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Toxic cardiac effects of catecholamines: role of β-adrenoceptor downregulation

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Abstract

The aim of our study was to analyse the mechanisms underlying cardiac toxicity caused by β-adrenoceptor stimulation and the relationships with their associated downregulation during heart failure. We used the experimental model of coronary artery ligation-induced myocardial infarction in male Wistar rats. In order to increase β-adrenergic stimulation, rats were subjected to a 15-day chronic isoprenaline administration (30 μg/kg/h). Isoprenaline administration induced haemodynamic inotropic compensation, almost abolished in vitro inotropic response to isoprenaline on papillary muscle ($P<0.005$) but promoted fibrosis. Isoprenaline treatment markedly reduced the B_{max} of β_2-adrenoceptors (by 53% in sham and 44% in infarcted rats) but not that of β_1-adrenoceptors. These results suggest that β_1-adrenoceptors rather than β_2-adrenoceptors underlie the deleterious effects of chronic β-adrenergic stimulation on cardiac fibrosis and are in agreement with the demonstrated benefit induced in human heart failure by β_1-adrenoceptor antagonists.

Keywords: β-Adrenoceptor; Autonomic nervous system; Contractility; Fibrosis; Heart failure

1. Introduction

Despite dramatic therapeutic improvements during the last decades, heart failure remains a major cause of morbidity and mortality (Garg et al., 1993; Gillum, 1993). The neurohumoral compensatory mechanisms such as those implicating the sympathetic nervous system allow the maintenance of blood pressure and organ perfusion but also lead to some deleterious effects such as interstitial fibrosis replacement that contribute to the progressive deterioration of left ventricular function. Benefit resulting from prevention of such cardiac toxicity by β-adrenoceptor antagonists (CIBIS II, 1999; MERIT-HF, 1999) has clearly been demonstrated by large-scale clinical trials. The mechanisms of the cardiac toxicity induced by stimulation of the β-adrenoceptors remain, however, to be elucidated and especially the role of downregulation of such β-adrenoceptors which should partly prevent such toxicity.

The aim of our study was to analyse the mechanisms underlying cardiac toxicity caused by β-adrenoceptor stimulation in an experimental model of heart failure, with special attention to the relationships between β-adrenoceptor downregulation and modifications of cardiac function and structure, particularly fibrosis replacement that occurs in response to chronic treatment by lma β-adrenoceptor agonist in this model.

2. Materials and methods

2.1. Experimental model and study design

Experiments were performed on 97 male Wistar normotensive rats (centre d'Elevage R. Janvier, Saint Berthevin, France) weighing 220–230 g at the beginning of the study. 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

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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, permission #0299 to MH). Rats were anaesthetized with sodium pentobarbital (60 mg/kg i.p.), and experimental heart failure was induced by myocardial infarction after coronary artery ligation (Pfeffer et al., 1979). Sham-operated animals were subjected to a thoracotomy without coronary ligation.

In order to induce chronic stimulation of β-adrenoceptor starting 10 weeks after coronary artery ligation, rats were subjected to a 2-week administration of isoprenaline through an osmotic mini-pump. ALZET osmotic mini-pumps (model 2002, ALZA, Palo Alto, CA, USA) that delivered saline (0.9% NaCl) or isoprenaline (30 μg/kg/h) were implanted subcutaneously in the back. Animals were divided in two groups: one group ($n = 66$) was used for haemodynamic and morphometric studies, and the other group ($n = 31$) was used for the measurement of papillary muscle contractility and binding studies.

2.2. Haemodynamic evaluation

Twelve weeks after myocardial infarction, animals were anaesthetized with sodium pentobarbital (60 mg/kg i.p.). Left ventricular haemodynamics and blood pressure were recorded using a micro-tip pressure transducer catheter (Millar Instrument) introduced into the left carotid artery. Once haemodynamic study was achieved, the heart was removed, weighed and prepared for morphometric studies.

2.3. Fibrosis quantification

After fixation with formalin and inclusion in paraffin, the heart was cut into coronal slices at three levels, 200 μm apart, from the apex to the base of each ventricle (using a Stiassnie microtome). Transverse sections (6 μm thick) were made at each level and stained with Sirius red for morphometric studies of ventricles and collagen identification.

Quantitative morphometric analyses were performed using an image analyser computer system (Leica Q500IW, Cambridge, UK) coupled to a high-resolution colour camera (JVC KYF 3 ccd, Japan) and to a macroviewer (Kaiser RS1, Germany). Data were further processed with a special computer program conceived from the interactive software QUIPS (Quantimet Image Processing Software; Leica). Several parameters were measured at the three different levels of each ventricle: transverse section area (including the cavities), right and left ventricle cavity area, fibrosis area for each ventricle, interventricular septum and right ventricular wall thickness. Extent of infarcted myocardium was calculated using the mean percentage of infarcted internal and external circumference according to the Pfeffer method (Pfeffer et al., 1979).

2.4. β-Adrenoceptor study

2.4.1. Preparation of left ventricle membranes

The protocol of Communal et al. (1998) was used with slight modifications. All steps were performed at 0–4 °C. Briefly, left ventricles were dissected out and homogenised with a Polytron PT100D apparatus (Touzart-Matignon, Paris, France) at setting 3–4, three times for 10 s each, in 15 volumes (v/w) of 0.05 M Tris–HCl, pH 7.4. Homogenates were centrifuged at $1000 \times g$ for 10 min and supernatants were collected for a second centrifugation at $40{,}000 \times g$ for 10 min. The resulting pellets were resuspended in 15 volumes (v/w) of 0.05 M Tris–HCl, pH 7.4 and centrifuged again at $40{,}000 \times g$ for 10 min. Final pellets were suspended in 5 volumes (v/w) of (in mM) 50 Tris–HCl, 150 NaCl, 10 $MgCl_2$, pH 7.4, and aliquots (0.5 ml) of membrane suspensions (~ 0.4 mg protein/ml) were kept at − 80 °C until use for binding assays.

2.4.2. Binding assay

After thawing at room temperature, aliquots (50 μl) of membrane suspensions were mixed with (in mM) 50 Tris–HCl, 150 NaCl, 10 $MgCl_2$, pH 7.4, in a total volume of 0.5 ml. Samples were supplemented with increasing concentrations (10–200 pM) of [^{125}I]iodocyanopindolol ([^{125}I]ICYP, 2000 Ci/mmol) without (total binding) or with 0.3 μM of the selective β_1-adrenergic receptor 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]ICYP 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. All samples were incubated for 60 min at 37 °C, and assays were stopped by rapid vacuum filtration through Whatman GF/B filters, followed by three times 3-ml washes of the filters with ice-cold incubation buffer. Filters were then dried and put into vials for counting entrapped γ radioactivity. 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) with bovine serum albumin as standard. K_d and B_{max} values were calculated from saturation curves using Graphpad and Inplot 4 programs (Fabre et al., 1997).

2.5. Isometric muscle contraction

Once dissected free from the ventricle, the papillary muscle strip was transferred to an oxygenated (95% O_2–5% CO_2, 30 °C) modified Krebs buffer (in mM): NaCl, 113.5; KCl, 4; $CaCl_2$, 1.3; NaH_2PO_4, 1.1; $MgCl_2$, 1.2; $NaHCO_3$, 24; glucose, 11; pH 7.4. The papillary muscle

Table 1
Haemodynamics parameters of sham-operated (sham), isoprenaline-treated sham-operated (Sham + I), myocardial infarcted (MI) and isoprenaline-treated MI (MI + I) rats

Heamodynamic parameters	Sham	Sham + I	MI	MI + I
Heart rate (beats/min)	383 ± 14	465 ± 8[a]	377 ± 13	443 ± 9[a]
Systolic aortic blood pressure (mm Hg)	122 ± 4	142 ± 4[a]	122 ± 6	137 ± 5[a]
Diastolic aortic blood pressure (mm Hg)	103 ± 4	115 ± 3[a]	102 ± 5	113 ± 5[a]
End-diastolic left ventricular blood pressure (mm Hg)	0.6 ± 0.5	3.0 ± 2.0	9.0 ± 3.0[b]	9.0 ± 3.0[b]
Positive dp/dt (mm Hg/s)	7766 ± 402	13600 ± 921[a]	6200 ± 563[b]	9746 ± 701[a,b]
Negative dp/dt (mm Hg/s)	7466 ± 363	9661 ± 810[a]	4584 ± 463[b]	7280 ± 701[a,b]
n	14	15	19	18

Two-way analysis of variance (ANOVA) was performed to determine the statistical significance of differences caused by myocardial infarction and/or isoprenaline administration as well as the interaction between them.
[a] F test: $P<0.05$ for isoprenaline factor.
[b] F test: $P<0.05$ for infarction factor.

was stimulated at 1 Hz (isometric force transducer, E.M.K. Technology), and once stabilized, it was stretched to the length at which contractile force was maximal (L_{max}). All subsequent experiments were performed at L_{max}. Isometric tension was recorded (DATAPAC software) for the quantification of twitch tension, time-to-peak tension and relaxation time. After stabilisation of the muscle, a recording of twitch tension before and after a 3-min rest interval without stimulation was performed and mean contraction and relaxation velocity ratio were calculated. Potentiation of isometric force of contraction after a rest interval is an index for the Ca^{2+}-uptake capacity of the sarcoplasmic reticulum (Terracciano et al., 1995). β-adrenoceptor sensitivity was assessed by an isoprenaline dose–response study (from 10^{-9} to 3×10^{-6} M). Assuming cylindrical geometry and a specific gravity of 1.05, papillary muscle cross-sectional area (CSA) was calculated as CSA = muscle mass/1.05/L_{max}, and the contractile force was expressed as g/mm^2. The maximal force developed under isoprenaline was measured and calculated from each dose–response experiment, using an E_{max} model equation: $E=(E_{max}+C)/(C+EC_{50})$, with sigma-plot software.

2.6. Statistics

Analysis of the different parameters was performed by analysis of variance with two factors: infarction and isoprenaline administration, and interaction between them. Results are expressed as means ± S.E.M. A difference was considered as being statistically significant at $P<0.05$.

2.7. Chemicals

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

3. Results

3.1. Haemodynamic parameters

Myocardial infarction significantly reduced first positive and negative derivates of left ventricular pressure (+ or − dp/dt_{max}) and increased left ventricular end-diastolic pressure. Isoprenaline treatment increased heart rate, systolic and diastolic aortic pressure, left ventricular systolic pressure, positive and negative dp/dt, in both sham-operated and infarcted groups. No interactions between infarction and isoprenaline effects were found: isoprenaline effects were similar in sham-operated and infarcted rats. However, indices of contractility such as dp/dt^+ and dp/dt^- after isoprenaline treatment remained much lower in infarcted rats than in sham-operated animals (see Table 1).

3.2. Morphometric parameters

The extent of myocardial infarction was similar in both infarcted controls and isoprenaline-treated infarcted rats. Area of infarcted tissue was 47% and 46% of left ventricle,

Table 2
Morphometric parameters of sham-operated (sham), isoprenaline-treated sham-operated (Sham + I), myocardial-infarcted (MI) and isoprenaline-treated MI (MI + I) rats

	Sham	Sham + I	MI	MI + I
Body weight (g)	517 ± 10	538 ± 13	548 ± 12[a]	581 ± 11[a]
Heart weight (g)	1.45 ± 0.02	1.74 ± 0.05[b]	2.00 ± 0.10[a]	2.20 ± 0.10[a,b]
Left ventricular weight (g)	1.26 ± 0.02	1.50 ± 0.03[b]	1.53 ± 0.04[a]	1.76 ± 0.05[a,b]
Atrial weight (g)	0.16 ± 0.01	0.23 ± 0.02	0.36 ± 0.04[a]	0.45 ± 0.06[a]
Heart-to-body ratio	0.28 ± 0.01	0.32 ± 0.01[b]	0.35 ± 0.01[a]	0.39 ± 0.02[a,b]
Left ventricular infarction (%)			47.4 ± 2.7	46.4 ± 4.0
Left ventricular fibrosis (%)	0.16 ± 0.02	3.00 ± 0.40[b]	1.30 ± 0.30[a]	2.50 ± 0.30[a,b,c]
Right ventricular fibrosis (%)	0.14 ± 0.03	1.05 ± 0.14[b]	0.97 ± 0.25	1.40 ± 0.20[b]
n	14	15	19	18

Two-way analysis of variance (ANOVA) was performed to determine the statisitical significance of differences caused by myocardial infarction and/or isoprenaline administration as well as the interaction between them.
[a] F test: $P<0.05$ for infarction factor.
[b] F test: $P<0.05$ for isoprenaline factor.
[c] F test: $P<0.05$ for interaction between both.

Table 3
Mean characteristics of β_1- and β_2-adrenoceptor binding sites in left ventricle membranes homogenates from sham-operated (sham) and myocardial infarcted (MI) rats, and of sham and myocardial infarcted rats treated with isoprenaline (Sham + I, MI + I)

	n	β_1		β_2	
		B_{max}	K_d	B_{max}	K_d
Sham	6	28.1 ± 2.5	0.04 ± 0.01	13.3 ± 1.3	0.025 ± 0.003
Sham + I	5	0.036 ± 0.008	23.3 ± 1.5	6.3 ± 1.8[a]	0.034 ± 0.003[a]
MI	6	28.0 ± 3.0	0.02 ± 0.01	16.0 ± 2.0	0.027 ± 0.006
MI + I	6	26.0 ± 2.5	0.050 ± 0.005	9.0 ± 2.0[a]	0.042 ± 0.004[a]

K_d (in nM) and B_{max} (in fmol/mg membrane protein) values are the means ± S.E.M. of five to six independent determinations.
Two-way analysis of variance (ANOVA) was performed to determine the statistical significance of differences caused by myocardial infarction and/or isoprenaline administration as well as the interaction between them.
Other differences are not significant.
[a] F test: $P < 0.05$ for isoprenaline factor.

respectively. Both myocardial infarction and isoprenaline treatment were found to increase left ventricle mass and interstitial fibrosis. A significant interaction was found at this level between isoprenaline and myocardial infarction factors, indicating that the response to isoprenaline administration was different in sham-operated rats (Table 2). This difference appeared to be due to fibrosis, which was already important in the left ventricle of myocardial infarcted animals. In contrast, in the right ventricle, myocardial infarction did not significantly affect fibrosis (Table 2).

3.3. β-adrenoceptor binding sites

Binding assays were carried out in order to measure separately β_1-adrenoceptor and β_2-adrenoceptor (Communal et al., 1998). Data in Table 3 show that myocardial infarction did not significantly alter the characteristics of β-adrenoceptor in the left ventricle. In addition, neither the number of β_1-adrenoceptor nor their affinity (K_d) were changed in rats subjected to chronic isoprenaline treatment. In contrast, this treatment markedly reduced β_2-adrenoceptor density (by about 53% in the sham group and by about 44% in the infarcted group, $P < 0.05$) and increased the K_d value for this receptor subtype (Table 3).

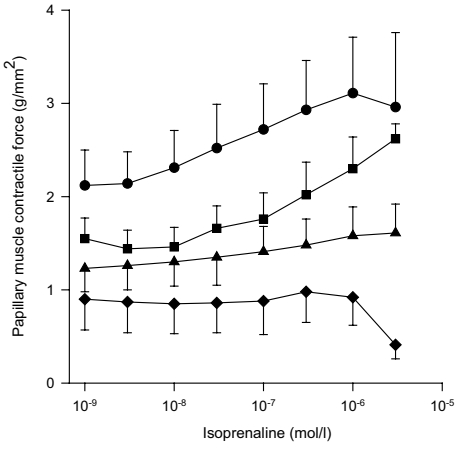

Fig. 1. Papillary muscle contractile response (mean ± S.E.M.) to cumulative isoprenaline concentration in isoprenaline-treated sham ($n = 8$, ▲) compared with control sham ($n = 8$, ●) and in isoprenaline-treated myocardial infarcted rats ($n = 7$, ◆) compared with control myocardial infarcted rats ($n = 8$, ■).

Table 4
Papillary muscle characteristics and contractile amplitude after sham surgery (sham), myocardial infarction (MI) and isoprenaline administration (Sham + I, MI + I)

	n	Papillary muscle cross-sectional area (mm^2)	Contractile force (g/mm^2)	
			Baseline	E_{max}
Sham	8	1.6 ± 0.2	2.0 ± 0.3	3.1 ± 0.6
Sham + I	9	2.0 ± 1.0	1.2 ± 0.3[a]	1.6 ± 0.3[a]
MI	8	2.0 ± 0.3[b]	1.4 ± 0.2	2.3 ± 0.3
MI + I	7	3.0 ± 0.5[b]	0.9 ± 0.3[a]	1.0 ± 0.3[a]

Two-way analysis of variance (ANOVA) was performed to statisitical significance of differences caused by myocardial infarction and/or isoprenaline administration as well as the interaction between them.
[a] F test: $P < 0.05$ for isoprenaline factor.
[b] F test: $P < 0.05$ for infarction factor.

3.4. Papillary muscle contractility

Papillary muscle cross-sectional area was increased by infarction and tended to be increased by isoprenaline ($P = 0.08$). The baseline contractile force tended to be reduced in untreated infarcted group compared to untreated sham group (Fig. 1 and Table 4). In vivo administration of isoprenaline induced an important reduction of the baseline contractile force ($P = 0.03$) and dramatically reduced the in vitro isoprenaline contractile responsiveness ($P = 0.0009$) in both sham and infarcted groups (Fig. 1 and Table 4).

As indicated in Table 5, post-rest potentiation of contractile force was markedly attenuated by infarction ($P = 0.02$) and infarction tended also to attenuate post-rest potentiation of relaxation velocity. Isoprenaline did not modify these parameters.

Table 5
Post-potentiation of papillary muscle contractile force, of contraction and relaxation velocity

	Sham	Sham + I	MI	MI + I
Contractile amplitude ratio	3.1 ± 0.3	3.2 ± 0.3	2.7 ± 0.4[a]	1.8 ± 0.3[a]
Mean contractile velocity ratio	2.8 ± 0.6	3.0 ± 1.0	2.2 ± 0.8[a]	1.7 ± 0.6[a]
Mean relaxation velocity ratio	2.4 ± 0.8	2.9 ± 1.3	2.6 ± 1.1	1.7 ± 0.8
n	8	9	8	7

Ratios of the values recorded before and after 3-min rest interval without stimulation.
Two-way analysis of variance (ANOVA) was performed to determine statistical significance of differences caused by myocardial infarction and/or isoprenaline administration as well as the interaction between them.
[a] F test: $P < 0.05$ for infarction factor.

4. Discussion

Heart failure is characterized in this experimental model of myocardial infarction by an increased end-diastolic left ventricular pressure, an alteration of contractility, left ventricular and atrial remodeling with compensatory hypertrophy and fibrosis. Administration of isoprenaline partially corrected haemodynamic alterations but increased hypertrophy and especially extent of left and right ventricular fibrosis despite the occurrence of a downregulation of the β_2-adrenergic subtype. In addition, contractile responsiveness to in vitro β-adrenoceptor stimulation was almost abolished after chronic in vivo administration of isoprenaline.

It may be considered that β-adrenoceptor desensitization in congestive heart failure is a protective mechanism that counteracts deleterious effects induced by chronic sympathetic activation (Sato et al., 1997) and that blockade of β-adrenoceptors in heart failure reinforces the protective effect of desensitization which occurs early during chronic β-adrenoceptor stimulation (Vatner et al., 1999). In control infarcted animals, the sympathetic tone increase assessed by plasma norepinephrine is not important and does not induce any modification of β-adrenoceptor density (Bonnefont-Rousselot et al., 2002). Therefore, we induced a chronic catecholamine administration. However, in our experiments, β_1-adrenoceptor density was not significantly reduced after a 2-week treatment with isoprenaline which corresponds to a high level of β-adrenoceptor stimulation. In contrast, β_2-adrenoceptor density was greatly reduced (about -50%) with decreased affinity.

We can conclude, therefore, that the cardiac toxicity induced by β-adrenoceptor stimulation on this experimental model was predominantly related to stimulation of β_1-adrenoceptors. Such β_1-adrenoceptor-related fibrosis appears to be the consequence of chronic ischaemia secondary to the high energetic cost leading to necrosis and fibrosis replacement process. Since β_2-adrenergic subtype is stimulated by epinephrine during heart failure, it was not clear, up to now, whether toxic effects such as myocardial fibrosis, could be rather induced by β_1- or β_2-adrenergic subtype. Our experiments clearly demonstrate that fibrosis can occur despite the desensitisation of β_2-adrenoceptors. Such results are in agreement with the demonstrated benefit obtained in human heart failure with selective β_1-adrenoceptor antagonists such as bisoprolol or metoprolol (CIBIS II, 1999; MERIT-HF, 1999).

The mechanisms involved in desensitization in heart failure include a decrease in β-adrenoceptor density (Bristow et al., 1986) caused by a reduction of mRNA encoding the receptor (Ungerer et al., 1993), the uncoupling of β-adrenoceptor from Gs, resulting in a decrease in high-affinity agonist binding (Kiuchi et al., 1993), an increase in the levels of inhibitory $G_{\alpha i2}$ protein (Feldman et al., 1988; Bohm et al., 1990; Eschenhagen et al., 1992), a decrease in adenylyl cyclase activity involving the catalytic unit (Ishikawa et al., 1994) and an increase in β-adrenoceptor kinase which phosphorylates and downregulates the β-adrenoceptor (Benovic et al., 1988). Most studies indicate that in failing human hearts, downregulation seems to be more prominent for the β_1-adrenergic subtype, because norepinephrine is the physiologic neuromediator. However, our study failed to show any significant downregulation of β_1-adrenoceptor or alteration in their affinity in isoprenaline-treated animals. In contrast, β_2-adrenoceptors were markedly downregulated. This differential behavior of β_1-adrenoceptor and β_2-adrenoceptor could not be explained by some difference in their affinity for isoprenaline, because both receptor subtypes share the same affinity for this agonist (Nanoff et al., 1989). Nevertheless, numerous studies suggested that β_1-adrenoceptors are more resistant to downregulation than β_2-adrenoceptors. Thus, Nanoff et al. (1989), looking at β-adrenergic receptor subtypes after in vivo infusion of isoprenaline in rats, found that the resulting downregulation was markedly larger for β_2-adrenoceptor (72%) than for β_1-adrenoceptor (40%) in crude myocardial membrane preparations. Molenaar et al. (1990) reported that 7-day infusion with isoprenaline in the guinea pig reduced β_2-adrenoceptor density by 80% in all tissue compartments of the heart (including atrial and ventricular myocardium, cardiac valves and aorta), whereas no changes in β_1-adrenoceptor density were observed in these tissues. Another study (Zhao and Muntz, 1993) applying the same treatment to rats also showed a 60–70% decrease in β_2-adrenoceptor in atrial and ventricular myocytes, coronary arterioles and connective tissue, but no changes in β_1-adrenoceptor density in any of these tissues.

The significant loss of in vitro effect of isoprenaline on the contractility of papillary muscle from rats that had been treated with this mixed β_1, β_2 and β_3 agonist is in contrast with the absence of β_1-adrenergic desensitisation and could result from different mechanisms. Firstly, it might be due to uncoupling of the remaining (not downregulated) β-adrenoceptors. However, in both sham and infarcted animals, in vivo isoprenaline administration produced marked increases in heart rate, dp/dt^+ and dp/dt^- and left ventricular systolic pressure which do not support this hypothesis. Alternatively, the loss of contractile response of papillary muscle to isoprenaline in vitro after in vivo treatment with this agonist might be related to ischaemia (Ward and Moffat, 1996; Harding et al., 1995; Van Den Ende et al., 1991). Indeed, in vitro preparation of papillary muscle is very sensitive to ischemia and oxygen diffusion. Since papillary muscle taken from rats previously treated with isoprenaline which was hypertrophic (as shown by the increased cross-sectional area) exhibited an increased content of fibrosis tissue throughout the entire ventricular muscle, oxygen diffusion might have been much more limited in such papillary muscles in vitro, thereby explaining, at least partly, their altered contractile force and maximal response to isoprenaline. Another hypothesis could be a predominant distribution of β_2-adrenoceptor on epicardial layers which are first reached by diffusion of isoprenaline in vitro on such isolated papillary muscle preparation. The high level of downregulation of β_2-adrenoceptor

could then participate in the loss of contractile response. In addition, sarcoplasmic reticulum function appeared altered in papillary muscles from rats with myocardial infarction, as assessed by the contractile force ratio after rest of papillary muscle without any stimulation for 3 min. Such an alteration of sarcoplasmic reticulum function in heart failure is in accordance with previous demonstration of a decreased expression of specific ATPase in this tissue (sarcoplasmic reticulum ATPase or SERCA) (Keller et al., 1995; Schomisch-Moravec et al., 1995). In contrast, sarcoplasmic reticulum function was not altered by isoprenaline infusion in sham animals and was not further altered by this treatment in infarcted rats. Therefore, chronic β-adrenoceptor stimulation does not seem to affect the functional characteristics of the sarcoplasmic reticulum.

The isoprenaline dose–response curve showed a negative inotropic effect at a concentration of 3×10^{-7} mol/l in isoprenaline-treated infarcted group. This negative inotropic effect may be due to β_3-adrenergic receptor activation (at this concentration) through the stimulation of a nitric oxide synthase that leads to an increase in nitric oxide production and intracellular cGMP as shown in human heart (Gauthier et al., 1998) and through a decrease in the calcium transient as shown in guinea pig heart (Kitamura et al., 2000). However, rat and guinea pig are classified as hyporesponders to β_3-adrenoceptor agonist stimulation in the myocardium (Gauthier et al., 1998). Moreover, the β_3-adrenoceptor study revealed a complexity in its pharmacology in the myocardium illustrated by a pronounced interspecies variability and heterogenous pharmacological profiles of β_3-adrenoceptor agonists in a given species (Gauthier et al, 1998).

Because the benefit of β-adrenoceptor antagonists treatment in heart failure could be related to the antagonism of the toxicity induced by chronic stimulation of β-adrenoceptors, the purpose of our study was to investigate further the mechanism of such toxicity and the relationship with the desensitisation process. Our results strongly suggest that deleterious effects of chronic β-adrenoceptor stimulation, such as fibrosis development, are mediated, for a major part, by β_1-adrenoceptor stimulation.

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References

Benovic, J.L., Bouvier, M., Caron, M.G., Lefkowitz, R.J., 1988. Regulation of adenylyl cyclase-coupled beta-adrenergic receptors. Annu. Rev. Cell Biol. 4, 405–428.

Bohm, M., Gierschik, P., Jakobs, K.H., Pieske, B., Schnabel, P., Ungerer, M., Erdmann, E., 1990. Increase of G_i alpha in human hearts with dilated but not ischemic cardiomyopathy. Circulation 82, 1249–1265.

Bonnefont-Rousselot, D., Mahmoudi, A., Mougenot, N., Varoquaux, O., Le Naour, G., Fouret, P., Lechat, Ph., 2002. Catecholamine effects on cardiac remodeling, oxidative stress, fibrosis and apoptosis in experimental heart failure. Redox Rep. 3, 145–151.

Bristow, M.R., Ginsburg, R., Umans, V., Fowler, M., Minobe, W., Rasmussen, R., Zera, P., Menlove, R., Shah, P., Jamieson, S., Stinson, E.B., 1986. β_1- and β_2-adrenergic receptor subpopulations in nonfailing and failing human ventricular myocardium: coupling of both receptor subtypes to muscle contraction and selective β_1-receptor down-regulation in heart failure. Circ. Res. 59, 297–309.

Communal, C., Ribuot, C., Durand, A., Demenge, P., 1998. Myocardial β-adrenergic reactivity in pressure overload-induced cardiac hypertrophy in the rat. Fundam. Clin. Pharmacol. 12, 590–598.

Effect of metoprolol CR/XL in chronic heart failure: Metoprolol CR/XL Randomised Intervention Trial in Congestive Heart Failure (MERIT-HF), 1999. Lancet 353, 2001–2007.

Eschenhagen, T., Mende, U., Nose, M., Schmitz, W., Scholz, H., Haverich, A., Hirt, S., Doring, V., Kalmar, P., Hoppner, W., 1992. Increased messenger RNA level of the inhibitory G protein α subunit $G_{i\alpha 2}$ in human end-stage heart failure. Circ. Res. 70, 688–696.

Fabre, V., Boni, C., Mocaer, E., Lesourd, M., Hamon, M., Laporte, A.M., 1997. [^{3}H]Alnespirone: a novel specific radioligand of 5-HT1A receptors in the rat brain. Eur. J. Pharmacol. 337, 297–308.

Feldman, A.M., Cates, A.E., Veazey, W.B., Hershberger, R.E., Bristow, M.R., Baughman, K.L., Baumgartner, W.A., Van Dop, C., 1988. Increase of the 40,000-mol wt pertussis toxin substrate (G protein) in the failing human heart. J. Clin. Invest. 82, 189–197.

Garg, R., Packer, M., Pitt, B., Yusuf, S., 1993. Heart failure in the 1990s: evolution of a major public health problem in cardiovascular medicine. J. Am. Coll. Cardiol. 22, 3A–5A.

Gauthier, C., Leblais, V., Kobzik, L., Trochu, J.N., Khandoudi, N., Bril, A., Balligand, J.-L., Le Marec, H., 1998. The negative inotropic effect of β_3-adrenoceptor stimulation is mediated by activation of a Nitric Oxide Synthase Pathway in human ventricle. J. Clin. Invest. 102, 1377–1384.

Gillum, R.F., 1993. Epidemiology of heart failure in the United States. Am. Heart. J. 126, 1042–1047.

Harding, S.E., MacLeod, K.T., Davies, C.H., Wynne, D.G., Poole-Wilson, P.A., 1995. Abnormalities of the myocytes in ischaemic cardiomyopathy. Eur. Heart J. 16 (Suppl. I), 74–81.

Ishikawa, Y., Sorota, S., Kiuchi, K., Shannon, R.P., Komamura, K., Katsushika, S., Vatner, D.E., Vatner, S.F., Homcy, C.J., 1994. Downregulation of adenylyl cyclase types V and VI mRNA levels in pacing-induced heart failure in dogs. J. Clin. Invest. 93, 2224–2229.

Keller, E., Schomisch-Moravec, C., Bond, M., 1995. Altered subcellular Ca^{2+} regulation in papillary muscles from cardiomyopathic hamster hearts. Am. J. Physiol. 268, H1875–H1883.

Kitamura, T., Onishi, K., Dohi, K., Okinaka, T., Isaka, N., Nakano, T., 2000. The negative inotropic effect of β_3-adrenoceptor stimulation in the beating guinea pig heart. J. Cardiovasc. Pharmacol. 35, 786–790.

Kiuchi, K., Shannon, R.P., Komamura, K., Cohen, D.J., Bianchi, C., Homcy, C.J., Vatner, S.F., Vatner, D.E., 1993. Myocardial β-adrenergic receptor function during the development of pacing-induced heart failure. J. Clin. Invest. 91, 907–914.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 19, 265–275.

Molenaar, P., Smolich, J.J., Russell, F.D., McMartin, L.R., Summers, R.J., 1990. Differential regulation of beta1- and beta2-adrenoceptors in guinea pig atrioventricular conducting system after chronic (−)-isoproterenol infusion. J. Pharmacol. Exp. Ther. 255, 393–400.

Nanoff, C., Freissmuth, M., Tuisl, E., Schutz, W., 1989. A different desensitization pattern of cardiac β-adrenoceptor subtypes by prolonged in vivo infusion of isoprenaline. J. Cardiovasc. Pharmacol. 13, 198–203.

Pfeffer, M.A., Ofeffer, J.M., Fishebein, M.C., Fletcher, P.J., Spardaro, J., Kloner, R.A., Braunwald, E., 1979. Myocardial infarct size and ventricular function in rats. Circ. Res. 44, 503–512.

Sato, N., Vatner, S.F., Shen, Y.-T., Kudej, R.K., Ghaleh-Marzban, B., Uechi, M., Asai, K., Mirsky, I., Patrick, T.A., Shannon, R.P., Vatner, D.E., 1997. Effects of cardiac denervation on development of heart failure and catecholamine desensitization. Circulation 95, 2130–2140.

Schomisch-Moravec, C., Keller, E., Bond, M., 1995. Decreased inotropic response to beta-adrenergic stimulation and normal sarcoplasmic reticulum calcium stores in the spontaneously hypertensive rat heart. J. Mol. Cell. Cardiol. 27, 2101–2109.

Terracciano, C.M., Naqvi, R.U., MacLeod, K.T., 1995. Rest interval on the release of calcium from the sarcoplasmic reticulum in isolated guinea pig ventricular myocytes. Circ. Res. 77, 354–630.

The Cardiac Insufficiency Bisoprolol Study II (CIBIS II): a randomized trial, 1999. Lancet 353, 9–13.

Ungerer, M., Bohm, M., Elce, J.S., Erdmann, E., Lohse, M.J., 1993. Altered expression of beta-adrenergic receptor kinase and beta1-adrenergic receptors in the human heart. Circulation 87, 454–463.

Van Den Ende, R., Batink, H.D., Pfaffendorf, M., Van Zwieten, P.A., 1991. Discrepancies between inotropic responses and beta-adrenoceptor characteristics after global ischemia in isolated hearts. J. Cardiovasc. Pharmacol. 18, 679–686.

Vatner, D.E., Asai, K., Iwase, M., Ishikawa, Y., Shannon, R.P., Homcy, C.J., Vatner, S.F., 1999. Beta-adrenergic receptor-G protein-adenylyl cyclase signal transduction in the failing heart. Am. J. Cardiol. 83, 80H–85H.

Ward, C.A., Moffat, M.P., 1996. Signal transduction mechanisms in the ischemic and reperfused myocardium. EXS 76, 191–207.

Zhao, M., Muntz, K.H., 1993. Differential downregulation of β_2-adrenergic receptors in tissue compartments of rat heart is not altered by sympathetic denervation. Circ. Res. 73, 943–951.