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Blockade of β_1- and desensitization of β_2-adrenoceptors reduce isoprenaline-induced cardiac fibrosis

Fazia Brouri[a], Naima Hanoun[b], Odile Mediani[a], Françoise Saurini[b], Michel Hamon[b], Paul M. Vanhoutte[c], Philippe Lechat[a,*]

[a] Service de Pharmacologie, Hôpital de la Pitié-Salpêtrière, 47-91, Boulevard de l'Hôpital, 75651 Paris Cedex 13, France
[b] INSERM U288, Faculté de médecine Pitié-Salpêtrière, 47-91, Boulevard de l'Hôpital, 75651 Paris Cedex 13, France
[c] Department of Pharmacology, University of Hong Kong, 21 Sassoon Road, Hong Kong, China

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Abstract

The aim of the present study was to analyse the role of β_1- and β_2-adrenoceptors in the catecholamine-induced myocardial remodeling, especially the interstitial fibrosis. Wistar rats were subjected to a 2-week chronic isoprenaline administration (30 μg/kg/h). Rats received a concomitant treatment with the selective β_1-adrenoceptor antagonist, bisoprolol (50 mg/kg/day p.o.) or were chronically pretreated with the selective β_2-adrenoceptor agonist salbutamol (40 μg/kg/h) for 1 week to induce β_2-adrenoceptor desensitization. The pretreatment with salbutamol induced a 59% down-regulation of left ventricular β_2-adrenoceptors compared to control. The extent of the isoprenaline-induced left ventricular fibrosis was significantly reduced in both the bisoprolol and salbutamol groups compared with the control isoprenaline-treated group especially in the apical region ($1.7 \pm 0.6\%$ and $1.4 \pm 0.3\%$ versus $6.0 \pm 1.3\%$, respectively, $P < 0.005$). β_1-adrenoceptor blockade and β_2-adrenoceptors down-regulation provided similar protection against isoprenaline-induced cardiac interstitial fibrosis suggesting that both β-adrenoceptors are involved in such cardiac remodeling process.

Keywords: β-Adrenoceptor; β-Adrenoceptor antagonist; Catecholamine; Fibrosis

1. Introduction

Experimental and clinical trials have clearly demonstrated the beneficial effect of β-adrenoceptor blockade in heart failure (Waagstein et al., 1975; The MDC study, 1998; The CIBIS-II study, 1999; Lechat et al., 1998). Such benefit relies on protection against the toxic effects of catecholamine. The latter include left ventricular hypertrophy, oxidative stress, calcium overload and particularly fibrosis, which are responsible for left ventricular remodeling and dysfunction leading to progressive worsening of heart failure. However, the exact mechanism underlying this toxicity remains to be elucidated.

In the experimental model of heart failure in which myocardial infarction is caused by coronary artery ligation in the rat, the administration of noradrenaline did not increase oxidative stress but increased the extent of interstitial fibrosis, an effect attributed to β-adrenoceptor stimulation (Bonnefont-Rousselot et al., 2002). Another study using the same model suggested that the cardiac fibrosis observed after chronic administration of isoprenaline (a combined β_1- and β_2-adrenoceptor agonist) was mediated by the β_1-adrenoceptor subtype since β_2-adrenoceptors were preferentially down-regulated by this treatment (Brouri et al., 2002).

The aim of the present study was to further investigate the respective roles of β_1- and β_2-adrenoceptors in the catecholamine-induced cardiovascular remodeling and especially the fibrotic replacement of myocytes. For this purpose, normotensive unoperated rats were treated for 2 weeks under conditions ensuring the selective stimulation of β_1- and β_2-adrenoceptors and the resulting fibrosis was assessed using a quantitative image analysis system.

2. Materials and methods

The experiments were performed on adult male Wistar normotensive rats (Centre d'Elevage R. Janvier, Saint Ber-

* Corresponding author. Tel.: +33-142-16-16-82; fax: +33-1-42-16-16-88.
E-mail address: philippe.lechat@psl-ap-hop.paris.fr (P. Lechat).

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thevin, France). The animals were housed under controlled environmental conditions (22 ± 1 °C ambient temperature, 60% relative humidity, 12:12 h light–dark cycle, food and water ad libitum) for the whole study. All the procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national and international laws and policies (Council directive #87.848, October 19, 1987, Ministère de l'Agriculture et de la Forêt, Service Vétérinaire de la Santé et de la Protection Animale).

2.1. Chronic stimulation of β_1- and β_2-adrenoceptors (Fig. 1)

Rats weighing 300–320 g were subjected to a 2-week administration of isoprenaline (30 μg/kg body weight per h) through osmotic minipumps (model 2002, ALZA, Palo Alto, CA, USA) implanted subcutaneously in the back as previously used (Brouri et al., 2002) (Fig. 1). Minipumps delivering saline (0.9% NaCl) were implanted for the same period in control rats.

2.2. Preliminary experiments

2.2.1. Selective stimulation of the β_2-adrenoceptors

To stimulate only the β_2-adrenoceptors, rats were treated with the selective β_1-adrenoceptor antagonist, bisoprolol (50 mg/kg body weight per day, in drinking water).

To test for the efficacy of such bisoprolol dose regimen in selectively blocking β_1-adrenoceptors, dose-response curves of the effect of i.v. isoprenaline on heart rate and of i.v. salbutamol on diastolic blood pressure, were drawn. For this purpose, animals were divided into two groups including untreated control rats and rats that were treated with bisoprolol for 2 weeks. On the day of the study, rats were anesthetized with sodium pentobarbital (60 mg/kg i.p.). In a first set of experiments, animals' (five in control and five in bisoprolol group) electrocardiogram monitoring allowed measurement of heart rate changes in response to increasing doses (0.0025–30 μg/kg) of isoprenaline. In a second set of experiments, animals' (six in control and six in bisoprolol group) blood pressure values were recorded in response to increasing doses (0.05–10 μg/kg) of salbutamol, a selective β_2-adrenoceptor agonist, using a micro-tip pressure transducer catheter (Millar Instrument, Houston, TX, USA) introduced into the right carotid artery. The maximal response was calculated from each dose-response curve, using the following E_{max} model equation: $E=(E_{max} \times C)/(C+EC_{50})$, with the sigma-plot software.

2.2.2. Selective β_1-adrenoceptor stimulation, β_2-adrenoceptor desensitization

To avoid β_2-adrenoceptor stimulation and to preferentially stimulate β_1-adrenoceptors, we used a model of β_2-adrenoceptor desensitization induced by the selective β_2-adrenoceptor agonist, salbutamol (Finney et al., 2000). Dose-response curves of the effect of acute administration of i.v. salbutamol on arterial blood pressure were drawn in rats which had been previously subjected to a 1-week continuous treatment with salbutamol (40 μg/kg/h) using a subcutaneous osmotic minipump. Such salbutamol dose regimen was found by Finney et al. (2000) to induce a 30% desensitization of pulmonary β_2-adrenoceptors. The maximal responses were calculated from dose-response curves to salbutamol (0.05 μg/kg to 10 μg/kg i.v) measuring the decrease in diastolic blood pressure caused by the drug.

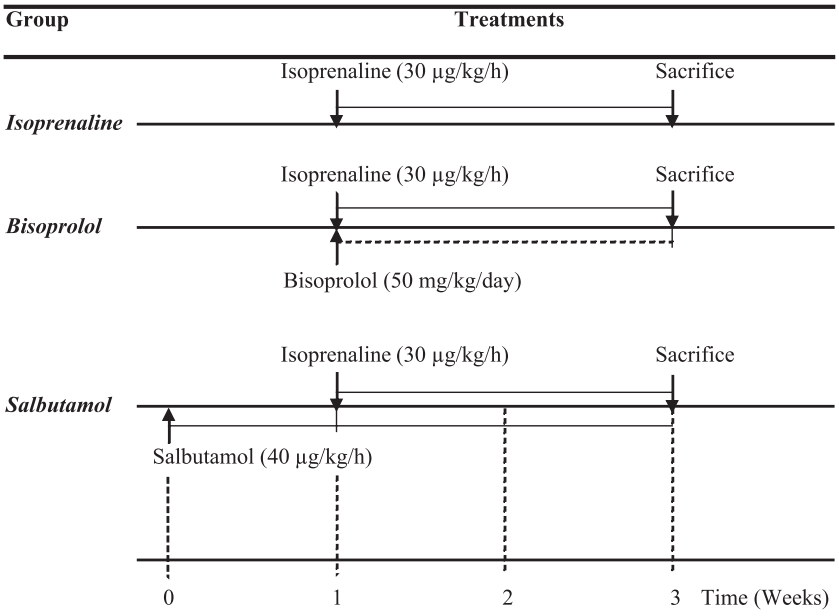

Fig. 1. Pharmacological protocols for the chronic stimulation (for 2 weeks) of $\beta_1 + \beta_2$-adrenoceptors (isoprenaline group), β_2-adrenoceptors (bisoprolol group) or β_1-adrenoceptors (salbutamol group) alone.

Diastolic blood pressure appeared indeed sensitive to β_2-adrenoceptor action as opposed to heart rate which was not significantly altered by i.v. salbutamol.

2.2.3. β-adrenoceptor binding studies to assess β-adrenoceptor desensitization

In order to establish the level of down-regulation of β_2-adrenoceptors induced, on the one hand, by the 1-week treatment by salbutamol, and, on the other hand, by isoprenaline during the 2-week period of treatment [as used in previous experiments, (Brouri et al., 2002) and planned to be used in the main study of cardiac fibrosis induction], the density and affinity of β_1- and β_2-adrenoceptors were estimated in binding experiments using crude membranes from the left ventricle at the end of each treatment periods. For this purpose, animals were divided into three groups: control saline (0.9% NaCl)-treated rats, salbutamol (40 μg/kg/h)-treated rats (1-week period) and isoprenaline (30 μg/kg/h)-treated rats (2-week period). Binding assays were performed using left ventricle membrane from rats sacrificed at the end of the treatment.

Membrane preparations and incubations with [^{125}I]iodocyanopindolol ([^{125}I]ICYP, 2000 Ci/mmol) were performed as previously described (Brouri et al., 2002). Samples were supplemented with increasing concentrations (10–200 pM) of [^{125}I]ICYP without (total binding) or with 0.3 μM of the selective β_1-adrenoceptor antagonist CGP 20712A (nonspecific binding) for the determination of the radioligand binding to β_1-adrenoceptor. Other samples supplemented with the same concentrations of [^{125}I]iodocyanopindolol plus 0.3 μM CGP 20712A were prepared for the measurement of the radioligand binding to β_2-adrenoceptor. In that case, nonspecific binding was determined in the presence of 1 μM propranolol. The specific binding was calculated as total binding minus nonspecific binding, and expressed as femtomoles [^{125}I]ICYP specifically bound per milligram of membrane protein, measured according to Lowry et al. (1951). K_d and B_{max} values were calculated from saturation curves using Graphpad and Inplot 4 programs (see Fabre et al., 1997).

We hypothesised that salbutamol treatment for 1 week could induce a similar level of β_2-adrenoceptor down-regulation compared to that induced by isoprenaline (2 weeks, Brouri et al., 2002) and that administration of isoprenaline after a 1-week pretreatment by salbutamol would take place on desensitized β_2-adrenoceptors. In such conditions, we could evaluate the effects of isoprenaline administration on previously desensitized β_2-adrenoceptors.

2.3. Main study (see Fig. 1)

2.3.1. Quantification of fibrosis

Rats were subjected to a 2-week treatment with isoprenaline (30 μg/kg/h). The first group ($n=9$) did not receive any other treatment, the second group ($n=9$) received a concomitant treatment with bisoprolol (50 mg/kg/day) and the third group ($n=9$) was pretreated with salbutamol (40 μg/kg/h) for 1-week immediately before starting the 2-week treatment with isoprenaline. At the end of these treatments, rats were anaesthetized deeply (sodium pentobarbital 60 mg/kg i.p.), and their hearts were removed and weighted. After fixation with formalin and inclusion in paraffin, the hearts were cut into coronal slices at three levels, 200 μm apart, from the apex to the base of each ventricle (using a H.M.340 E. Microtome, Microm France, Francheville). Transverse sections (6 μm thick) were made at each level and stained with Sirius red for morphometric studies and identification of the collagen.

Quantitative morphometric analysis was performed using an image analyser software (Lucia) coupled to a high-resolution colour camera (Nikon, Champigny/Marne, France) and to a macroviewer (Nikon). Left ventricle percentage of fibrosis was calculated at the three different levels for each heart.

2.4. Statistics

Analysis of the different parameters was performed by analysis of variance with one or two factors and comparisons between group were performed using the Neuman–Keuls test. Results are expressed as means ± S.E.M. A difference was considered to be statistically significant when the P value was less than 0.05.

2.5. Chemicals

Isoprenaline, salbutamol, bisoprolol and propranolol were from Sigma (St. Louis, MO, USA). [^{125}I]Iodocyanopindolol was purchased from Amersham-Pharmacia Biotech (Buckinghamshire, UK). CGP 20712A ((±)-2hydroxy-5-[2-[[2-hydroxy-3[4-[1-methyl-4(trifluoromethyl)-1*H*-imidazol-2-yl]phenoxy]propyl]amino]ethoxyl-benzamide]methanesulfonate) was a gift from Novartis (Basel, Switzerland).

3. Results

3.1. Preliminary experiments

3.1.1. β_1-adrenoceptor blockade by bisoprolol

At baseline, the heart rate was significantly reduced in animals treated with bisoprolol (50 mg/kg body weight per day) compared to control animals (respectively 208.0 ± 23.2 beats/min versus 312.0 ± 17.2 beats/min, $P<0.005$). The maximal increase in response to isoprenaline averaged 126.9 ± 14.2 beats/min in the control group versus 96.0 ± 8.1 beats/min in the bisoprolol group (NS), and the isoprenaline dose-response curve for bisoprolol treated animals was shifted rightward relative to that of control animals ($EC_{50}=0.13\pm0.08$ μg/kg for the bisoprolol group and $EC_{50}=2.22\pm0.78$ μg/kg for the control group, $P<0.05$).

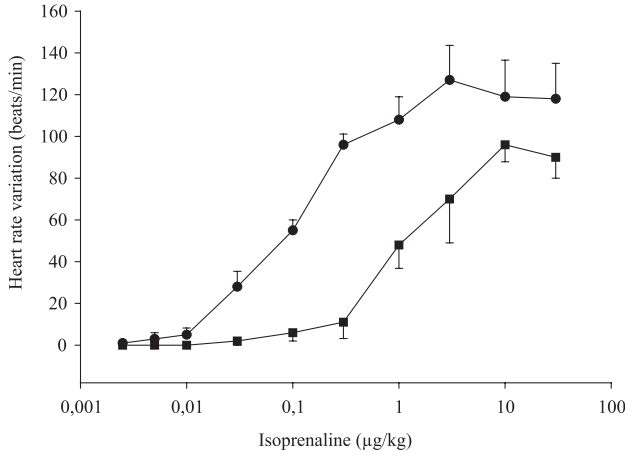

Fig. 2. Effects of chronic treatment with bisoprolol on heart rate variations (mean ± S.E.M.) in response to i.v. administration of increasing doses of isoprenaline. Animals were divided into two groups: untreated control rats ($n = 5$, ●) and rats treated with bisoprolol at the dose of 50 mg/kg body weight per day, in drinking water, for 2 weeks ($n = 5$, ■).

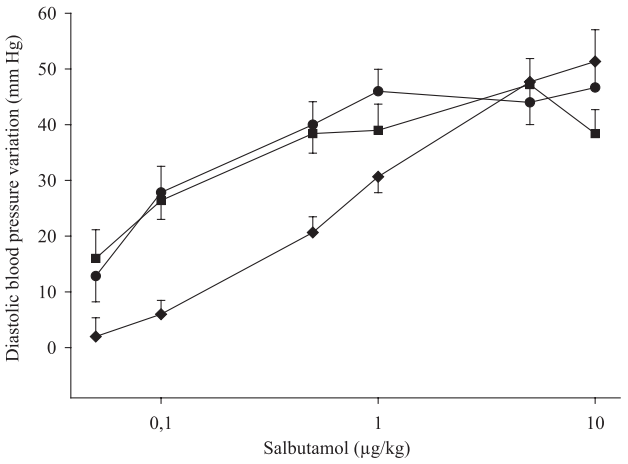

Fig. 3. Effects of chronic treatment with bisoprolol or salbutamol on the decrease of diastolic arterial blood pressure evoked by acute intravenous administration of increasing doses of salbutamol. Animals were divided into three groups: untreated control animals ($n = 6$, ●); animals treated with bisoprolol (50 mg/kg body weight per day) for 2 weeks ($n = 6$, ■), and animals treated with salbutamol (40 µg/kg/h) for 1 week ($n = 6$, ♦).

Reductions in arterial blood pressure caused by increasing doses of salbutamol (0.05 µg/kg to 10 µg/kg, i.v.) obtained in control and bisoprolol-treated animals were not significantly different (see Figs. 2 and 3 and Table 1). As shown in Fig. 3, the dose-response curve to salbutamol on diastolic blood pressure fall in bisoprolol treated animals was not shifted relative to that of control animals ($EC_{50} = 0.11 \pm 0.05$ µg/kg for the bisoprolol group and $EC_{50} = 0.11 \pm 0.05$ µg/kg for the control group). These results confirm the selectivity of β_1-adrenoceptor blockade by such bisoprolol dose regimen.

Table 1
EC_{50} and E_{max} values for the effects of acute administration of isoprenaline or salbutamol on heart rate and arterial blood pressure in rats chronically treated with bisoprolol or pretreated with salbutamol

		n	E_{max} (beats/min)	EC_{50} (µg/kg)
Increase in heart rate in response to intravenous isoprenaline	Control	5	126.9 ± 14.23	0.13 ± 0.08
	Bisoprolol (50 mg/kg/day)	5	96.0 ± 8.1	2.22 ± 0.78[a]
		n	E_{max} (mm Hg)	EC_{50} (µg/kg)
Decrease in diastolic blood pressure in response to intravenous salbutamol	Control	6	46.66 ± 4.58	0.11 ± 0.05
	Bisoprolol (50 mg/kg/day)	6	38.4 ± 4.31	0.11 ± 0.05
	Salbutamol (40 µg/kg/h)	6	51.3 ± 5.7	0.9 ± 0.2[b]

One-way analysis of variance (ANOVA) was performed to determine the statistical significance of the changes caused by bisoprolol treatment in isoprenaline-induced increase in heart rate and of those caused by bisoprolol or salbutamol pretreatment in the decrease in arterial blood pressure in response to acute intravenous administration of salbutamol. Between groups, comparisons were performed using the Neuman–Keuls test.

[a] $P < 0.05$ for bisoprolol versus control.

[b] $P < 0.05$ for salbutamol versus control.

3.1.2. β_2-adrenoceptor desensitization

Baseline diastolic arterial blood pressure was slightly but nonsignificantly higher in salbutamol group compared to control group (100.0 ± 2.7 mm Hg versus 87.7 ± 6.2 mm Hg, respectively, $P = 0.09$). The dose-response curve to acute administration of salbutamol on diastolic arterial blood pressure in animals already subjected to a 1-week chronic treatment with this drug (40 µg/kg/h) was shifted rightward relative to that of control animals (see Fig. 3 and Table 1), with an EC_{50} of 0.90 ± 0.20 and 0.11 ± 0.05 µg/kg, respectively ($P < 0.05$). Binding studies revealed a significant down-regulation of β_2-adrenoceptors averaging 59% in salbutamol-treated group ($P < 0.005$), and averaging 51% in isoprenaline-treated animals ($P < 0.005$) with a trend toward an increase in the K_d value for this receptor subtype in the isoprenaline-treated animals ($P = 0.05$, see Table 2).

Table 2
Characteristics of β_1- and β_2-adrenoceptor binding sites in left ventricle membranes from rats treated chronically with saline (controls), isoprenaline (30 µg/kg/h) or salbutamol (40 µg/kg/h)

	β_1		β_2	
	B_{max}	K_d	B_{max}	K_d
Controls	30.4 ± 1.6	0.040 ± 0.010	21.4 ± 0.5	0.013 ± 0.003
Isoprenaline	28.7 ± 3.0	0.033 ± 0.003	10.5 ± 0.7[a]	0.033 ± 0.007
Salbutamol	36.4 ± 3.2	0.023 ± 0.003	8.8 ± 0.4[b]	0.016 ± 0.003

K_d (in nM) and B_{max} (in fmol [^{125}I]ICYP specifically bound/mg membrane protein) values are the means ± S.E.M. of five to seven independent determinations.

One-way analysis of variance (ANOVA) was performed to determine the overall statistical significance. Between groups, comparisons were performed using the Neuman–Keuls test of changes caused by chronic administration of isoprenaline or salbutamol.

[a] $P < 0.05$ for isoprenaline compared to control.

[b] $P < 0.05$ for salbutamol compared to control.

In contrast, neither the number of β_1-adrenoceptors nor their affinity were significantly changed in the isoprenaline and salbutamol groups. These results confirm the similar level of β_2-adrenoceptor selective desensitization with the chosen dose regimen of salbutamol and of isoprenaline.

3.2. Cardiac remodeling and fibrosis study

Animals that received only a 2-week administration of isoprenaline displayed a great extent of fibrosis in the apical and middle region of their left ventricle compared with the basal region (about $5.99 \pm 1.27\%$ and $3.07 \pm 0.64\%$, respectively versus $1.45 \pm 0.43\%$, see Figs. 4A and 5). This fibrosis was mainly located in the subendocardial area.

The extent of such isoprenaline induced left ventricular fibrosis was dramatically and significantly reduced in both bisoprolol treated animals and in salbutamol pretreated animals compared to the control isoprenaline-treated group. Statistical analysis revealed a highly significant difference between control and both bisoprolol and salbutamol

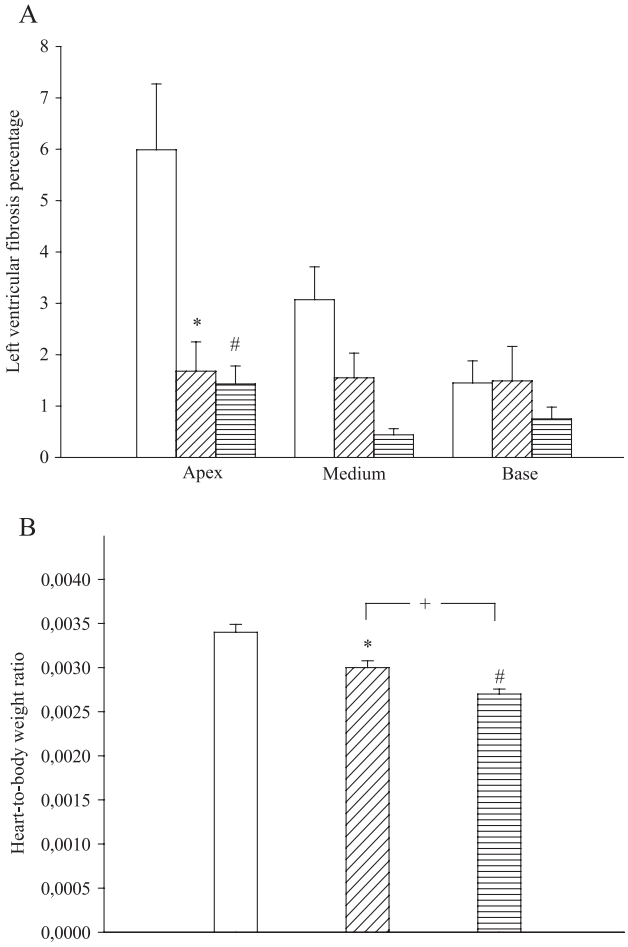

Fig. 4. Morphometric analysis performed at three levels of the heart in rats treated chronically with isoprenaline alone (30 μg/kg/h, $n=9$, □), in combination with bisoprolol (50 mg/kg/day, $n=9$, ▨), or after a 1-week pretreatment with salbutamol (40 μg/kg/h, $n=9$, ▤). (A) Left ventricular fibrosis expressed as percent of left ventricular area. (B) Heart-to-body weight ratio. Each bar is the mean ± S.E.M. $^{*}P<0.05$ for bisoprolol compared to control. $^{\#}P<0.05$ for salbutamol compared to control. $^{+}P<0.05$ for bisoprolol compared to salbutamol.

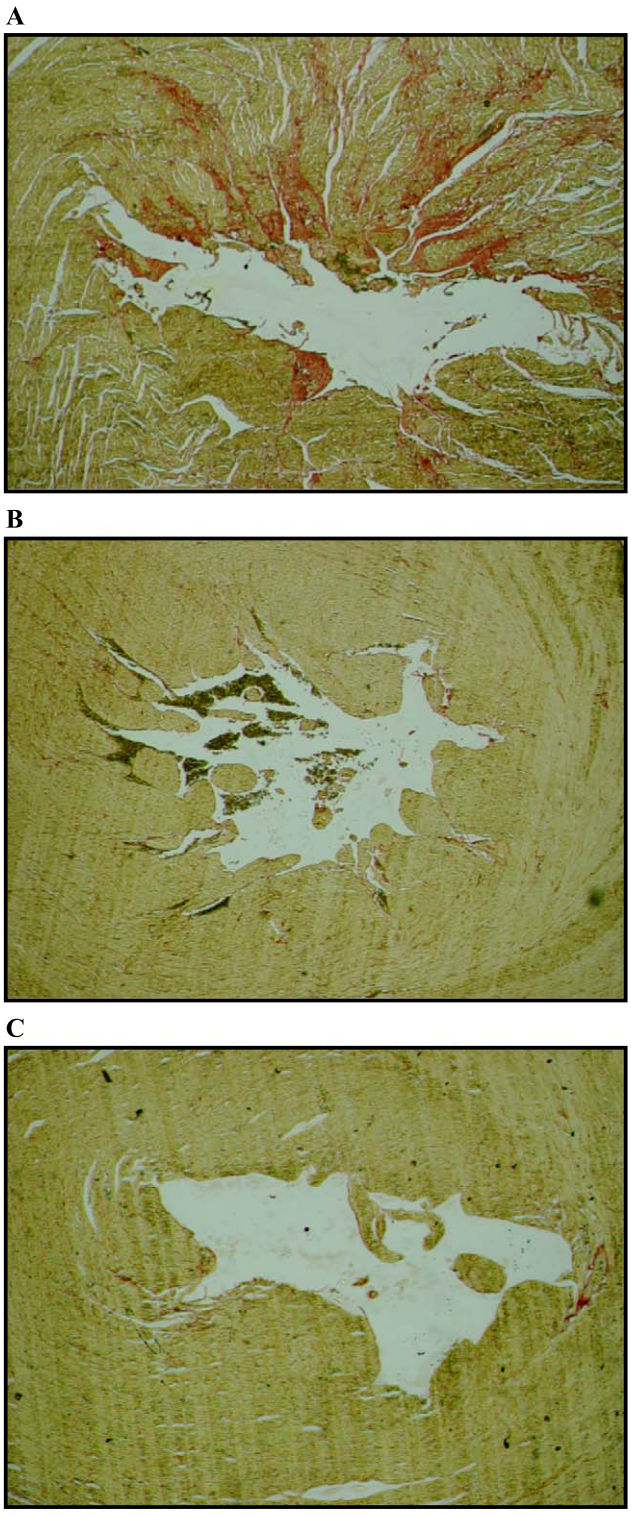

Fig. 5. Illustrations of left ventricular slices from rats treated chronically with isoprenaline alone (30 μg/kg/h, $n=9$, A), in combination with bisoprolol (50 mg/kg/day, $n=9$, B), or after a 1-week pretreatment with salbutamol (40 μg/kg/h, $n=9$, C).

($P<0.05$) groups on the left ventricular fibrosis extent. Such difference was maximal in the apical region since extent of fibrosis is the highest at this level (Figs. 4A and 5). Analysis of variance indicated a significant interaction between groups and cardiac levels (apical, median and basal). No difference could be detected between bisoprolol and salbutamol groups (Figs. 4A and 5).

Heart-to-body weight ratio were significantly reduced in both bisoprolol and salbutamol groups compared with control isoprenaline-treated group ($0.30 \times 10^{-2} \pm 7.66 \times 10^{-5}$, $0.27 \times 10^{-2} \pm 5.70 \times 10^{-5}$, respectively versus $0.34 \times 10^{-2} \pm 9.00 \times 10^{-5}$, $P<0.005$). This reduction was more pronounced in salbutamol group ($P<0.005$, see Fig. 4B).

4. Discussion

The aim of our study was to further investigate the respective roles of β_1- and β_2-adrenoceptors in the catecholamine-induced cardiovascular remodeling and especially the fibrotic replacement of myocytes. We used isoprenaline, a β-adrenoceptor agonist devoided of α-adrenoceptor stimulatory properties. Indeed, catecholamine-induced α-adrenoceptor stimulation induces vasoconstriction (α_1 in coronary arteries and α_2 in microvascular vessels) and potentially induces myocardial ischemia, then leading to tissular toxic effects such as cardiomyocytes apoptosis, necrosis and fibrosis. Our study selectively evaluated the effects of the stimulation of the different β_1- and β_2-adrenoceptors avoiding α-adrenoceptor-mediated vasoconstriction.

The main finding of the present study was that β_1-adrenoceptor blockade as well as down-regulation of β_2-adrenoceptors provided a similar protection against cardiac remodeling (fibrosis formation) induced by administration of isoprenaline in the rat.

Our preliminary experiments demonstrated that at the dose used bisoprolol selectively blocked β_1-adrenoceptors and that pretreatment with salbutamol, a selective β_2-adrenoceptor agonist causes desensitization of β_2-adrenoceptors. Binding experiments showed that pretreatment with salbutamol decreased by 59% β_2-adrenoceptor density, without affecting β_1-adrenoceptor density. Since our previous experiments (Brouri et al., 2002) suggested that the isoprenaline-induced cardiac fibrosis was mainly related to β_1-adrenoceptor stimulation, we anticipated that β1-adrenoceptor blockade would prevent such cardiac fibrosis while β_2-adrenoceptor desensitization would not affect it. Indeed, in our previous experiments, cardiac fibrosis induced by isoprenaline was obtained despite the down-regulation of β_2-adrenoceptors without significant density reduction of β_1-adrenoceptors, suggesting the proeminent role of β_1-adrenoceptors for the induction of cardiac fibrosis. Our present experiments confirm that β_1-adrenoceptor blockade indeed may prevent the isoprenaline induced cardiac fibrosis, but also, that down-regulation of β_2-adrenoceptors provides a similar protection. Such result was unexpected.

The results of binding experiments are consistent with those obtained in our previous study (Brouri et al., 2002) in which chronic isoprenaline administration down-regulated preferentially β_2-adrenoceptors (-50%) without affecting β_1-adrenoceptors. The underlying mechanism of differential regulation of β-adrenoceptor subtypes remains unclear at present, but is likely to be based on the fact that β_1- and β_2-adrenoceptors on the same cell type responded differently to agonist stimulation. Implicitly, different amino acid sequences in the receptors allow for differential protein structure, function, and regulation. Mutagenesis studies have established that tyrosine residues in the carboxy terminal tail of cell surface β_2-adrenoceptors are implicated in receptors endocytosis through coated pits (Ktistakis et al., 1990). Valiquette et al. (1990) reported that when two tyrosine residues in the carboxyl tail of the human β_2-adrenoceptors are replaced with alanine, β_2-adrenoceptors down-regulation is significantly blunted. In contrast, human β_1-adrenoceptors lack any tyrosine residues in the cytoplasmic tail (Suzuki et al., 1992). One inherent difference is the efficacy of β_1- and β_2-adrenoceptor coupling to Gs and adenylate cyclase that has been identified and mapped, at least in part, to the third intracellular loop (Bristow et al., 1989; Levy et al., 1993; Green et al., 1992). This region of the β_1-adrenoceptor is considerably longer than the corresponding region of the β_2-adrenoceptor because of the presence of an unusually proline-rich sequence that has been implicated as a negative modulator of β_1-adrenoceptor-Gs coupling (i.e., its removal improves β_1-adrenoceptor-Gs coupling, whereas its insertion into the β_2-adrenoceptor impairs β_2-adrenoceptor-Gs coupling; Green and Liggett, 1994). In fact β_2-adrenoceptors are more tightly coupled to Gs and physiological responsiveness than the β_1-adrenoceptors (Bristow et al., 1989; Levy et al., 1993; Green et al., 1992). Another mechanism that could explain subtype differences in β-adrenoceptor down-regulation is differences in the rate of synthesis or degradation between subtypes. In this regard, the half-life for β_1-adrenoceptor is two times greater than for β_2-adrenoceptor in unstimulated C6 glioma cells (Neve and Molinoff, 1986).

The left ventricular fibrosis was mainly extended in the apical myocardium, and in the subendocardial region of the left ventricle. In a previous study (Mori et al., 1993), in which contractile and metabolic responses to various *adrenoceptor* stimuli and β-adrenoceptor density were compared between left ventricular basal and apical regions in mongrel dogs, constant infusion of noradrenaline produced a greater response in normalised end-systolic length in the apical myocardium than in basal region and a greater increase in tissue cyclic AMP. In the rat, β-adrenoceptor density in the apical region is greater than in the basal region (Mori et al., 1993). In the normal human ventricle, the greatest β-adrenoceptor density was observed autoradiographically in the subendocardial myocytes (Murphree and Saffitz, 1989). Therefore, such higher density of β-adrenoceptors in the apical and subendocardial myocardial area mainly explain

the preferential fibrosis localization observed under isoprenaline administration in our experiments.

The morphometric study revealed a protection from left ventricular fibrosis and cardiac hypertrophy in rats that received the selective β_1-adrenoceptor antagonist bisoprolol (50 mg/kg/day) concomitantly with the 2-week chronic isoprenaline treatment (30 μg/kg/h) and in rats that were chronically pretreated with salbutamol (40 μg/kg/h) immediately before the administration of isoprenaline. The reduction of fibrosis extent and heart-to-body weight ratio in the bisoprolol group was expected since several clinical trials studies have suggested that β_1-adrenoceptor antagonists improve cardiac performance and significantly reduce mortality during heart failure (CIBIS II, 1999; Merit-HF, 1999). In contrast, the decrease of both fibrosis extent in left ventricle and heart-to-body weight ratio of rats pretreated with salbutamol is unexpected. In our previous study, an increase of fibrosis extent was obtained in animals subjected to a 2-week administration of isoprenaline despite a β_2-adrenoceptor down-regulation of about 53% induced by this compound (Brouri et al., 2002). In the present study, the same chronic isoprenaline administration was performed once the β_2-adrenoceptors were down-regulated by about 59%. What emerges from these two studies is that the protective effect of the β_2-adrenoceptor desensitization was obtained only when it occurred before the exposure to isoprenaline. It suggests that, in the previous case, the level of β_2-adrenoceptor desensitization did not reach a sufficient level early enough to be protective. Previous studies performed in the rat (Lau et al., 1980; Long et al., 1993; Gustafsson and Brunton, 2000) and more recently in humans (Turner et al., 2003) revealed that cultured cardiac fibroblasts express predominantly the β_2-adrenoceptor subtype. Since cardiac fibroblasts are responsible for synthesis and deposition of fibrillar collagens, proliferation of these cells can be considered to be an important mechanism that adds to the remodeling process. The proliferation of human (Turner et al., 2003) and adult rat (Leicht et al., 2000) cultured cardiac fibroblasts is enhanced in response to catecholamines via the stimulation of β_2-adrenoceptors. Nevertheless, the blockade of β_1-adrenoceptors by the selective antagonist bisoprolol prevented this fibrosis replacement. These results strongly suggest an involvement of both β_1- and β_2-adrenoceptors in the development of interstitial cardiac fibrosis and further complementary roles. The proliferation of human and rat fibroblasts depends on a comitogenic action of a β_2-adrenoceptor signaling pathway and growth factors such as *epidermal growth factor* (EGF), *platelet-derived growth factor* (PDGF), *transforming growth factor* β (TGF-β) and angiotensinogen (Turner et al., 2003; Leicht et al., 2000). Furthermore, human and rat fibroblasts produce these growth factors (Turner et al., 2003; Long et al., 1993; Fisher and Absher, 1995; Dostal et al., 2000). Since the stimulation of β_1-adrenoceptor induces the synthesis of renin which induces the production of angiotensin II, and knowing that angiotensin II is directly responsible for the fibrosis development (Robert et al., 1999) a possible explanation for our results is that the stimulation of both the β_1- and the β_2-adrenoceptors is necessary to induce the proliferation of cardiac fibroblasts and the collagen synthesis. In addition, several experimental models of β_2-adrenoceptor overexpression are associated with a rapidly progressive fibrosis (Liggett et al., 2000; Du et al., 2000) and cardiac hypertrophy (Liggett et al., 2000). The present experiments do not allow to speculate further concerning the causes of protection by previous desensitization of β_2-adrenoceptors.

The present study is the first to show a protective action of previous β_2-adrenoceptor desensitization in a model of chronic exposure to catecholamines. Moreover, it provides the evidence that both β_1- and β_2-adrenoceptors are involved in the fibrosis process during cardiac remodeling induced by catecholamines. A possible relevance of the observed desensitization of β_2-adrenoceptor could be a potential difference in efficacy (or toxicity) of β-adrenoceptor blockers, used for treatment in heart failure, according to their pharmacological profile, in particular their degree of selectivity for β_1-adrenoceptors. Selective β_1-adrenoceptor blockers would allow β_2-adrenoceptor stimulation (and subsequent desensitization) by endogenous catecholamines, while nonselective β-adrenoceptor antagonists would block equally both β_1- and β_2-adrenoceptors, thus curtailing the beneficial effect of β_2-adrenoceptor desensitization. This interpretation is supported by the results of the published COMET trial (Poole-Wilson et al., 2002), which compared carvedilol (nonselective) and metoprolol (β_1 selective).

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