Myocardial hypertrophy, cardiac β -adrenoceptors and adenylate cyclase activity during sinoaortic denervation in dogs

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1 The long-term effects of sinoaortic denervation on the development of left ventricular hypertrophy (assessed by the measurement of the ratio (R): heart weight/total body weight and LVT: left ventricular thickness), myocardial β -adrenergic receptivity (measured by [¹²⁵I]-cyanopindolol binding and adenylate cyclase activity) and plasma catecholamine levels (measured by h.p.l.c.) were investigated in three groups of dogs: normotensive controls (group 1), dogs made hypertensive by sinoaortic denervation and evaluated 1 (group 2) and 18 months (group 3) later.

2 Noradrenaline (NA) and adrenaline (A) plasma levels were 461 ± 54 and 85 ± 45 pg ml⁻¹ in controls, 861 ± 185 and 191 ± 23 pg ml⁻¹ in group 2 ($P < 0.05$). They were normal in group 3 (426 ± 132 and 110 ± 16 pg ml⁻¹).

3 R and LVT values were significantly ($P < 0.05$) higher in sinoaortic denervated dogs ($R = 7.7 \pm 0.1$ and 7.8 ± 0.2 ; $LVT = 13.6 \pm 1.3$ and 14.2 ± 0.9 mm in groups 2 and 3 respectively) than in normotensive dogs (group 1: $R = 6.7 \pm 0.1$, $LVT = 9.3 \pm 0.8$ mm).

4 In group 1, the total number of β -adrenoceptors (B_{max}) was 37 ± 11 and 29 ± 6 fmol mg⁻¹ protein in the left ventricle (LV) and right auricle (RA) respectively. In group 2, B_{max} was significantly lower (10 ± 3 in LV and 13 ± 2 fmol mg⁻¹ protein in RA, $P < 0.05$) than in group 1. There was no difference between group 1 and group 3 (37 ± 3 fmol mg⁻¹ prot in LV and 31 ± 3 fmol mg⁻¹ protein in RA).

5 The percentage of β_1 -adrenoceptors was 82 ± 4 in LV and 75 ± 5 in RA in group 1. It was significantly lower ($P < 0.05$) in groups 2 (LV: 33 ± 6 and RA: 33 ± 5) and 3 (LV: 59 ± 3 and RA: 55 ± 4).

6 Basal values of adenylate cyclase activity in LV significantly decreased after sinoaortic denervation. Isoprenaline alone or in the presence of a β_2 -adrenoceptor antagonist (ICI 118551, 1 μ M) failed to stimulate adenylate cyclase activity 1 (but not 18) month after sinoaortic denervation.

7 These data show that sinoaortic denervation is associated with left ventricular hypertrophy which appears early (1 month) and persists until 18 months despite the normalization of plasma catecholamine levels. The total number of myocardial β -adrenoceptors is closely related to catecholamine levels but a selective decrease in β_1 -adrenoceptors is observed during cardiac hypertrophy. The fall in basal adenylate cyclase activity suggests that cardiac hypertrophy is associated with an impairment of transmembrane signalling.

Keywords: Sinoaortic denervation; cardiac hypertrophy; catecholamines; β -adrenoceptors; adenylate cyclase

Introduction

In arterial hypertension, pressure overload is associated with left ventricular hypertrophy. The β -adrenergic receptivity plays an important role in myocardial function and its alteration during heart hypertrophy may be involved in the process leading to congestive cardiac failure (Saragoca & Tarazi, 1981). Although many authors have investigated β -adrenergic receptivity during left ventricular hypertrophy due to pressure overload, the data remain controversial. Most of the earlier studies concluded that a decrease in myocardial β -adrenoceptor density occurred with a decrease in the inotropic response to catecholamines in rat pressure overload models (Ayobe & Tarazi, 1983). In contrast, in other species an increased β -adrenoceptor number was reported (Vatner *et al.*, 1985). These conflicting results may be explained by differences in the experimental models as well as in the mechanisms of pressure overload.

However, when investigating β -adrenergic receptivity in heart, it must be kept in mind that β -adrenoceptors are submitted to an homologous regulation by catecholamine plasma levels and sympathetic nervous system activity (Hoffmann & Lefkowitz, 1982). Thus, chronic sinoaortic denervation in the

dog may represent an interesting model for the study of β -adrenergic regulation during left ventricular hypertrophy. In fact, we previously found that sinoaortic denervation in the dog induces sustained hypertension and a transient increase in catecholamine plasma levels during the first two months (Valet *et al.*, 1988; 1989). Moreover, in this experimental model, a negative correlation exists between plasma catecholamine levels and β -adrenoceptor number in lymphocytes (Valet *et al.*, 1988; 1989).

Since no data are available on myocardial β -adrenoceptors and cardiac hypertrophy in sinoaortic denervated dogs, the aim of the present study was to investigate plasma catecholamine levels, myocardial hypertrophy, cardiac β -adrenoceptors and adenylate cyclase activity 1 and 18 months after sinoaortic denervation.

Methods

Sinoaortic denervation in dogs

Eighteen beagle dogs of either sex weighing 12–25 kg were studied. Sinoaortic denervation involved two successive surgical procedures as previously described (Valet *et al.*, 1988; 1989). On the day of the first procedure the animals were anaesthetized with alpha-chloralose (80 mg kg⁻¹, intravenously (i.v.)). After intubation of the trachea, they were

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ventilated with a Palmer respirator (London, England). Under sterile surgical conditions, a lateral neck incision was made on the right side. The carotid arteries and sinuses were isolated, the carotid sinus nerve identified and transected with an ophthalmic cauteriser. Moreover, in order to prevent any reinnervation, the carotid bifurcation was destroyed by sectioning the common artery (proximal to the bifurcation) and both internal and external carotids (above their origin). The unilateral surgical procedure did not induce neurological disturbances since, in dogs, the blood supply to the brain mainly comes from the vertebral arteries. During the same experimental procedure, the aortic depressor nerve in the cervical region was also sectioned on the same side: the sheath of the vagus was dissected, the aortic depressor nerve isolated and identified by electrical stimulation and a 4 cm segment of aortic depressor nerve was removed to prevent any reinnervation. During this surgical procedure care was taken to keep intact both the vagal and the sympathetic fibres in the vagus. This procedure was repeated for the left side seven weeks later. The effectiveness of baroreceptor denervation was assessed every month after sinoaortic denervation by the failure of noradrenaline (0.5, 1 and $2 \mu\text{g kg}^{-1}$, i.v.) and phenylephrine (0.1, 1 and $10 \mu\text{g kg}^{-1}$, i.v.) to induce bradycardia. Moreover, nitroglycerin (1, 3, 10 and $30 \mu\text{g kg}^{-1}$, i.v.) induced a dose-dependent decrease in blood pressure without any change in heart rate, thus confirming the absence of baroreceptor pathways. Six sham-operated beagle dogs (group 1) weighing 10 to 25 kg were also prepared: under chloralose anaesthesia, two successive lateral neck incisions without lesion of the aortic depressor or carotid sinus nerves were performed. They were killed 18 months later using an overdose of pentobarbitone. Dogs subjected to sinoaortic denervation were killed under similar experimental conditions 1 month (group 2, $n = 6$) and 18 months (group 3, $n = 6$) after surgery. All investigations described in this paper were conducted in conformity with the guide for the care and use of laboratory animals as adopted and promulgated by the National Institute of Health.

Blood pressure and heart parameters

Blood pressure and heart rate were recorded in conscious animals by means of a catheter introduced into the abdominal aorta via the femoral artery under local anaesthesia (xylocaine 5%) and connected to a Gould P23ID transducer on a Honeywell recorder. Heart rate was obtained by use of a heart period meter triggered by blood pressure.

Heart weight was determined on freshly collected organs. The results are expressed using the ratio (R): heart weight (g)/total body weight (kg). The left ventricle was then dissected and left ventricular thickness (LVT) was measured with a vernier between the epicardial and the endocardial borders along the left ventricle free wall, below the mitral leaflets and above the mitral pillar.

Plasma catecholamine determination

For plasma catecholamine determinations, 5 ml of fresh blood were collected from conscious dogs on lithium heparin with 10 mM sodium metabisulphite and centrifuged (2000 g, 10 min, 0°C). Plasma was stored at -80°C . Catecholamines were selectively isolated from the sample at 4°C , in the darkness, by adsorption on activated alumina, then eluted with 0.1 M perchloric acid. Dihydroxybenzylamine was used as an internal standard to monitor recovery from this extraction step. Noradrenaline and adrenaline were assayed by high performance liquid chromatography (h.p.l.c.) using electrochemical (amperometric) detection: the working electrode potential was set at 0.65 V against a Ag/AgCl reference electrode. Catecholamines were separated on a C18 Waters column (3.9×150 mm) at a constant flow rate of 1 ml min^{-1} . Under these conditions, the detection limit was 10 pg ml^{-1} (Senard *et al.*, 1990).

Preparation of cardiomyocyte membranes

Muscular tissue was obtained from the 4 cardiac cavities after the dogs were killed. After accessory tissues were discarded by dissection, cardiomyocytes were minced and homogenized in an ice-cold lysing buffer containing 5 mM Tris-HCl, 5 mM EDTA (pH 7.5) with an Ultraturrax device. The homogenate was then resuspended in a solution containing 320 mM sucrose, 50 mM Tris-HCl, 0.5 mM MgCl_2 (pH 7.5) and centrifuged (100 g, 10 min, 4°C). The supernatant was then carefully collected and centrifuged (31 000 g, 10 min, 4°C). The resultant pellet was resuspended and washed twice in binding buffer (50 mM Tris-HCl, 120 mM NaCl, 0.5 mM MgCl_2 , pH 7.5). The membranes were frozen at -80°C until analysis. Assays were generally performed within 48 h after freezing.

Binding studies

All experiments were performed in binding buffer (see above) in a total volume of 200 μl . Membranes were incubated under constant shaking in the presence of increasing [^{125}I]-cyanopindolol concentrations ranging from 10 pM to 400 pM for 60 min at 37°C . For determination of total β -adrenoceptor number, non-specific binding was defined in the presence of (–)-adrenaline 10^{-4} M. Determination of β_1 - and β_2 -adrenoceptor subtypes was obtained through competition experiments. A fixed concentration of [^{125}I]-cyanopindolol (at least twice the K_d) was added to each sample in the presence of increasing concentrations of displacing drug. Two selective antagonists were used: bisoprolol (a β_1 -adrenoceptor selective antagonist) and ICI 118551 (a selective β_2 -adrenoceptor antagonist). Ritanserin (10^{-5} M) was added to all samples. The reaction was stopped by addition of 4 ml of ice cold buffer. The separation of free and bound ligand was carried out by filtration through GF/C glass fibre filters under vacuum. The filters were washed twice with 10 ml of buffer and then placed in vials. The radioactivity retained on the filters was counted in a Beckmann scintillation counter at an efficiency of 82%.

Computer-assisted analysis of binding data was performed using the EBDA-Ligand programme (McPherson, 1985). Analysis of competition curves was performed by use of a two-site model according to nH value. The protein concentration was determined as described by Bradford (1976) with serum albumin used as standard.

Adenylate cyclase activity

Adenylate cyclase activity was measured in left ventricle membranes by the method of Salomon *et al.* (1974). Briefly, 20 to 30 μg of membrane proteins were incubated in a 100 μl final volume of 40 mM Tris-HCl (pH 7.4) containing 100 μM EGTA, 0.1 μM guanosine 5'-triphosphate (GTP), 1.5 mM MgCl_2 , 1 mM adenosine 3':5'-cyclic monophosphate (cyclic AMP), 0.5 mM isobutylmethylxanthine (IBMX), 0.2 mM adenosine 5'-triphosphate (ATP), 5 mM creatine phosphate, 70 iu ml^{-1} creatine kinase, 0.2% bovine serum albumin and 0.5–1 μCi [$\alpha^{32}\text{P}$]-ATP. The stimulation of adenylate cyclase activity was obtained either with increasing doses of isoprenaline alone or in the presence of 1 μM ICI 118551. Incubations were carried out during 10 min at 30°C and the reaction was stopped by adding 20 μl HCl 2.2 N and 10 nCi [^3H]-cyclic AMP (pH 7.4). The incubates were poured on to Dowex columns and eluted with 100 mM ammonium acetate buffer (pH 7.4). The radioactivity contained in the eluate was counted in liquid scintillation spectrophotometer. The results are expressed as pmol of formed cyclic AMP per mg of protein.

Data analysis

Results are presented as mean values $\pm$ s.e.mean. Statistical analysis was performed by the non parametric Mann-Whitney U test. A P value <0.05 was considered as significant.

Drugs and reagents

[¹²⁵I]-cyanopindolol (RAS: 2000 Ci mmol⁻¹) and [³H]-cyclic AMP were purchased from Amersham (Amersham, England), [^α-³²P]-ATP from New England Nuclear (England). Bisoprolol was obtained from Merck Clevenot laboratories, ICI 118551 (erythro-DL-(7-methylindan-4-yloxy)-3-isopropyl-aminobutan-2-ol) from ICI (Cheshire, U.K.), (–)-adrenaline from Sigma laboratories (Sigma France) ketanserin from Research Biochemical Inc (Ma, USA) and ritanserin from Janssen laboratories. All other drugs were of reagent grade.

Results

Blood pressure and catecholamine plasma levels

Cardiovascular parameters were measured in conscious dogs after a resting period of at least 60 min. As previously reported (Valet *et al.*, 1988; 1989), bilateral sinoaortic denervation induced a significant rise in blood pressure and heart rate after one month which persisted 18 months later (Table 1). Noradrenaline and adrenaline plasma levels were significantly higher one month after sinoaortic denervation than before surgery. In contrast, 18 months later, both plasma noradrenaline and adrenaline levels were in the normal range (Table 1).

Ratio heart weight/total body weight

In normotensive controls, R ratio (heart weight/total body weight) was 6.7 ± 0.1 . In dogs studied 1 month after sinoaortic denervation, R was significantly greater than in controls (7.7 ± 0.1 , $P < 0.05$). Eighteen months later, it remained significantly higher than in controls (7.8 ± 0.2 , $P < 0.05$) but did not differ from the value observed after 1 month. LVT rose significantly in sinoaortic denervated dogs after a 1 (13.6 ± 1.3 mm, $P < 0.05$) or 18 (14.2 ± 0.9 mm, $P < 0.05$) months follow-up when compared with controls (9.3 ± 0.8 mm). Moreover, the weight of the dogs was not significantly modified one and eighteen months after the surgical procedure.

Cardiac β-adrenoceptors

Preliminary experiments were performed in order to check the selectivity of [¹²⁵I]-cyanopindolol binding on myocardial membranes. When saturation experiments were realized in standard conditions (i.e. in the presence of (–)-adrenaline for non-specific binding determination). Scatchard plots were hardly interpretable because of high non-specific binding values (around 75% at K_d value). This high non-specific binding can be explained by the fact that, first [¹²⁵I]-cyanopindolol is retained on the GF/C filters (this could be abolished by incubating the filters in presence of 1 mM polyethylene-imine) and secondly [¹²⁵I]-cyanopindolol binds to additional binding sites different from β-adrenoceptors. Competition experiments carried out with ritanserin and ketanserin in presence of 100 μM (–)-adrenaline indicated that the two drugs were able to inhibit [¹²⁵I]-cyanopindolol binding but with very low affinity, suggesting the existence of

Figure 1 Inhibition of [¹²⁵I]-cyanopindolol binding in membranes from left ventricle of normotensive dogs by increasing concentrations of ritanserin (●), ketanserin (○), bisoprolol (□) and ICI 118551 (■). The experiments were performed in the presence of adrenaline (10 μM) in order to avoid any interaction of the radioligand with β-adrenoceptors. Each point is the mean of six separate experiments performed in duplicate.

a non β-adrenoceptor specific binding. These data disagree with the pharmacological definition of 5-HT₂ receptors. Moreover, under these selective conditions ICI 118551 (but not bisoprolol) inhibited [¹²⁵I]-cyanopindolol binding (Figure 1). Thus, further experiments were performed in the presence of 10 μM ritanserin in all samples in order to avoid [¹²⁵I]-cyanopindolol interacting with this non β-adrenoceptor specific binding.

In dogs studied 1 month after sinoaortic denervation, the number of β-adrenoceptors in the four measured cardiac cavities was significantly lower than that measured in controls. The decrease was around 50% except in the left ventricle where it reached 75%. In contrast, the number of cardiac β-adrenoceptors in dogs submitted to sinoaortic denervation for 18 months was similar to control values. No change in K_d values was observed in any of the groups (Table 2). Under these experimental conditions, the Hill number was always near unity indicating that [¹²⁵I]-cyanopindolol interacts with a homogeneous class of binding sites.

The respective percentage of β₁- and β₂-adrenoceptors was calculated in the right auricle and the left ventricle. In controls, β₁-adrenoceptors represent $75 \pm 5\%$ of the whole number of β-adrenoceptors in the right auricle and $82 \pm 4\%$ in the left ventricle. One month after sinoaortic denervation, the percentage of β₁-adrenoceptors was $33 \pm 5\%$ in the right auricle and $33 \pm 6\%$ in the left ventricle ($P < 0.05$ when compared with controls). After 18 months, the percentage was $55 \pm 4\%$ in the right auricle and $59 \pm 3\%$ in the left ventricle ($P < 0.05$ when compared with controls).

Adenylate cyclase activity

In the left ventricle of normotensive dogs, isoprenaline increased adenylate cyclase activity in a dose-dependent

Table 1 Systolic (SABP) and diastolic (DABP) arterial blood pressures, heart rate (HR) and plasma catecholamine levels (NA: noradrenaline, A: adrenaline) in normotensive controls (group 1) and sinoaortic denervated dogs studied 1 month (group 2) and 18 months (group 3) after sinoaortic denervation

	SABP (mmHg)	DABP	HR (b min ⁻¹)	NA (pg ml ⁻¹)	A (pg ml ⁻¹)
Group 1	148 ± 11	63 ± 5	85 ± 8	461 ± 54	85 ± 45
Group 2	202 ± 12*	101 ± 9*	130 ± 7*	861 ± 185*	191 ± 23*
Group 3	209 ± 11*	119 ± 5*	123 ± 5*	426 ± 132	110 ± 16

All measurements were obtained in conscious animals after a 60 min rest. Results are expressed as mean values ± s.e.mean; $n = 6$ in each group; * $P < 0.05$ when compared with group 1.

Table 2 Maximal number of β -adrenoceptors ($B_{\max}$, fmol mg⁻¹ protein) and dissociation constant at equilibrium (K_d , pM) in the four cardiac cavities in normotensive control (group 1) and sinoaortic denervated dogs studied 1 month (group 2) and 18 months (group 3) after sinoaortic denervation

	Group 1		Group 2		Group 3	
	$B_{\max}$	K_d	$B_{\max}$	K_d	$B_{\max}$	K_d
Right auricle	29 ± 6	68 ± 5	13 ± 2*	40 ± 4	31 ± 3	55 ± 10
Left auricle	43 ± 3	61 ± 6	20 ± 4*	68 ± 8	42 ± 2	68 ± 5
Right ventricle	22 ± 3	61 ± 9	10 ± 2*	45 ± 10	21 ± 2	70 ± 6
Left ventricle	37 ± 11	72 ± 5	10 ± 3*	66 ± 8	37 ± 3	69 ± 4

The results are expressed as mean ± s.e.mean; $n = 6$ in each group; * $P < 0.05$ when compared with group 1.

manner (Figure 2a). The activity of adenylate cyclase following selective β_1 -adrenoceptor activation was measured in the presence of ICI 118551 (1 μ M), a selective β_2 -adrenoceptor blocker. In the presence of ICI 118551, isoprenaline stimulated adenylate cyclase with a similar affinity ($EC_{50} = 268 \pm 36$ nM) and efficiency as isoprenaline alone ($EC_{50} = 195 \pm 29$ nM) (Figure 2b).

In dogs studied one month after sinoaortic denervation, basal activity of adenylate cyclase was significantly ($P < 0.05$) decreased (129 ± 12 pmol mg⁻¹ protein) when compared with controls (178 ± 14 pmol mg⁻¹ protein). Stimulation of adenylate cyclase activity by isoprenaline was significantly lower than in controls (Figure 2a), however, a slight increase in the EC_{50} value was observed (660 ± 79 nM). In the presence of 1 μ M ICI 118551, isoprenaline failed to induce any activation of adenylate cyclase (Figure 2b).

In dogs studied 18 months after sinoaortic denervation, basal activity of adenylate cyclase was dramatically decreased

(14 ± 2 vs 178 ± 14 pmol mg⁻¹ protein in controls). However, isoprenaline alone or in the presence of ICI 118551 induced a similar increase of adenylate cyclase activity with no significant difference in the EC_{50} (248 ± 47 and 288 ± 52 nM respectively) values when compared to controls (Figure 3).

Discussion

The present results demonstrate that (i) chronic sinoaortic denervation leads to an early development of left ventricular hypertrophy and (ii) total cardiac β -adrenoceptor number is negatively correlated with plasma catecholamine levels but apparently seems unrelated to the cardiac hypertrophy; (iii) the percentage of β_1 -adrenoceptors dramatically decreases 1 month after sinoaortic denervation and does not return to normal values after 18 months and (iv) the basal values of adenylate cyclase activity in LV were significantly decreased after sinoaortic denervation. Isoprenaline alone or in the presence of a β_2 -adrenoceptor antagonist (ICI 118551 1 μ M) failed to stimulate adenylate cyclase activity 1 (but not 18) month after sinoaortic denervation.

The occurrence of left ventricular hypertrophy after sinoaortic denervation suggests that this experimental model is adequate for studying the visceral complications of arterial hypertension. In fact, recent data have shown that this experimental model is associated with renal vascular and glomerular lesions (Orfila *et al.*, 1990). Moreover, the early development of cardiac hypertrophy suggests that this phenomenon does not only depend on pressure overload. Other mechanisms may be involved, especially the rise in catecholamine plasma levels. In fact, *in vitro* data indicate that noradrenaline is able to induce protein synthesis in cultured rat myocytes (Simpson, 1983). In human essential arterial hypertension, there is a direct correlation between noradrenaline plasma levels and

Figure 2 Effects of isoprenaline alone (a) or isoprenaline plus 1 μ M ICI 118551 (b) on adenylate activity (in pmol mg⁻¹ of protein) measured in membranes from the left ventricle of 6 normotensive controls (■) and 5 dogs studied 1 month after sinoaortic denervation (●). The results are expressed as mean with s.e.mean shown by vertical bars. * $P < 0.05$ using the Mann-Whitney U test when control animals were compared to sinoaortic denervated dogs.

Figure 3 Effect of isoprenaline (□) or isoprenaline plus 1 μ M ICI 118551 (▲) on adenylate activity (in pmol mg⁻¹ of protein) measured in membranes from the left ventricle of five dogs studied 18 months after sinoaortic denervation. The results are expressed as mean with s.e.mean shown by vertical bars.

left ventricular mass (Corea *et al.*, 1983). In sinoaortic denervated dogs, cardiac hypertrophy can be initiated by the transient rise in catecholamine plasma levels observed in the early stage of hypertension. However, the role of arterial hypertension probably remains important, since, in this model, plasma catecholamines return to normal values within 2 months after surgical procedure (Valet *et al.*, 1988; 1989) whereas cardiac hypertrophy persists 18 months after sinoaortic denervation. At the end of the study, the dogs with left ventricular hypertrophy appeared fully compensated and none of them developed congestive heart failure.

Myocardial β -adrenoceptors were investigated by use of [125 I]-cyanopindolol, a non selective β -adrenoceptor antagonist. This ligand was used because of its high specific radioactivity which allows binding studies to be carried out on small myocardial fragments. Moreover, the β -adrenoceptor number identified with [3 H]-dihydroalprenolol is identical to that obtained with [125 I]-cyanopindolol (data not shown). Preliminary experiments indicated that [125 I]-cyanopindolol binds to β -adrenoceptors but also possesses affinity for additional binding sites. Competition experiments performed with 5-hydroxytryptamine receptor antagonists indicated that these additional binding sites probably correspond to non β -adrenoceptor specific binding sites. Thus, experiments were carried out in the presence of ritanserin (10 μ M) in order to avoid any interaction of [125 I]-cyanopindolol with these additional sites.

Saturation experiments show that the number of myocardial β -adrenoceptors is decreased one month after sinoaortic denervation. In contrast, it returned to normal values after 18 months. These changes can be related to plasma catecholamine levels and not to myocardial hypertrophy. In fact, during the hyperadrenergic state (i.e. after 1 month), the density of β -adrenoceptors is decreased in the four cardiac cavities whereas only the left ventricle is subjected to pressure overload. Although we did not measure the number of β -adrenoceptors per ventricular myocardial cell, the present data suggest that the decreased β -adrenoceptor density is due to a down-regulation secondary to the rise in plasma catecholamines. In the same experimental model, Valet *et al.* (1989) reported a decrease in lymphocyte β -adrenoceptors in the early stage of hypertension followed by a return to normal values when plasma levels of catecholamines are normal. The lack of direct relationship between myocardial hypertrophy induced by pressure overload and the number of β -adrenoceptors is supported by the fact that $B_{\max}$ returned to normal values after 18 months in spite of unmodified left ventricular hypertrophy.

These data could explain the controversial results found in the literature concerning β -adrenoceptors and myocardial hypertrophy. In experimental models of arterial hypertension associated with increased sympathetic activity, β -adrenoceptor number was usually found to be decreased. This 'down-regulation' was reported in spontaneously hypertensive rats (Limas & Limas, 1978; Mukherjee *et al.*, 1980; Kuchii *et al.*, 1981; Kumano *et al.*, 1983; Yamada *et al.*, 1984), in rats with renal hypertension (Giachetti *et al.*, 1979; Woodcock & Johnston, 1980; Ayobe & Tarazi, 1983), and in the adrenaline-induced model of cardiac hypertrophy (Upsher & Khairallah, 1985). In contrast, after aortic banding in dogs (Vatner *et al.*, 1984) or in guinea-pigs (Karliner *et al.*, 1980), i.e. two experimental conditions where myocardial catecholamine stores are decreased (Fischer *et al.*, 1955), cardiac hypertrophy is associated with an increase of β -adrenoceptors. In rats with aortic banding, the results still remain controversial. β -Adrenoceptor number was reported to be either increased (Limas, 1979), decreased (Will-Shahab *et al.*, 1986; Chevalier *et al.*, 1989), or

unchanged (Cervoni *et al.*, 1981). In man, no data are available in cardiac hypertrophy due to pressure overload. In hypertrophic cardiomyopathy, a disorder characterized by a non-dilated, hypertrophied left ventricle in the absence of any overt cause, the density of β -adrenoceptors in the right atrial tissue was found to be normal (Wagner *et al.*, 1989) but increased in the left ventricular endomyocardium (Takita *et al.*, 1990). Abnormal adrenergic function has been proposed as a cause of this cardiomyopathy.

In control normotensive dogs, β_1 -adrenoceptors represent 75–80% of the total β -adrenoceptors. In the early stage of sinoaortic denervation a dramatic decrease in the number of β_1 -adrenoceptors was observed with no change in the number of β_2 -adrenoceptors. After an 18 month follow-up, the percentage of β_1 -adrenoceptors increased when compared to values obtained 1 month after sinoaortic denervation but did not return to control values. Thus, the increase in catecholamine plasma levels after sinoaortic denervation induced a specific down-regulation of β_1 -cardiac adrenoceptors. However, the fact that the β_1 -adrenoceptor percentage did not return to basal values after 18 months despite the catecholamine normalization suggests that cardiac hypertrophy is also involved in the observed changes. Similar variations of myocardial β -adrenoceptor subtypes were previously reported after aortic banding in rats (Chevalier & Swynghedauw, 1990) and in spontaneously hypertensive rats (Michel *et al.*, 1987). In man at the final stage of cardiac heart failure (where plasma catecholamines are increased), myocardial β -adrenoceptor number and overall β_1 -adrenoceptor subtypes were found to be significantly reduced (Bristow *et al.*, 1982; Fowler *et al.*, 1986). In other tissues (adipocytes and lymphocytes) from sinoaortic denervated dogs, down regulation preferentially concerns β_2 -adrenoceptors (Valet *et al.*, 1988). These results indicate that the differential regulation of the subtypes of β -adrenoceptor is not only related to plasma catecholamine levels but also varies according to their location. In fat cells the relative proportions of β -adrenoceptor subtypes is very different from that in myocardial cells (respectively 67 and 18% of β_2 -adrenoceptors).

The determination of adenylate cyclase activity first allows further insight into results obtained in the binding studies. In dogs submitted to sinoaortic denervation from 1 month, the stimulation of adenylate cyclase activity induced by either the whole β -adrenoceptor population (isoprenaline alone) or by β_1 -adrenoceptors (isoprenaline plus ICI 118551) was significantly decreased. This phenomenon is no longer observed 18 months later. Taken together (Figure. 2), these data suggest that the stimulation of adenylate cyclase activity in myocardial cells involves mainly β_1 -adrenoceptors. In fact, in the presence of isoprenaline plus ICI 118551, adenylate cyclase activity reached the same maximal values. Basal values of adenylate cyclase activity are lower than controls in 1 month sinoaortic denervated dogs and dramatically decreased 18 months after sinoaortic denervation. These results suggesting that adenylate cyclase activity falls in parallel with the development of left ventricular hypertrophy agree with the recent data of Chen *et al.* (1990) after aortic banding in dogs.

In conclusion, chronic sinoaortic denervation in dogs appears to be a convenient model for studying the regulation of β -adrenoceptors in the hypertrophied left ventricle. Homologous regulation by the increase in endogenous catecholamines during the early stage of sinoaortic denervation would seem to be the main factor for the down-regulation of cardiac β_1 -adrenoceptors. Whilst left ventricular hypertrophy does not appear to regulate β -adrenoceptors directly, it could be involved in the alterations of transmembrane signalling (adenylate cyclase and/or G protein expression).

References

- AYOBE, M.H. & TARAZI, R.C. (1983). Beta-receptors and contractile reserve in left ventricular hypertrophy. *Hypertension*, **5**, 1192–1197.
- BRADFORD, M.M. (1976). A rapid sensitive method for the quantitation of protein utilizing the principle of protein dye binding. *Anal Biochem.*, **72**, 248–254.
- BRISTOW, M.R., GINSBERG, R., MINOBE, W., CUBICCIOTTI, R.S.,

- SAGEMAN, W.S., LURIE, K., BILLINGHAM, M.E., HARRISON, D.C. & STINSON, E.B. (1982). Decreased catecholamine sensitivity and beta-adrenergic receptor density in failing human hearts. *N. Engl. J. Med.*, **307**, 205–211.
- CERVONI, P., HERZLINGER, H., LAI, F.M. & TANIKELLA, T. (1981). A comparison of cardiac reactivity and β -adrenoceptor number and affinity between aorta-coarcted hypertensive and normotensive rats. *Br. J. Pharmacol.*, **74**, 517–523.
- CHEN, L., VATNER, D.E., VATNER, S.F., HITTINGER, L. & HOMCY, C.J. (1990). Decreased Gs α mRNA levels accompany the fall in Gs and adenyl cyclase activities in compensated left ventricular hypertrophy. *J. Clin. Invest.*, **87**, 293–298.
- CHEVALIER, B., MANSIER, P., CALLENS-EL AMRANI, F. & SWYNGH-EDAUW, B. (1989). The beta-adrenergic system is modified in compensatory pressure cardiac overload in rats. Physiological and biochemical evidence. *J. Cardiovasc. Pharmacol.*, **13**, 412–420.
- CHEVALIER, B. & SWYNGHEDAUW, B. (1990). Récepteurs beta-adrénergiques dans l'hypertrophie et dans l'insuffisance cardiaques. In *Hypertrophie et Insuffisance Cardiaques*. ed. Swynghedauw B. pp. 205–216. Paris, INSERM, John Libbey Eurotext.
- COREA, L., BENTIVOGLIO, M. & VERDECCHIA, P. (1983). Echocardiographic left ventricular hypertrophy as related to arterial pressure and plasma norepinephrine concentration in arterial hypertension: reversal by atenolol treatment. *Hypertension*, **5**, 837–843.
- FISHER, J.E., HORST, W.D. & KOBIN, I.J. (1955). Norepinephrine metabolism in hypertrophied rat hearts. *Nature*, **207**, 952–953.
- FOWLER, M.B., LASER, J.A., HOPKINS, G.I., MINOBE, W. & BRISTOW, M.R. (1986). Assessment of the beta-adrenergic receptor pathway in the intact failing human heart: progressive receptor down-regulation and subsensitivity to agonist response. *Circulation*, **74**, 1290–1302.
- GIACHETTI, A., CLARK, T.L. & BERTI, F. (1979). Subsensitivity of cardiac beta-adrenoceptors in renal hypertensive rats. *J. Cardiovasc. Pharmacol.*, **1**, 467–471.
- HOFFMANN, B.B. & LEFKOWITZ, R.J. (1982). Adrenergic receptors in the heart. *Annu. Rev. Physiol.*, **44**, 475–484.
- KARLINER, J.S., BARNES, P., BROWN, M. & DOLLERY, C. (1980). Chronic heart failure in the guinea pig increases cardiac alpha1- and beta-adrenoceptors. *Eur. J. Pharmacol.*, **67**, 115–118.
- KUCHII, M., FUKUDA, K., HANAU, T., OHTANI, H., MOHARA, O., NISCHIO, T. & MASUYAMA, Y. (1981). Changes in cardiac beta-adrenoceptor concentrations in spontaneously hypertensive and experimental renal hypertensive rats. *Jpn. Circ. J.*, **45**, 1104–1110.
- KUMANO, K., UPSHER, M.E. & KHAIRALLAH, P.A. (1983). Beta-adrenergic receptor response coupling in hypertrophied hearts. *Hypertension*, **5**, 1175–1183.
- LIMAS, C. & LIMAS, C.J. (1978). Reduced number of beta-adrenergic receptors in the myocardium of spontaneously hypertensive rats. *Biochem. Biophys. Res. Commun.*, **83**, 710–714.
- LIMAS, C.J. (1979). Increased number of beta-adrenergic receptors in the hypertrophied myocardium. *Biochim. Biophys. Acta*, **588**, 174–178.
- McPHERSON, G.A. (1985). Analysis of radioligand binding experiments: a collection of computer programs for the IBM PC. *J. Pharmacol. Meth.*, **14**, 213–218.
- MICHEL, R.C., WANG, X.L. & SCHLICKER, E. (1987). Increased beta2-adrenoceptor density in heart, kidney and lung of spontaneously hypertensive rats. *J. Auton. Pharmacol.*, **7**, 41–51.
- MUKHERJEE, A., GRAHAM, R.M., SAGALOWSKY, A.I., PETTINGER, W. & MCCOY, K.E.A.I. (1980). Myocardial beta-adrenergic receptors in the stroke prone spontaneously hypertensive rat. *J. Mol. Cell. Cardiol.*, **12**, 1263–1272.
- ORFILA, C., DAMASE-MICHEL, C., RAKOTOARIVONY, J., MONTASTRUC, J.L., MONTASTRUC, P., SUC, J.M. & GIROLAMI, J.P. (1990). Etude des lésions morphologiques rénales au cours de l'hypertension artérielle provoquée par la défenation sinoaortique chez le chien. *Arch. Mal. Coeur*, **83**, 543.
- SALOMON, V., LONDOS, C. & ROBBELL, M. (1974). A highly sensitive adenylate cyclase assay. *Anal. Biochem.*, **58**, 541–548.
- SARAGOCA, M.A. & TARAZI, R.C. (1981). Left ventricular hypertrophy in rats with reno-vascular hypertension. Alterations in cardiac functions and adrenergic responses. *Hypertension*, **3**, II171–II178.
- SENARD, J.M., BARBE, P., ESTAN, L., GUIMBAUD, R., LOUVET, J.P., BERLAN, M., TRAN, M.A. & MONTASTRUC, J.L. (1990). Decreased high affinity state in platelet alpha2-adrenoceptors from diabetic patients with orthostatic hypotension. *J. Clin. Endocrinol. Metab.*, **71**, 311–317.
- SIMPSON, P. (1983). Norepinephrine-stimulated hypertrophy of cultured rat myocardial cells is an alpha1-adrenergic response. *J. Clin. Invest.*, **72**, 732–738.
- TAKITA, T., IKEDA, J., SAKUMA, T. & ASUGI, M. (1990). The relation between human myocardial hypertrophy and beta-adrenergic receptors evaluated by autoradiography. *Circulation*, **82**, III-389 (Abst. 1544).
- UPSHER, M.E. & KHAIRALLAH, P.A. (1985). Beta-adrenergic receptors in rat myocardium during the development and reversal of hypertrophy and following chronic infusions of angiotensin II and epinephrine. *Arch. Int. Pharmacodyn. Ther.*, **274**, 65–79.
- VALET, P., DAMASE-MICHEL, C., MONTASTRUC, J.L. & MONTASTRUC, P. (1989). Plasma catecholamines and adrenoceptors after chronic sinoaortic denervation in dogs. *Fundam. Clin. Pharmacol.*, **3**, 307–320.
- VALET, P., MONTASTRUC, J.L., BERLAN, M., TRAN, M.A., LAFONTAN, M. & MONTASTRUC, P. (1988). Differential regulation of fat cell beta2- and beta1-adrenoceptors by endogenous catecholamines in dog. *J. Pharmacol. Exp. Ther.*, **249**, 271–277.
- VATNER, D.E., HOMCY, C.J., SIT, S.P., MANDERS, W.T. & VATNER, S.F. (1984). Effects of pressure overload, left ventricular hypertrophy on beta-adrenergic receptors and responsiveness to catecholamines. *J. Clin. Invest.*, **73**, 1473–1482.
- VATNER, D.E., VATNER, S.F., FUJII, A.M. & HOMCY, C.J. (1985). Loss of high affinity cardiac beta-adrenergic receptors in dogs with heart failure. *J. Clin. Invest.*, **76**, 2259–2264.
- WAGNER, J.A., SAX, F.L., WEISMAN, H.F., PORTEFIELD, J., McINTOSH, C., WEISFELDT, M.L., SNYDER, S.H. & EPSTEIN, S.E. (1989). Calcium-antagonist receptors in the atrial tissue of patients with hypertrophic cardiomyopathy. *N. Engl. J. Med.*, **320**, 755–761.
- WILL-SHAHAB, L., KÜTTNER, I. & WARBANOW, W. (1986). Signal transfer in cardiac muscle. Alteration of the beta-adrenoceptor adenylate cyclase system in the hypertrophied myocardium. *Biomed. Biochim. Acta*, **45**, S199–S204.
- WOODCOCK, E.A. & JOHNSTON, C.I. (1980). Changes in tissue alpha- and beta-adrenergic receptors in renal hypertension in the rat. *Hypertension*, **2**, 156–161.
- YAMADA, S., ISCHIMA, T., TOMITA, T., HAYASHI, M., OKADA, T. & HAYASHI, E. (1984). Alterations in cardiac alpha- and beta-adrenoceptors during the development of spontaneous hypertension. *J. Pharmacol. Exp. Ther.*, **228**, 454–459.

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