Time course and extent of α_1 -adrenoceptor density changes in rat heart after β -adrenoceptor blockade

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1 It has been suggested that impaired β -adrenoceptor stimulation is a condition under which the functional role of cardiac α_1 -adrenoceptors is enhanced. We therefore investigated the extent and time course of changes in α_1 -adrenoceptor characteristics after chronic treatment with the β -adrenoceptor blocker propranolol in rat heart. For comparison β -adrenoceptors were also studied. The mechanism of the changes in adrenoceptor density was investigated with cycloheximide, an inhibitor of protein synthesis. The functional significance of an increased α_1 -adrenoceptor density was tested by measuring isometric force of contraction in the presence of phenylephrine or isoprenaline in right ventricular papillary muscles.

2 Rats were treated with propranolol (9.9 mg kg^{-1} daily) or 0.9% NaCl, applied with osmotic minipumps for 1, 2, 3 or 7 days. Propranolol treatment resulted in a maximally 28% increase of α_1 -adrenoceptor density after 3 days (NaCl 95.9 ± 3.5 vs. propranolol $123.0 \pm 1.6 \text{ fmol mg}^{-1}$ protein, $n = 6$, $P < 0.01$). This up regulation reached significant levels after 2 days of treatment and was reversible after cessation of treatment within two days. K_D -values were the same for NaCl- and propranolol-treated rats. Changes of B_{max} and K_D in β -adrenoceptor binding assays did not reach significant levels.

3 Cycloheximide (1.5 mg kg^{-1} i.p. daily for 3 days) inhibited the propranolol-induced increase in B_{max} of α_1 -adrenoceptors completely. In addition, cycloheximide also decreased the density of α_1 - and β -adrenoceptors also under control conditions.

4 pD_2 -values for the positive inotropic effect of phenylephrine and isoprenaline in isolated electrically driven papillary muscle were similar in NaCl- and propranolol-treated rats (phenylephrine: 5.41 ± 0.11 vs. 5.41 ± 0.19 , $n = 7$; isoprenaline: 6.31 ± 0.18 vs. 6.65 ± 0.19 , $n = 7$). The observed increase in α_1 -adrenoceptor density in healthy rat heart may therefore not be high enough to enhance the phenylephrine-induced increase in force of contraction.

5 In conclusion, time course and effects of cycloheximide indicate that the increase in B_{max} of myocardial α_1 -adrenoceptors is due to *de novo* synthesis of receptors. However, at least for the rat heart model, a functional significance of this increase could not be demonstrated.

Introduction

Radioligand binding studies and functional experiments have given evidence for the coexistence of myocardial α_1 - and β -adrenoceptors in hearts of various species, including rat (Alexander *et al.*, 1975; Williams & Lefkowitz, 1978; Wagner & Brodde, 1978; Winek & Bhalla, 1979; Mukherjee *et al.*, 1983; Brückner *et al.*, 1985). Though different effector

enzymes and different second messengers seem to be involved (Endoh, 1986; Schmitz *et al.*, 1987; Scholz *et al.*, 1988), both adrenoceptors mediate positive inotropic effects (Benfey, 1973; 1987; Scholz, 1980). Since during β -adrenoceptor blockade the importance of β -adrenoceptors for regulation of cardiac performance is greatly reduced, it is conceivable that this enhances the significance of α_1 -adrenoceptor-mediated effects. Indeed, previous studies have

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demonstrated increased α_1 -adrenoceptor density in rat heart after chronic propranolol treatment (Mügge *et al.*, 1985; 1986). But the time course and the functional importance of the increase in α_1 -adrenoceptor density under chronic β -adrenoceptor blockade is unknown. Our aim was to study the extent and the time course of changes in α_1 -adrenoceptor density (B_{max}) and affinity (K_D), measured by radioligand binding in rat heart ventricular membranes, after chronic propranolol-treatment. For comparison, the influence of propranolol on B_{max} and K_D of β -adrenoceptors was also studied. Since changes in receptor characteristics may be accompanied by altered responses to sympathomimetic drugs, the influence of the α_1 -adrenoceptor agonist phenylephrine and the β -adrenoceptor agonist isoprenaline, respectively, on myocardial force of contraction was also investigated. The mechanism of the possible changes in adrenoceptor density was studied with cycloheximide, an inhibitor of protein synthesis.

Methods

Male Wistar rats (250–300 g), fed with altromin 1324 (Altromin, Lage, FRG) and water *ad libitum*, were treated with ($\pm$)-propranolol (9.9 mg kg⁻¹ body weight daily) or 0.9% NaCl for control, supplied by osmotic minipumps (OMPS, ALZET, Palo Alto U.S.A., model 2ML2) for 1, 2, 3 or 7 days. OMPS were implanted on day zero under the neck skin during short ether anaesthesia. Assays were performed after 1, 2, 3 or 7 days of treatment. In addition, OMPS from rats receiving 7 days of either propranolol- or NaCl-treatment were explanted and these animals were killed 1, 2, 3 or 7 days following explantation. The effectiveness of propranolol-treatment was controlled by heart rate measurements with an ECG Cardioscript III (Schwarzer, Munich, FRG). The rats were killed by cervical dislocation, bled from the carotid arteries, the hearts quickly removed and dissected free of pericardium, atria, fat and large vessels. Ventricles (0.65–0.75 g) were weighed and placed in 20 volumes of ice-cold incubation buffer (50 mmol l⁻¹ Tris-HCl, 10 mmol l⁻¹ MgCl₂, pH 7.5). Crude membrane homogenates were prepared according to the method of Baker *et al.* (1980) with minor modifications. Briefly, the hearts were minced and homogenized in a precooled Ultra-Turrax tissue homogenizer (3 $\times$ 10 s bursts). The suspension was diluted with an equal volume of ice-cold 1 mol l⁻¹ KCl solution and left on ice for 10 min before centrifugation at 48,000 *g* for 10 min at 4°C. Pellets were

resuspended and homogenized with a motor driven glass-teflon homogenizer (Colora, Lorch, FRG) for 1 min at setting 6 and recentrifuged. This procedure was repeated twice. The final pellet was resuspended in 14 volumes of incubation buffer (to adjust the protein content in incubation assays to approximately 0.250 mg), passed through 4 layers of gauze and kept on ice under stirring. The radioligands [³H]-prazosin and (–)-[³H]-CGP-12177 were used to label myocardial α_1 - and β -adrenoceptor binding sites, respectively. Assays were carried out in duplicate. Aliquots of 250 μ l of the membrane preparations were incubated in a total volume of 1000 μ l with either (–)-[³H]-CGP-12177 (0.05–11.0 nmol l⁻¹) or [³H]-prazosin (0.025–1.0 nmol l⁻¹). Time and temperature during incubation was 90 min at 35°C for β - and 60 min at 25°C for α_1 -adrenoceptor binding, respectively. Conditions chosen allowed complete equilibration of radioligands with binding sites. Incubation was terminated by rapid vacuum filtration through Whatman GF/C filters and two 5 ml washes with ice-cold incubation buffer. Filters were dried at room temperature and radioactivity was counted by liquid scintillation spectrometry. Specific binding was calculated by subtracting non-specific binding (in the presence of 0.1 μ mol l⁻¹ prazosin for α_1 - or 5 μ mol l⁻¹ propranolol for β -adrenoceptor binding) from total binding. Protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

The contraction experiments were performed on electrically driven papillary muscles isolated from the right ventricles of propranolol- and NaCl-treated rats, respectively. One of two papillary muscles from each rat was exposed to isoprenaline (concentration range from 1 nmol to 10 μ mol l⁻¹) and the other to phenylephrine (concentration range from 0.1 μ mol to 100 μ mol l⁻¹). Preparations were attached to a bipolar platinum stimulating electrode and mounted individually in 10 ml glass tissue chambers for recording isometric contractions (for details see Böhm *et al.*, 1984). The bathing solution was a modified Tyrode solution containing (mmol l⁻¹): NaCl 119.8, KCl 5.4, MgCl₂ 1.05, CaCl₂ 1.8, NaH₂PO₄ 0.42, NaHCO₃ 22.6, glucose 5.0, ascorbic acid 0.28 and Na₂-EDTA 0.05. The bathing solution was continuously aerated with 95% O₂ and 5% CO₂ (pH 7.4). Temperature was kept constant at 35°C. Force of contraction was measured with an inductive force transducer (W. Fleck, Mainz, FRG) attached to a Hellige Helco Scriptor recorder. Each muscle was stretched to the length at which force of contraction was maximal. The resting force (approximately 5 mN) was kept constant throughout the experiment. Stimulation frequency was 1 Hz (duration 5 ms, intensity 10–20% above threshold). All preparations were allowed to equilibrate for at least 60 min until

Figure 1 Effect of propranolol-treatment on heart rate of rats, determined by ECG-measurement. Ordinate scale: heart rate in beats min^{-1} ; abscissa scale: days of propranolol- or NaCl-treatment (control), respectively. Open columns, control; hatched columns, ($\pm$)-propranolol 9.9 mg kg^{-1} daily. Numbers of animals are displayed in the columns. * $P < 0.01$.

complete mechanical stabilization before any drug addition. Concentration-response curves were obtained cumulatively. Time of exposure to each concentration of isoprenaline and phenylephrine was

Figure 2 Total (●), specific (○) and non-specific (▲) [^3H]-prazosin binding in a single saturation experiment of rat heart ventricular membranes after propranolol-treatment for 3 days. Ordinate scale: [^3H]-prazosin bound in fmol mg^{-1} protein; abscissa scale: concentration of radioligand in nmol l^{-1} . Inset: Linearized binding plot of specific binding. [^3H]-prazosin bound in fmol mg^{-1} protein (B) was plotted as a function of the ratio (B/F) of bound [^3H]-prazosin to free [^3H]-prazosin in nmol l^{-1} . The intercept with the ordinate scale (108.1) represents the maximal number of binding sites in fmol mg^{-1} protein (B_{max}), the slope represents the K_D (0.04 nmol l^{-1}).

5 and 15 min, respectively. To avoid any interference with β -adrenoceptor activation, the effects of phenylephrine were studied in the presence of $1 \mu\text{mol l}^{-1}$ propranolol added 30 min before phenylephrine. In all experiments, only one concentration-response curve was determined in each preparation.

In some experiments NaCl- and propranolol-treated rats were additionally treated with cycloheximide (1.5 mg kg^{-1} body weight daily, i.p.) for 3 days, starting on the morning of OMPS implantation. This dose was shown to inhibit protein synthesis markedly (Yeh & Shils, 1969). Animals were killed on day 3 and binding assays performed as described above.

Drugs

The drugs used were from the following sources: ($\pm$)-propranolol hydrochloride (Rhein-Pharma, Heidelberg, FRG); prazosin hydrochloride (Pfizer, Karlsruhe, FRG); ($\pm$)-isoprenaline hydrochloride (Boehringer, Ingelheim, FRG); (–)-phenylephrine hydrochloride (Boehringer, Ingelheim, FRG); cycloheximide (Sigma, Deisenhofen, FRG); [^3H]-prazosin (specific activity $80.9 \text{ Ci mmol}^{-1}$, New England Nuclear, Dreieich, FRG); (–)-[^3H]-CGP-12177 ((–)-4-(3-*t*-butylamino-2-hydroxypropoxy)-[5,7- ^3H]benzimidazol 2-one); specific activity $53.1 \text{ Ci mmol}^{-1}$, Amersham Buchler, Braunschweig, FRG).

Statistics

Values presented are means $\pm$ s.e.mean. Statistical significance was estimated by Student's *t* test for unpaired observations. A *P* value of less than 0.05 was considered significant. The maximal density (B_{max}) and apparent affinity (K_D) of binding sites were obtained in individual experiments from Scatchard plots determined by linear regression analysis as described by Winek & Bhalla (1979). In contraction experiments, drug concentrations that produced 50% of the maximal effect (EC_{50}) were determined graphically in each experiment and are presented as pD_2 -values.

Results

Heart rate

Compared to control animals, heart rates of propranolol-treated rats were reduced by 25%

Figure 3 Time course of α_1 -adrenoceptor density (B_{max}) on rat heart ventricular membranes, recorded on days 1, 2, 3 and 7 after beginning of propranolol- or NaCl-treatment, respectively. Ordinate scale: [3 H]-prazosin bound in fmol mg^{-1} protein; abscissa scale: days of treatment. Numbers of animals are displayed in the columns; open columns, control; hatched columns, ($\pm$)-propranolol 9.9 mg kg^{-1} daily. K_D -values (nmol l^{-1}) of control and propranolol-treated rats, respectively, were ($n = 5-6$): day 1: 0.05 ± 0.002 vs. 0.05 ± 0.003 ; day 2: 0.08 ± 0.024 vs. 0.05 ± 0.003 ; day 3: 0.07 ± 0.018 vs. 0.05 ± 0.005 ; day 7: 0.06 ± 0.013 vs. 0.05 ± 0.001 . * $P < 0.05$.

(Figure 1). The reduction remained constant on day 1, 2, 3 or 7 after beginning of propranolol-treatment indicating effective β -adrenoceptor blockade during the entire treatment period.

Figure 4 Time course of α_1 -adrenoceptor density (B_{max}) changes in rat heart ventricular membranes, recorded on day 0, 1, 2, 3 and 7 after withdrawal of a 7 day treatment period with propranolol or NaCl, respectively. Ordinate scale: [3 H]-prazosin bound in fmol mg^{-1} protein; abscissa scale: days after end of treatment. Numbers of animals are displayed in the columns; open columns, control; hatched columns, ($\pm$)-propranolol 9.9 mg kg^{-1} , daily. K_D values (nmol l^{-1}) of control and propranolol-treated rats, respectively, were ($n = 8-9$): day 0: 0.04 ± 0.002 vs. 0.04 ± 0.002 ; day 1: 0.04 ± 0.003 vs. 0.04 ± 0.004 ; day 2: 0.04 ± 0.002 vs. 0.04 ± 0.002 ; day 3: 0.04 ± 0.002 vs. 0.04 ± 0.004 ; day 7: 0.04 ± 0.003 vs. 0.04 ± 0.003 . * $P < 0.05$.

Figure 5 Influence of cycloheximide (1.5 mg kg^{-1} daily) on α_1 -adrenoceptor density in rat heart ventricular membranes (B_{max}) without and with propranolol(Prop)-treatment (9.9 mg kg^{-1} daily) over a period of 3 days. Numbers of animals are displayed in the columns. K_D values (nmol l^{-1} ; $n = 7-8$) were: column (a): 0.04 ± 0.003 ; column (b): 0.04 ± 0.001 ; column (c): 0.04 ± 0.002 ; column (d): 0.04 ± 0.002 . * $P < 0.05$ vs. (a); † $P < 0.001$ vs. (b).

α_1 -Adrenoceptor binding

Scatchard analysis of the saturation curves of all experiments showed a single component, high affinity binding of [3 H]-prazosin in NaCl- and propranolol-treated rats. Non-specific binding at the K_D -value averaged 4.0% of total binding. Figure 2 shows an example of concentration-dependent ($0.025-1.0 \text{ nmol l}^{-1}$) [3 H]-prazosin-binding to cardiac α_1 -adrenoceptors after 3 days of propranolol-treatment. B_{max} and K_D in this experiment, calculated from linearized binding plot (inset of Figure 2), were $108.1 \text{ fmol mg}^{-1}$ protein and 0.04 nmol l^{-1} , respectively. Whereas propranolol-treatment did not affect K_D , α_1 -adrenoceptor density was increased following propranolol-treatment. The time course of this propranolol-induced up regulation is shown in Figure 3. The effect became significant after 2 days and reached a maximal increase of 28% after 3 days of treatment. Thereafter α_1 -adrenoceptor density remained constant throughout 7 days of treatment.

Withdrawal of propranolol was followed by decreased α_1 -adrenoceptor density reaching pretreatment levels two days after cessation of propranolol-uptake (Figure 4). Thus, the time courses for up- and down regulation of myocardial α_1 -adrenoceptors were similar.

Influence of cycloheximide

Impairment of protein synthesis by cycloheximide led to a significant decrease in the α_1 -adrenoceptor

Figure 6 Total (●), specific (○) and non-specific (▲) (–)[³H]-CGP-12177 ([³H]-CGP) binding in a single saturating experiment of rat heart ventricular membranes after propranolol-treatment (3 days). Ordinate scale: [³H]-CGP bound in fmol mg^{−1} protein; abscissa scale: concentration of radioligand in nmol l^{−1}. Inset: linearized binding plot of specific binding. [³H]-CGP bound in fmol mg^{−1} protein (B) was plotted as a function of the ratio (B/F) of bound [³H]-CGP to free [³H]-CGP in nmol l^{−1}. The intercept with the ordinate scale (49.9) represents the maximal number of binding sites in fmol mg^{−1} protein (B_{max}), the slope represents the K_D (0.45 nmol l^{−1}).

density (Figure 5). When NaCl-treated rats were also given cycloheximide, α_1 -adrenoceptor binding sites were reduced by 22.9% (Figure 5c), indicating a marked influence of cycloheximide on the turnover

process of α_1 -adrenoceptors. The propranolol-induced increase of α_1 -adrenoceptors was inhibited completely by cycloheximide (Figure 5d).

β -Adrenoceptor binding

Scatchard analysis of the saturation curves of all experiments showed a single component, high affinity binding of (–)[³H]-CGP-12177 in NaCl- and propranolol-treated rats. Non-specific binding at the K_D -concentration averaged 10.8% of total binding. Figure 6 shows an example of concentration-dependent (0.1–11.0 nmol l^{−1}) (–)[³H]-CGP-12177-binding to cardiac β -adrenoceptors after 3 days of propranolol-treatment with the linearized binding plot depicted in the inset. Treatment with propranolol did not yield significant increases of B_{max} nor did it influence K_D . Table 1 lists B_{max} and K_D values of β -adrenoceptor binding assays after the beginning and the end of propranolol treatment. Similar results were obtained in the presence of cycloheximide (Table 2).

Force of contraction

Phenylephrine produced increases in force of contraction in control rats from a threshold concentration of 3 μ mol l^{−1} to a maximal concentration of 30 μ mol l^{−1}. Similar results were obtained for propranolol-treated rats (Figure 7a). The pD₂ values were the same for both groups (5.41 $\pm$ 0.11 in

Table 1 B_{max} and K_D values from β -adrenoceptor binding experiments with (–)[³H]-CGP-12177 in rat heart ventricular membranes after beginning or end of propranolol-treatment (Prop: 9.9 mg kg^{−1} daily)

	After beginning of treatment				After end of treatment			
	n	Day	Control	Prop	n	Day	Control	Prop
B_{max} (fmol mg ^{−1} protein)					7	0	38.1	46.0
							± 3.3	± 7.0
	6	1	33.3	30.8	5	1	34.7	40.5
			± 1.3	± 1.4			± 2.0	± 6.2
	6	2	34.1	36.8	5	2	36.2	33.7
			± 2.1	± 1.8			± 2.7	± 4.1
	6	3	31.6	35.6	5	3	31.8	31.8
			± 2.0	± 1.9			± 3.7	± 5.4
	6	7	32.2	36.1	5	7	35.0	33.0
			± 1.2	± 2.4			± 2.2	± 5.5
K_D (nmol l ^{−1})					7	0	0.32	0.38
							± 0.09	± 0.13
	6	1	0.27	0.23	5	1	0.27	0.33
			± 0.03	± 0.01			± 0.10	± 0.11
	6	2	0.24	0.25	5	2	0.25	0.27
			± 0.05	± 0.04			± 0.02	± 0.02
	6	3	0.18	0.19	5	3	0.21	0.20
			± 0.02	± 0.03			± 0.04	± 0.04
	6	7	0.28	0.30	5	7	0.43	0.39
			± 0.10	± 0.07			± 0.15	± 0.18

n = number of experiments.

Table 2 $B_{\max}$ and K_D values of β -adrenoceptor binding experiments with (–)-[^3H]-CGP-12177 in rat heart ventricular membranes after propranolol-treatment (Prop: 9.9 mg kg^{-1} daily) without or with additional treatment with cycloheximide (1.5 mg kg^{-1} daily): both drugs were given simultaneously for 3 days

$B_{\max}$ (fmol mg^{-1} protein)				K_D (nmol l^{-1})			
Control		Cycloheximide		Control		Cycloheximide	
NaCl(7)	Prop(8)	NaCl(7)	Prop(6)	NaCl(7)	Prop(8)	NaCl(7)	Prop(6)
41.5	44.8	35.4*	39.1*	0.22	0.26	0.23	0.22
± 1.4	± 1.6	± 2.0	± 2.1	± 0.02	± 0.04	± 0.02	± 0.02

Numbers of animals are shown in parentheses. (* $P < 0.05$ vs. control).

control rats, $n = 7$; 5.41 ± 0.19 in propranolol-treated rats, $n = 7$).

Isoprenaline produced increases in force of contraction in control rats from a threshold concentration of $1\text{ }\mu\text{mol l}^{-1}$ to a maximal concentration of $10\text{ }\mu\text{mol l}^{-1}$. In propranolol-treated rats the positive inotropic effect started at $0.1\text{ }\mu\text{mol l}^{-1}$ and was also maximal at $10\text{ }\mu\text{mol l}^{-1}$ (Figure 7b). The pD_2 values were not significantly different in both groups (6.31 ± 0.18 in control rats, $n = 7$; 6.65 ± 0.19 in propranolol-treated rats, $n = 7$).

Discussion

Catecholamine-induced increase in myocardial force of contraction is predominantly a result of β -adrenoceptor stimulation (Black & Prichard, 1973; Neil, 1975; Kaumann *et al.*, 1978; Kenakin, 1984). However, stimulation of myocardial α_1 -adrenoceptors also leads to a positive inotropic response (Benfey, 1973; 1987; Scholz, 1980; Endoh, 1986). Whereas agonist occupation of β -adrenoceptors leads to activation of adenylate cyclase and to a subsequent increase in cyclic AMP content, no elevation of cyclic AMP has been detected upon α_1 -adrenoceptor stimulation (Osnes & Øye, 1975; Schumann *et al.*, 1975; Brückner *et al.*, 1978; Homcy & Graham, 1985; Endoh, 1986; Scholz *et al.*, 1986). Since both receptor systems act via different pathways (Endoh, 1986; Schmitz *et al.*, 1987; Scholz *et al.*, 1988), it is conceivable that in the diseased state they may also be influenced in a different manner. Thus chronic heart failure with down regulation of β -adrenoceptors (Bristow *et al.*, 1982; 1986; Brodde *et al.*, 1986; Fowler *et al.*, 1986) or chronic β -adrenoceptor blockade with impaired β -adrenoceptor stimulation could enhance the significance of myocardial α_1 -adrenoceptors in order to maintain cardiac function (Osnes *et al.*, 1985).

In this study, we observed in rats chronically treated with propranolol an increase in α_1 -adrenoceptor density two days after beginning of treatment without change in affinity. Since this elevation was maintained throughout the duration of a

seven day treatment period it may be considered as an adaptive process that is maintained for the same period of time that β -adrenoceptors are blocked with propranolol.

Figure 7 Cumulative concentration-response curves for the effects of phenylephrine (a) and isoprenaline (b) on isometric force of contraction in right ventricular papillary muscles isolated from control rats (●) and from rats after propranolol-treatment (○) 9.9 mg kg^{-1} daily for 3 days. Ordinate scale: force of contraction in mN; abscissa scale: concentration range of phenylephrine and isoprenaline, respectively, expressed in $\mu\text{mol l}^{-1}$. Values for phenylephrine were obtained in the presence of $1\text{ }\mu\text{mol l}^{-1}$ propranolol. In (a), pD_2 for control = 5.41 ± 0.11 ($n = 7$); pD_2 for propranolol-treatment = 5.41 ± 0.19 ($n = 9$); * $P < 0.05$ vs. predrug value. In (b) pD_2 for control = 6.31 ± 0.18 ($n = 7$); pD_2 for propranolol-treatment = 6.65 ± 0.19 ($n = 7$). * $P < 0.05$ vs. predrug value.

The observed increase in the density of α_1 -adrenoceptors was reversible within two days after withdrawal of propranolol. This demonstrates similar time courses for up- and down regulation of myocardial α_1 -adrenoceptors.

The increase of α_1 -adrenoceptors in rat heart after chronic propranolol-treatment could either be the result of new synthesis of adrenoceptors or externalization of adrenoceptors located within the cell (Lefkowitz *et al.*, 1983; Sibley & Lefkowitz, 1985; Fratelli & De Blasi, 1987). We therefore treated rats under identical experimental conditions with cycloheximide. This substance is a strong inhibitor of protein synthesis (Trakatellis *et al.*, 1965; Korner, 1966; Jondorf & Grünberger, 1967; Yeh & Shils, 1969). The propranolol-induced increase of myocardial α_1 -adrenoceptor density was inhibited completely by cycloheximide. This indicates that new receptor synthesis is responsible for the increase in receptor density. Furthermore, the basal turnover rate of α_1 -adrenoceptor synthesis in rat heart may be decreased by cycloheximide since hearts from NaCl-treated rats also revealed a decreased number of binding sites after cycloheximide.

In contraction experiments, one might expect an increased potency or efficacy of the α -sympathomimetic drug phenylephrine in ventricular heart preparations of propranolol-treated rats, but no significant differences from preparations from NaCl-treated rats were obtained. The increase in α_1 -adrenoceptor density in rat heart after chronic propranolol-treatment can therefore not be correlated to higher potency or efficacy of phenylephrine in our experiments, and the biological significance of this effect thus remains an open question. However, it is also conceivable that the observed increase of α_1 -adrenoceptor density may have been too small to be of functional relevance.

Whether a similar adaptation in density of α_1 -adrenoceptors after chronic β -adrenoceptor blockade also occurs in other species has not been investigated. Species differences in the number of myocardial α -adrenoceptors are well known (Mukherjee *et al.*, 1983) and it remains speculative, whether our results are applicable e.g. to the human heart, where only a very low α_1 -adrenoceptor density was found (Ferry & Kaumann, 1987; Bevilacqua *et al.*, 1987). But the reason for the clinical improvement of certain patients with dilated cardiomyopathy after treatment with β -adrenoceptor blocking drugs is still uncertain (Waagstein *et al.*, 1975; Shanes, 1987) and it seems worth investigating whether the myocardial α_1 -adrenoceptor density is also increased in human heart after chronic β -adrenoceptor blockade.

The results from the β -adrenoceptor binding studies show a slight increase in binding sites after

propranolol-treatment without reaching significant levels. Other results have been reported by Glaubiger & Lefkowitz (1977) and Aarons & Molinoff (1982) who found a significantly increased number of β -adrenoceptor binding sites in rat heart after chronic treatment with propranolol. The reason for this discrepancy remains unclear. However, our results are in agreement with Baker & Potter (1980) who also did not detect significant increases of β -adrenoceptor binding sites in rat ventricular membranes after chronic propranolol-treatment. Similar results were presented for guinea-pig ventricular membranes by Chess-Williams & Broadley (1984).

In NaCl- and propranolol-treated rats, β -adrenoceptor densities were depressed to a similar extent by cycloheximide. Compared to α_1 -adrenoceptor binding, cycloheximide influence was less pronounced in β -adrenoceptor binding experiments and our results denote a stronger influence of cycloheximide on myocardial α_1 -adrenoceptors than on myocardial β -adrenoceptors. This is in agreement with studies on turnover rate of adrenoceptors in cell cultures (Bouhelal *et al.*, 1987) showing a considerable shorter half life for α_1 -adrenoceptors than for β -adrenoceptors. It is thus conceivable that the α_1 -adrenoceptor may have a faster turnover rate and hence is more sensitive to inhibition of protein synthesis.

Contraction experiments with isoprenaline did not display a significant difference between propranolol- and NaCl-treated rats although the concentration-response curve for propranolol-treated rats is shifted slightly to the left. These results are in agreement with the data obtained from our β -adrenoceptor binding where the difference in binding sites did not reach significance for propranolol-treated rats and where an unchanged affinity was found for the ligand used.

In conclusion, chronic β -adrenoceptor blockade with propranolol led to a compensatory increase in the number of myocardial α_1 -adrenoceptors which was apparently due to a new synthesis of receptors. Since this increase was not accompanied by an increased positive inotropic effect of the α_1 -adrenoceptor agonist phenylephrine it may have been too small to be of functional importance. It was therefore not possible to determine an increased functional role of myocardial α_1 -adrenoceptors during chronic β -adrenoceptor blockade, at least not for the rat heart. Whether myocardial α_1 -adrenoceptors are of greater importance during the development of cardiac failure with down regulation of β -adrenoceptors remains to be investigated.

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