation of adenylyl cyclase. Almost 80% of total β-adrenergic stimulation of adenylyl cyclase was mediated via β_2-adrenoceptors although the β_2-subtype represented only 30% of the cardiac β-adrenoceptor population. The functional uncoupling of β_1-adrenoceptors could not be reversed by 4 weeks' treatment with metoprolol and propranolol in low and high doses, respectively. Prolonged application of propranolol 40 mg/kg/day resulted in a reduced functional efficacy of cardiac β_2-adrenoceptors without affecting the β_1-subtype.

In human dilated cardiomyopathy, selective down-regulation of β_1-adrenoceptors has repeatedly been shown, which is thought to result from increased tone of the endogenous agonist noradrenaline. 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; Bristow et al., 1994; CIBIS Investigators and Committees, 1994). It has been speculated that beneficial effects of β-adrenoceptor antagonists in heart failure could reflect an up-regulation and re-sensitization of cardiac β_1-adrenoceptors. Some studies have indeed shown an up-regulation of myocardial β-adrenoceptors in patients chronically treated with β-adrenoceptor antagonists (Michel et al., 1988; Heilbrunn et al., 1989), while others did not find differences in β-adrenoceptor densities between treated and untreated patients (Kaumann et al., 1995; Molenaar et al., 1997).

In hamsters with hypertrophic cardiomyopathy, myocardial β-adrenoceptor density has been reported to be either increased (Karliner et al., 1981), unchanged (Witte et al., 1995) or decreased (Kaura et al., 1996). Since in BIO 8262-hamsters treatment with β-adrenoceptor antagonists had no effect on the densities of cardiac β_1- and β_2-adrenoceptors, agonist-dependent down-regulation of myocardial β-adrenoceptors does not seem to play an important role in this animal model of heart failure. The inability of both β-adrenoceptor antagonists to improve the functional efficacy of β_1-adrenoceptors indicates that even the uncoupling of the β_1-subtype from adenylyl cyclase does not depend on sympathetic tone, but rather reflects a specific alteration of receptor–effector coupling in myopathic hamster hearts. This assumption is supported by our earlier finding that already in 30 day-old BIO 8262-hamsters, i.e. at the pre-necrotic stage of the disease (Gertz, 1972), cardiac β_1-adrenoceptors are partially uncoupled from their signal transduction cascade (Witte et al., 1995).

It may be argued that higher doses of β-adrenoceptor antagonists than those applied in the present study are required for effects on receptor density and coupling. However, constant infusion of propranolol in doses of 9.9 and 29.7 mg/kg/day in rats has been found to prevent isoprenaline-induced increases in Giα-mRNA (Eschenhagen et al., 1992) and Giα-gene transcription (Müller et al., 1994), respectively. Infusion of propranolol 13.2 mg/kg/day resulted in β-adrenoceptor up-regulation in myocardial tissue of mature rats (Conlon et al., 1995). Oral doses of 80 mg/kg/day of propranolol and atenolol were able to reduce myocardial hypertrophy in rats with aortic coarctation (Östman-Smith, 1995a) and experimental hypoxia (Östman-Smith, 1995b), respectively. Thus, a daily dose of 40 mg/kg/day of propranolol could be expected to influence cardiac β-adrenergic signal transduction. The 2.5 fold higher dose of metoprolol was selected because propranolol, due to its higher lipophilicity, shows a 2–3 fold greater accumulation in cardiac tissue than metoprolol (Lemmer et al., 1985). The lower doses of propranolol and metoprolol were used to test the dose-dependence of the drug effects.

Interestingly, treatment with a high dose of metoprolol resulted in an increased mortality among myopathic hamsters, which cannot easily be explained. Sensitization of β_2-adrenoceptors has been observed in patients treated with β_1-selective β-adrenoceptor antagonists (Hall et al., 1990), which could increase the risk of β_2-adrenoceptor-mediated cardiac arrhythmias (Kaumann and Sanders, 1993). Since the present study with hamsters did not find there to be sensitized β_2-adrenoceptors in the metoprolol-treated group, other mechanisms are more likely to be involved in the increased mortality. The strain of myopathic hamsters used (BIO 8262) is known to develop atrioventricular and left bundle branch conduction defects (Lossnitzer et al., 1977), and metoprolol could have further impaired atrioventricular conduction, finally leading to cardiac arrest. However, in the absence of electrophysiological data for metoprolol-treated hamsters this hypothesis remains speculative.

Our observation that treatment with propranolol reduced

the functional efficacy of cardiac β_2-adrenoceptors cannot be explained at present. A contamination of the membrane preparation with relevant amounts of the highly lipophilic propranolol is unlikely to play a role, because the potencies of isoprenaline and noradrenaline did not differ between untreated and propranolol-treated hamsters. In order to clarify the paradoxical effects of propranolol on β_2-adrenergic coupling efficacy, further studies with myopathic BIO 8262-hamsters are needed that focus on treatment-dependent changes in other components of the β-adrenergic signal transduction cascade.

In conclusion, the present study confirms a disturbed functional coupling of cardiac β_1-adrenoceptors in myopathic hamsters, which could not be reversed by in vivo treatment with β-adrenoceptor antagonists and, therefore, does not seem to result from agonist-dependent desensitization.

Acknowledgements

The authors are grateful to Professor Dr. E. Mutschler, Pharmakologisches Institut für Naturwissenschaftler, Frankfurt, for supplying the animals.

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