

# Prevention of Heart Failure in Rats by Trimetazidine Treatment: A Consequence of Accelerated Phospholipid Turnover?

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Received July 23, 2002; accepted November 7, 2002

## ABSTRACT

Heart failure is known for alteration of cardiac catecholamine responsiveness involving adrenergic receptor (AR) down-regulation. Trimetazidine, a metabolically active anti-ischemic drug, accelerates the turnover of phospholipids. The present study evaluated the consequences of trimetazidine treatment (supposed to increase phospholipid synthesis) on AR in heart failure in rats. In control rats, trimetazidine (7.5 mg/day supplied in the diet) induced after 8 weeks a significant increase in both  $\beta$ - (+54%) and  $\alpha$ -AR (+30%) density, although after 12 weeks, the receptor density was normalized. Heart failure was obtained by ascending aortic banding. These heart failure rats developed a severe cardiac hypertrophy, mainly affecting the left ventricle, which was significantly reduced in the trimetazidine-treated group. The plasma level of brain natriuretic peptide (BNP), a

marker of heart failure severity, was significantly increased in the heart failure group as compared with the sham group (900 and 1200% after 8 and 12 weeks, respectively). In the trimetazidine-treated group, the plasma BNP increase was significantly lower. The development of heart failure was associated with a decrease in  $\beta$ - and  $\alpha$ -AR sites (−23 and −36% versus sham, respectively) after 8 weeks and continued to decrease after 12 weeks (−37 and −48% versus sham, respectively). This down-regulation was prevented by trimetazidine without alteration in affinity. These results suggest that trimetazidine prevents AR desensitization and cardiac hypertrophy, in a pressure-overload model of heart failure. This cytoprotection suggests that membrane homeostasis preservation may be considered as a therapeutic target in the treatment of heart failure.

In heart failure, excessive sympathetic activation is a characteristic feature leading to an alteration of the adrenergic function and therefore a decreased responsiveness to catecholamines. This desensitization is associated with a down-regulation of  $\beta$ -adrenergic receptors (ARs) (Bristow et al., 1982) that is involved in the progression from the compensated cardiac hypertrophy status to the heart failure status as reported in human and experimental animal models (Böhm et al., 1997; Joseph and Gilbert 1998; Anderson et al., 1999). The reduction of cardiac  $\beta$ -AR site number is usually considered to parallel the evolution of the disease and, particularly, the dramatic alteration in cardiac membrane homeostasis capacity. The relationship of adrenergic function and membrane lipid composition has been thoroughly investigated. Alterations in the fatty acid composition of the main phospholipids of rat myocardium have been observed during

aging and after repeated epinephrine administration in rats, and were associated with changes in AR properties (Benediktsdottir et al., 1995, 1999). The down-regulation of  $\beta$ -ARs (decrease in the density of binding sites) appears to be synchronized with the specific changes in the fatty acyl chain composition within the membrane bilayer (Gudbjarnason and Benediktsdottir, 1996). Trimetazidine (1-[2,3,4-trimethoxy-benzyl]piperazine 2 HCl; Laboratoires Servier, Courbevoie, France) is an anti-anginal drug devoid of hemodynamic properties. The drug is known for its cardioprotective effects in the treatment of ischemic cardiomyopathy (Bricaud et al., 1990) and was shown to exert its protective effect at the ventricular myocyte level (Lavanchy et al., 1987; Renaud, 1988; Fantini et al., 1994). Several publications reported that the cytoprotective properties of trimetazidine could be partly attributed to an effect on cellular lipid metabolism. The molecule was shown to decrease the utilization of fatty acids for energy production through a reduction of  $\beta$ -oxidation (Fantini et al., 1994; Kantor et al., 2000), resulting in an increased

Article, publication date, and citation information can be found at <http://jpet.aspetjournals.org>.  
DOI: 10.1124/jpet.102.042143.

**ABBREVIATIONS:** AR, adrenergic receptor; AS, aortic stenosis; Sh, sham; BNP, brain natriuretic peptide; TMZ, trimetazidine; Ct, control; CGP-12177, 4-[3-[(1,1-dimethylethyl)amino]2-hydroxypropoxy]-1,3-dihydro-2H-benzimidazol-2-one; ICI 118,551, ( $\pm$ )-1-[2,3-(dihydro-7-methyl-1H-inden-4-yl)oxy]-3-[(1-methylethyl)amino]-2-butanol hydrochloride; RIA, radioimmunoassay; LV, left ventricle; PI, phosphatidylinositol; PIP<sub>2</sub>, phosphatidylinositol bisphosphate.

contribution of nonlipid substrates, mainly glucose. Furthermore, Sentex et al. (1997) demonstrated that a significant increase of membrane phospholipid synthesis was a major effect of trimetazidine. This beneficial effect on membrane homeostasis through phospholipid turnover induced a significant increase of the incorporation of long-chain polyunsaturated fatty acids in membrane structures (Sentex et al., 1998). More recently, this effect on lipid metabolism was reported to occur in vitro as well as in vivo in the rat in several organs, such as retina, inner ear, and liver (Sentex et al., 2001). This study was designed to test the hypothesis that trimetazidine through acceleration of phospholipid turnover would result in a delayed development of heart failure in the rat, as investigated through AR down-regulation. The experiments were carried out in an animal model of pressure overload, induced by ascending aortic stenosis in rat (Feldman et al., 1993).

## Materials and Methods

### Rat Model of Congestive Heart Failure (Ascending Aortic Stenosis)

Male Wistar rats ( $\approx 60$  g; Iffa Credo, L'Arbresle, France) were anesthetized by i.p. injection of pentobarbital (60 mg/kg b.wt.). The aortic stenosis was induced (AS group) via a left thoracic incision by banding the ascending aorta with a titanium hemoclip (Weck Atraclip, 0.6-mm i.d.; Rüschi Pilling, Le Faget, France) (Feldman et al., 1993). Sham rats (Sh group) were prepared to serve as age-matched controls, by a similar surgical treatment without placement of the clip. Some of the Sh and AS rats were given a daily oral supplement of trimetazidine incorporated in a jellied diet (Rousseau et al., 2001). These groups are referred to as the Sh + TMZ group and the AS + TMZ group. This procedure was designed to avoid the 120 to 180 injections per rat required by the protocol, but did not allow the determination of a plasma peak concentration of trimetazidine, due to the food intake schedule of the rats. The dose of 7.5 mg/day was determined from the literature (Sentex et al., 2001) and a preliminary study of the dose leading to a plasma trimetazidine concentration close to 0.1 to 0.2  $\mu\text{M}$  as determined at 8:00 AM and 8:00 PM. Although different from the usual 1 to 10  $\mu\text{M}$  range used in vitro in acute investigations with trimetazidine (Kantor et al., 2000; Sentex et al., 2001), this concentration is relevant as compared with that reported for therapeutic administration in humans, 0.2 to 0.6  $\mu\text{M}$  for 60 mg/day (Sellier et al., 1987). The treatment was initiated 3 to 4 days after surgery and was maintained for an additional period of 8 or 12 weeks, leading the rats to the age of 12 or 16 weeks, respectively, similar to the literature (Sentex et al., 1998, 2001). In the sham rats, no mortality was observed, although in the AS rats the mortality was close to 25%. This mortality, observed during the 4th week after surgery, is known in this model to be related to the individual capacity to adapt to the pressure increase. It was similar in all the clipped groups. At the end of the 8- or 12-week treatment period, the rats were anesthetized (pentobarbital, 60 mg/kg b.wt. in the sham rats and 30 mg/kg in the AS rats, due to increased sensitivity to anesthetic drugs). Blood was collected in tubes containing EDTA, centrifuged (1000g for 15 min, 4°C), and stored at  $-20^\circ\text{C}$  for determination of plasma brain natriuretic protein (BNP), as a marker of heart failure severity (Hirata et al., 2001). Heart failure was also defined, as described by Desjardins et al. (1988), when the heart weight to body weight ratio in the AS rats was greater than the mean  $\pm 2$  S.D. of Sh rats. A macroscopic necropsy was realized for each animal. This was carried out for descriptive purposes only and was not statistically evaluated. The heart was then withdrawn and separated into ventricles, atria, and septum, which were also weighed (as well as the liver and kidneys). The membranes were isolated for ventricles for determination of AR characteristics. At the

end of the experiments the data were collected from 8 rats in the Sh and the Sh + TMZ groups (at 8 and 12 weeks), 8 and 11 rats in the AS group (8 and 12 weeks, respectively), and 8 and 9 rats in the AS + TMZ group (8 and 12 weeks, respectively).

### Membrane Preparation

The heart was removed and cut into different parts and weighed. The ventricles were homogenized with a Polytron (three times, for 10 s), in 10 ml of ice-cold buffer (50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, pH 7.4). A solution of 2.5 mM KCl (33  $\mu\text{l}/\text{ml}$  buffer solution) and the homogenates were incubated for 15 min at 4°C under agitation to solubilize the myofilaments. The samples were first centrifuged (1,000g, 10 min at 4°C), and the supernatant was removed and centrifuged again at 50,000g for 15 min at 4°C. The pellet was resuspended in buffer (500  $\mu\text{l}/600$  mg of heart weight) and the protein content was determined as described by Lowry et al. (1951). The membrane preparations were stored at  $-80^\circ\text{C}$  for the receptor binding assay.

### Determination of Myocardial $\alpha$ - and $\beta$ -Adrenoceptors

Two experiments were carried out to investigate the effect of trimetazidine on the evolution of ARs in heart failure. The first one was performed on two groups of control rats (Ct and Ct + TMZ), to evaluate the influence of increasing membrane turnover on AR characteristics. The second experiment was performed on three groups of rats: sham (Sh), AS, and AS + TMZ groups, as described above. The  $\beta$ - and  $\alpha$ -adrenoceptor binding assays were carried out on each heart homogenate. The membrane preparations were used at a final concentration of 3.6 mg protein/ml.

**Quantification of Total  $\beta$ -Adrenergic Receptors.** Fractions of 40  $\mu\text{l}$  of membrane preparation were incubated for 1 h at 37°C in the presence of 12 different concentrations of  $^3\text{H}$ -4-[3-[(1,1-dimethylethyl)amino]2-hydroxypropoxy]-1,3-dihydro-2H-benzimidazol-2-one ( $^3\text{H}$ -CGP-12177) (45 Ci/mmol; Amersham Biosciences, Inc., Les Ulis, France) ranging from  $6 \times 10^{-11}$  to  $2 \times 10^{-9}$  M, in a final volume of 400  $\mu\text{l}$  of Tris-HCl buffer. The binding reaction was terminated by the addition of 5 ml of ice-cold washing buffer (5 mM  $\text{KH}_2\text{PO}_4$ , 20 mM  $\text{Na}_2\text{HPO}_4$ , 100 mM NaCl, pH 7.4), and immediately vacuum-filtered through Millipore APFB 02500 glass fiber filters (Millipore, St-Quentin en Yvelines, France). The filters were then rinsed three times with 5 ml of the same buffer and dried, and the bound radioactivity was determined by liquid scintillation counting (Pico-Fluor 40; PerkinElmer Life Sciences, RunGIS, France). The specific binding was calculated by subtracting the nonspecific binding (as evaluated with  $10^{-4}$  M isoproterenol) from the total binding at each  $^3\text{H}$ -CGP-12177 concentration.

**$\beta$ -Adrenoceptor Subtypes.** A  $\beta_2$ -selective antagonist, ( $\pm$ )-1-[2,3-(dihydro-7-methyl-1H-inden-4-yl)oxy]-3-[(1-methylethyl)amino]-2-butanol hydrochloride (ICI 118.551), was used at a concentration of  $10^{-7}$  M to block the  $\beta_2$ -receptors and allow the estimation of the relative proportion of  $\beta_1$  in membranes (Mansier et al., 1993). This proportion was defined as the radioactivity bound to myocardial membranes that was not displaced by a high concentration of ICI 118.551 at a saturation concentration of  $^3\text{H}$ -CGP-12177 ( $10^{-9}$  M).

**Quantification of  $\alpha_1$ -Adrenergic Receptors.** For  $\alpha_1$ -adrenoceptor binding assays, a total of 40  $\mu\text{l}$  of membrane preparation was incubated for 30 min at 37°C, with 12 different concentrations of [ $^3\text{H}$ ]prazosin (80 Ci/mmol; Amersham Biosciences, Inc.) ranging from  $6 \times 10^{-11}$  to  $2 \times 10^{-9}$  M in a final volume of 400  $\mu\text{l}$  of Tris-HCl buffer. The incubation was followed by a rapid vacuum filtration as described above. The specific binding was calculated by subtracting the nonspecific binding (phentolamine,  $10^{-5}$  M) from the total binding. The maximal number of binding sites ( $B_{\text{max}}$ ) and the equilibrium dissociation constant ( $K_d$ ) were calculated by linear regression of the Gauss-Newton method using Micropharm software (Institut National de la Santé et de la Recherche Médicale, Paris, France).

## BNP Determination

The plasma BNP-32 concentration was determined by RIA. BNP-32 was extracted from 2 ml of plasma with 1 ml of Vycor glass suspension (60 mg of activated glass powder/ml deionized water). The absorbed BNP-32 was eluted with 2.5 ml of acetone-water (60:40), containing HCl (0.2%), and the elution fraction was evaporated to dryness. The resulting pellet was reconstituted in 0.5 ml of RIA buffer (0.1 M potassium phosphate, pH 7.4, containing 0.05 M NaCl, 0.1% bovine serum albumin, 0.1% Triton X-100, and 0.01% sodium azide). The BNP-32 antiserum (Peninsula Laboratories, Belmont, CA) showed no cross-reactivity with  $\alpha$ -atrial natriuretic peptide 1-28, endothelin-1, and angiotensin II. An aliquot (0.1 ml) of the extracted fraction was added to 0.1 ml of antiserum and 0.1 ml of RIA buffer. The mixture was incubated at 4°C for 24 h, and 6000 cpm of  $^{125}\text{I}$ -BNP-32 (Amersham Biosciences, Inc.) was added for another 24-h incubation period. The separation of free tracer from antibody-bound tracer was obtained by batch addition of dextran-charcoal and centrifugation at 1200g for 15 min. The radioactivity of supernatant was counted with a gamma counter. The assay detection limit was 10 pg/tube. The normal values for rat plasma were  $29.0 \pm 11.7$  pg/ml, and the interassay and intra-assay variations were 11% and 8%, respectively.

## Statistical Evaluation

The data were expressed as mean  $\pm$  S.E.M. According to the experiment, they were submitted to a two- or three-way analysis of variance, including banding (AS versus Sh) and trimetazidine treatment and, when necessary, duration (8 versus 12 weeks) as fixed factors (Dagnelie, 1975). When significantly different, the means were compared with the Newman-Keuls test.

## Results

### Anatomy Data

**Heart Weight.** In this study, 45 rats underwent ascending aortic banding. At the end of the experiment, 36 rats had survived and developed heart hypertrophy of variable severity. Whatever the group of rats, no significant difference appeared between the 8-week groups and the 12-week groups in heart weight and left ventricle (LV) weight, which indicates the absence of further progress in heart hypertrophy after 8 weeks. The whole heart wet weight was significantly ( $p < 0.01$ ) increased in the AS group versus the sham group ( $2.25 \pm 0.08$  versus  $1.13 \pm 0.02$  g including the 8- and 12-week groups). Similar results were obtained for the LV wet weight group ( $1.10 \pm 0.03$  versus  $0.57 \pm 0.01$  g). The treatment with trimetazidine led to a cardiac hypertrophy, which was significantly ( $p < 0.01$ ) less pronounced in both the whole heart and LV groups ( $1.96 \pm 0.08$  and  $0.92 \pm 0.06$  g, respectively). Conversely, trimetazidine did not influence the heart weight in sham rats. When expressed as heart weight to body weight ratio, the results were similar. However, the development of the cardiac disease induced a body weight progression that was significantly different between the sham and banded rats. Therefore, each of the results of

the 8-week and 12-week series, although statistically different, confirmed both the cardiac hypertrophy and the effect of trimetazidine treatment (Table 1). Moreover, the weight of the right ventricle and atria was also significantly increased in the AS group as compared with the Sh group ( $p < 0.01$ , data not shown).

**Necropsy.** As expected, the sham rats showed no pathological characteristics at necropsy. Conversely, the clinical and anatomical signs of heart failure were apparent in the AS group 8 weeks after banding and were more severe 12 weeks after banding. The rats displayed an accelerated respiration and hyperventilation. Necropsy revealed several trends of a multifocal failure, including congestive liver and renal hypotrophy, hydrothorax and/or ascites, and elevated sensitivity to anesthesia, consistent with a transition from compensated heart hypertrophy to congestive heart failure (Table 2). The number of rats displaying one or more of these pathological alterations was lower in the group of rats treated with trimetazidine (Table 2), suggesting a delayed transition to congestive heart failure. Moreover, the anatomy-based assessment of heart failure showed that 17 of 19 rats in the AS group could be considered as failing heart rats, versus 11 of 17 rats only in the AS + TMZ group (Table 2).

### Brain Natriuretic Peptide

The plasma BNP level was measured in the different groups of rats, and the results are presented in Fig. 1. The plasma BNP concentration was significantly increased in the AS group as compared with the sham group (900% and 1200% after 8 and 12 weeks, respectively). These data confirm the severity of heart failure in this animal model. In the AS + TMZ group, this increase in plasma BNP level was significantly lower after treatment ( $-30\%$  and  $-50\%$  after 8 and 12 weeks, respectively, as compared with the AS group). Moreover, the BNP release appeared to keep increasing between week 8 and week 12 in the AS group, but not in the AS + TMZ group.

### Adrenergic Receptor Study

The first experiment investigated the consequences of increasing lipid membrane turnover on the density of ARs. The receptor density was measured after 8 and 12 weeks in a control group (Ct) and a trimetazidine-treated group (Ct + TMZ). As shown in Fig. 2, the 8-week treatment with trimetazidine induced a significant increase ( $p < 0.05$ ) in both  $\beta$ - and  $\alpha$ -ARs ( $+54\%$  and  $+30\%$ , respectively). However, this increase was transient, since the receptor density returned to basal values after 12 weeks of treatment, as evidenced by the significant cross-interaction. The trimetazidine treatment increased the  $K_{d\alpha}$ . This effect was not statistically observed at either 8 or 12 weeks of treatment, but was significant ( $p < 0.05$ ) over the whole experiment. This effect on the  $\alpha$ -receptor affinity was not influenced by the treatment duration. Con-

TABLE 1

Heart and LV weight to body weight ratio ( $\times 1000$ ) in the four groups, 8 and 12 weeks after aortic banding  
The means displaying different letters in parentheses are significantly different ( $p < 0.01$ ).

	Sh	Sh + TMZ	AS	AS + TMZ
Heart (g)/body (g) after 8 weeks	$2.45 \pm 0.06$ (a)	$2.40 \pm 0.05$ (a)	$5.53 \pm 0.07$ (b)	$4.72 \pm 0.53$ (c)
LV (g)/body (g) after 8 weeks	$1.21 \pm 0.04$ (a)	$1.23 \pm 0.03$ (a)	$2.64 \pm 0.09$ (b)	$2.22 \pm 0.23$ (c)
Heart (g)/body (g) after 12 weeks	$2.23 \pm 0.05$ (a)	$2.36 \pm 0.07$ (a)	$5.00 \pm 0.36$ (b)	$4.14 \pm 0.54$ (c)
LV (g)/body (g) after 12 weeks	$1.14 \pm 0.02$ (a)	$1.22 \pm 0.04$ (a)	$2.47 \pm 0.18$ (a)	$1.93 \pm 0.23$ (c)

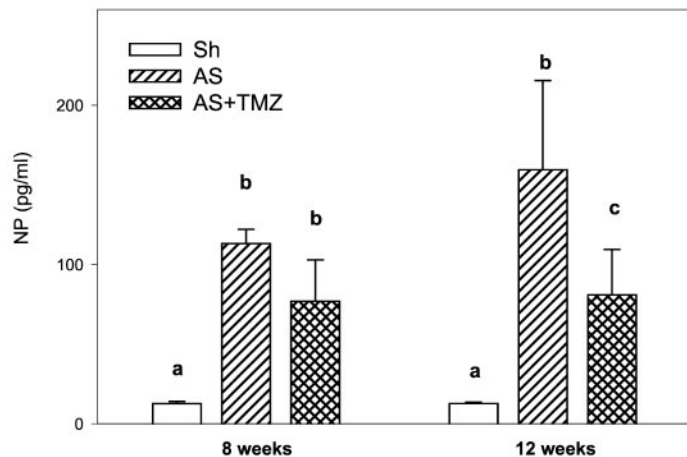
TABLE 2

Observations collected during the necropsy of the rats from the banded groups

The sham rats of the two groups Sh and Sh + TMZ showed no pathological characteristics.

Necropsy Findings	AS (n = 19)	AS + TMZ (n = 17)
Dead at anesthesia	5	1
Hyperventilation	18	13
Renal hypotrophy	11	8
Liver congestion	13	6
Hydrothorax and/or ascites	15	7
Failing heart*	17	11

\* Anatomic description assessed from heart weight, as described under *Materials and Methods*.



**Fig. 1.** Plasma BNP levels. Comparison between sham, AS, and AS + TMZ groups. Values are mean  $\pm$  S.E.M.,  $n = 8$  to 19. The means displaying different letters are significantly different ( $p < 0.01$ ).

versely, neither the affinity of the  $\beta$ -receptors (Fig. 2) nor the  $\beta_1/\beta_2$  proportion was affected by trimetazidine.

The second experiment compared the Sh, AS, and AS + TMZ groups. The data collected from the determinations of binding site density of the ARs 8 and 12 weeks after banding are showed in Fig. 3. In this model of heart failure, cardiac hypertrophy was associated with a marked decrease ( $p < 0.01$ ) in the density of ARs as soon as 8 weeks after banding. This decrease affected both the  $\beta$ - ( $-23\%$ ) and  $\alpha$ - ( $-36\%$ ) ARs when compared with the sham rats. This decrease tended to be more pronounced after 12 weeks and reached  $-37\%$  and  $-48\%$  for  $\beta$ - and  $\alpha$ -receptors, respectively. This effect of banding on receptor density was highly significant ( $p < 0.01$ ). Conversely, in the AS + TMZ group, no significant down-regulation of  $\beta$ -ARs was observed. Although a slight trend to decrease was observed after 12 weeks, the AS + TMZ group was never significantly different from the sham group but was at each time significantly different from the AS group. Similar results were obtained for the  $\alpha$ -ARs. The treatment with trimetazidine completely prevented the down-regulation observed after 8 weeks. At 12 weeks, the down-regulation of the  $\alpha$ -ARs became significant in the AS + TMZ group (versus Sh group) but remained significantly less pronounced than in the AS group.

The  $\beta$ -AR down-regulation was not specific to the  $\beta_1$ -receptor subtype, since the proportion of  $\beta_1$  among total  $\beta$ -adrenergic sites remained unchanged in the AS rats at 8 and 12 weeks of study, with or without trimetazidine (Table 3). Similarly, the aortic banding did not alter the  $K_d$  values of  $\beta$ - and  $\alpha$ -ARs (data not shown).

## Discussion

This study investigated the consequences of a trimetazidine treatment intended to increase the membrane phospholipid turnover on cardiac hypertrophy and AR down-regulation in a heart failure model (Brodde et al., 1986; Summers et al., 1995; Böhm et al., 1997). Myocardial hypertrophy resulting from chronic pressure overload is a major cause of heart failure (Kannel et al., 1972; Böhm et al., 1997). We used an animal model of chronic pressure overload due to ascending aortic stenosis in rats, which induced physiological and morphological signs of heart failure, including breathing acceleration in a quiet environment, hydrothorax and ascites, liver congestion, kidney hypotrophy, and possible renal failure. As a response to cardiac load increase, the rats developed cardiac hypertrophy, as already reported (Feldman et al., 1993). This hypertrophy was due to an increase in both atria and ventricle weight. BNP, a vasodilator peptide secreted in the left ventricle regardless of the degree of left ventricular dysfunction (Yasue et al., 1994), is a strong prognostic predictor and a sensitive marker of ventricular damage and heart failure severity when measured in plasma (Hirata et al., 2001). Plasma BNP increases in response to ventricular overload in patients with congestive heart failure or cardiac hypertrophy (Mukoyama et al., 1991). In patients with dilated cardiomyopathy with both atria and ventricle overload, plasma BNP level increases in proportion to the severity of New York Heart Association classification, the elevation ranging from 16- to 70-fold (Yoshimura et al., 1993). In the present study, the average plasma BNP concentration rose to 9-fold versus sham rats 8 weeks after surgery and reached an average of 12-fold (with a maximum of 30-fold) 12 weeks after surgery. These data confirm the validity of this model to investigate the progression from cardiac hypertrophy to heart failure. Half the animals in the sham and aortic banding groups received an oral dose of trimetazidine (7.5 mg/day), introduced in a specially designed jellied diet. This treatment was designed to avoid giving two i.p. injections per day during the 12-week duration of the experiments, which is stressful and painful especially in sick animals. In a preliminary experiment, we observed that this dietary intake led to a basal plasma trimetazidine concentration ranging from 0.1 to 0.2  $\mu\text{M}$ , a concentration relevant for a chronic trimetazidine supply, since patients receiving 60 mg of trimetazidine per day displayed individual plasma concentrations at peak in the range 0.2 to 0.6  $\mu\text{M}$  (Sellier et al., 1987). The trimetazidine treatment resulted in a significant decrease in cardiac hypertrophy and plasma BNP elevation, which was significant as soon as 8 weeks after surgery. Moreover, the increase in heart weight and BNP was significantly more pronounced at 12 weeks than at 8 weeks after surgery in the trimetazidine-free group, but not in the trimetazidine-treated group. These data suggest a prevention of the progression of cardiac dysfunction. In addition, we observed that the morphological alterations associated with heart failure were less severe in the trimetazidine-treated rats. Trimetazidine significantly lowered the incidence of hyperventilation and renal hypotrophy ( $-20\%$ ), liver congestion, hydrothorax, and/or ascites ( $-50\%$ ), and death induced by anesthesia ( $-80\%$ ).

Increased sympathetic activation contributes to the development of cardiac hypertrophy, and  $\beta$ -adrenergic alteration reflects the limits of the compensatory alterations. The de-

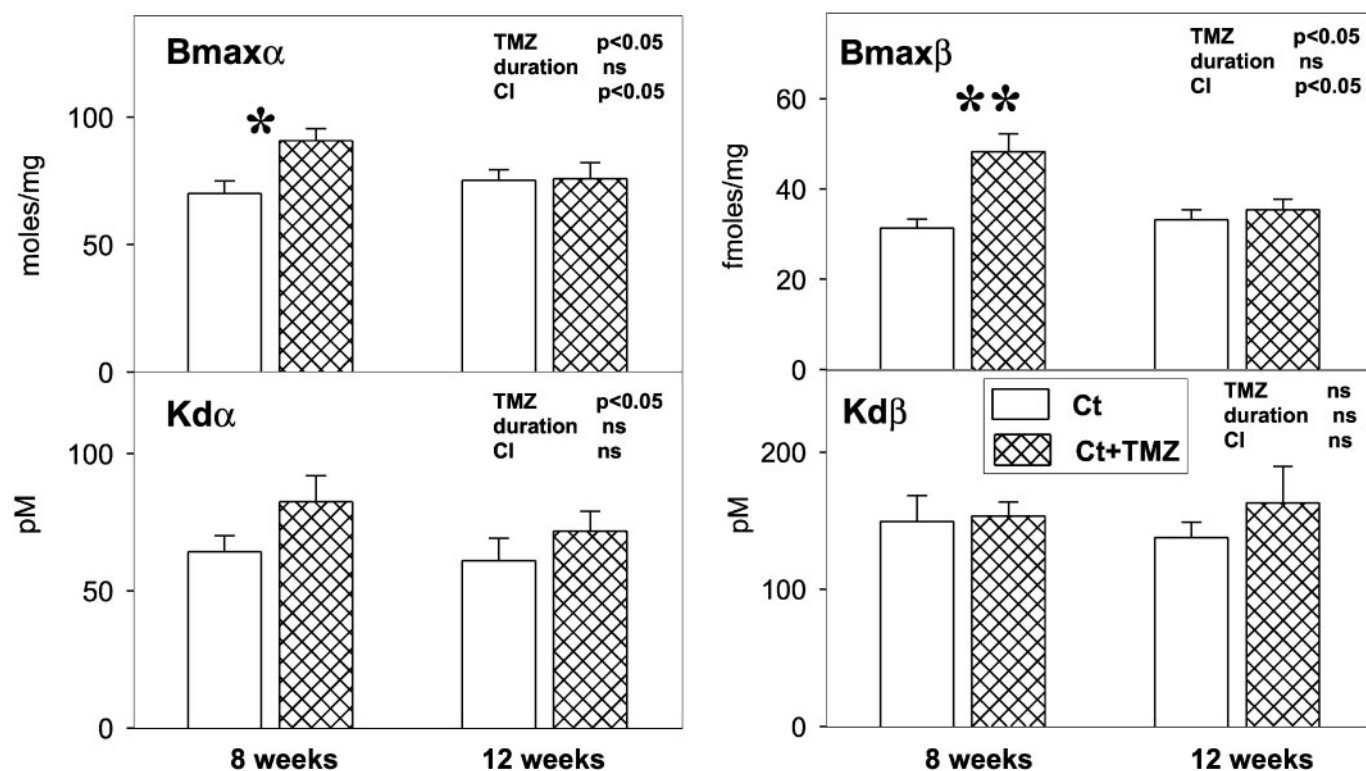


Fig. 2.  $\alpha$ - and  $\beta$ -Adrenergic receptor density ( $B_{max}$ ) and affinity ( $K_d$ ) in healthy control rats, after 8 and 12 weeks of study with or without TMZ treatment. Values are mean  $\pm$  S.E.M.,  $n = 8$ . ns, not significant; CI, cross-interaction; Duration is treatment duration.

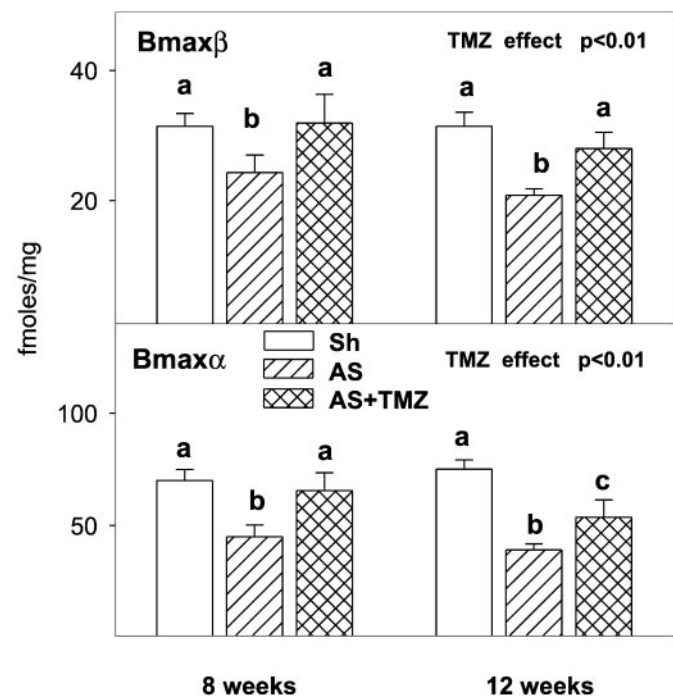


Fig. 3.  $\alpha$ - and  $\beta$ -Adrenergic receptor density ( $B_{max}$ ) 8 and 12 weeks after banding. Comparison between sham, AS, and AS + TMZ groups. Values are mean  $\pm$  S.E.M.,  $n = 8$  to 11. The means displaying different letters are significantly different ( $p < 0.01$ ).

creased  $\beta$ -AR density in heart failure was related to the severity of the disease (Böhm, 1995). In patients with end-stage congestive cardiomyopathy, the number of  $\beta$ -AR sites is markedly reduced, due to a selective down-regulation of  $\beta_1$ -AR, whereas  $\beta_2$ -ARs were not affected (Brodde et al., 1986).

In animal models, the  $\beta_1$ -AR density is altered, whereas a controversy still exists on  $\beta_2$ -AR alteration, based on the etiology of heart failure (Brodde, 1991; Summers et al., 1995). Moreover, although AR alterations were observed in numerous animal models, some differences were reported between humans and animals (Summers et al., 1995). In heart failure resulting from myocardial infarction in rat, the down-regulation is mainly due to a selective  $\beta_1$ -AR decrease without change in  $K_d$  (Rutger et al., 2000). In cardiac hypertrophy due to abdominal ascending aortic banding, the  $\beta_1$ -AR down-regulation occurs together with a reduced  $\beta_1/\beta_2$  ratio (Communal et al., 1998). In this study, as expected, we observed a significant decrease in  $\beta_1$ -AR density, which paralleled the rise in plasma BNP and thus the severity of heart failure. However, this  $\beta$ -AR down-regulation was not specific to the  $\beta_1$ -receptor subtype but affected also the  $\beta_2$ -subtype, since the  $\beta_1/\beta_2$  ratio remained unchanged. Alterations in  $\alpha$ -AR density are not usually considered to occur in the development of heart failure in humans (Bristow, 1993; Brodde et al., 1995; Li et al., 1997). Pressure overload due to abdominal aortic stenosis induces a decrease in  $\alpha_1$ -AR density associated with the earlier stages of cardiac hypertrophy, whereas in later stages no changes were detected (Martinez et al., 1999). To our knowledge, the evolution of  $\alpha_1$ -ARs during the progression from hypertrophy to failure was not documented in the model used in this study, and our results clearly indicate a marked decrease in  $\alpha_1$ -AR density, which parallels  $\beta$ -AR down-regulation.

The decrease in  $\beta$ -AR density was significantly limited by the trimetazidine treatment during the progression from hypertrophy to failure. A similar result was observed for  $\alpha_1$ -AR decrease in response to trimetazidine. The loss in  $\alpha_1$ -AR sites was completely prevented after 8 weeks, and more partially

TABLE 3  
Proportion of  $\beta_1$  subtype among the total  $\beta$ -adrenergic receptor density

	SH	SH + TMZ	AS	AS + TMZ	ANOVA
% $\beta_1$ , 8 weeks	66.7 $\pm$ 2.99	64.1 $\pm$ 2.69	56.7 $\pm$ 4.55	66.3 $\pm$ 4.36	N.S.
% $\beta_1$ , 12 weeks	63.6 $\pm$ 3.55	63.5 $\pm$ 2.33	62.1 $\pm$ 2.35	64.2 $\pm$ 1.99	N.S.

ANOVA, analysis of variance.

after 12 weeks, since the amount of sites was significantly higher than in nontreated rats, but significantly lower than in sham rats. The results of this study clearly demonstrate that the trimetazidine treatment prevented the down-regulation of ARs without any specificity for  $\alpha_1$ -,  $\beta_1$ -, or  $\beta_2$ -subtype, and without affecting their  $K_d$ .

This lack of specificity may be related to the mechanism of the drug, which was never reported for a direct effect on receptors but largely documented for its effect on the membrane phospholipids supporting the ARs. The action of trimetazidine on energy metabolism reported in cultured cells (Fantini et al., 1994) and isolated perfused rat heart (Kantor et al., 2000) could hardly account for the effects reported here. Conversely, the effect on membrane phospholipids may account for the nonspecific improvement of AR density, since the modification of cardiac complex lipid metabolism was reported in vivo in the range 3 to 15 mg/day (Sentex et al., 2001), in accordance with the dose used in this study (7.5 mg/day).

Two pathways are involved in cardiac phospholipid synthesis. The synthesis of phosphatidylcholine and phosphatidylethanolamine occurs partly at the membrane level and partly in the cytoplasm, although the synthesis of PI and cardiolipin occurs at the membrane level. Trimetazidine was shown to increase specifically the synthesis of PI and cardiolipin in isolated cardiomyocytes (Sentex et al., 1998), and in perfused rat heart at a concentration of 1  $\mu$ M (Sentex et al., 2001). The other pathway was stimulated only at higher concentrations.

Although the results of this in vivo study cannot be directly compared with in vitro data, the mechanism reported for an acute treatment with 1  $\mu$ M trimetazidine in the perfusate of isolated rat hearts may serve as a basis for the explanation of the results reported here in a chronic treatment in vivo at a baseline plasma trimetazidine concentration of 0.2  $\mu$ M. Unfortunately, the demonstration of the in vivo alteration in cardiac complex lipid metabolism can be shown in healthy rats (Sentex et al., 2001) but not in heart-failing rats, which do not withstand long-time anesthesia. The evaluation of the membrane phospholipid turnover is now ongoing in our laboratory in isolated perfused failing hearts. Nevertheless, the effect on phospholipid synthesis may account for the trimetazidine effect observed in this study. This view is supported by the results presented here showing that trimetazidine treatment in healthy control rats elicited a nonspecific transient up-regulation of  $\alpha_1$ -,  $\beta_1$ -, and  $\beta_2$ -ARs. Trimetazidine has been used for therapy and research for 35 years, and no effect on AR biology was, to our knowledge, reported. This transient change can be viewed as an adaptation process to the membrane turnover changes. The contribution of trimetazidine to membrane homeostasis during the transition from cardiac hypertrophy to heart failure may thus contribute to the nonspecific prevention of AR down-regulation observed in this study. In turn, these beneficial events can account for the im-

provement of the clinical markers of heart failure, including plasma BNP, and associated cardiac hypertrophy, hydrothorax, ascites, hyperventilation, and renal failure. Among the phospholipids, the increased synthesis of PI may play a specific role in this process. PIs are directly involved in  $\alpha$ -adrenergic signaling and are known to contribute to cell hypertrophy in chronic  $\alpha$ -adrenergic stimulation. In a recent paper, we reported that the increase in PI synthesis by trimetazidine in cultured cardiomyocytes, and the reduced cell availability of Ips, is due to their increased recycling as PI. This decrease in second-messenger availability prevented the cell hypertrophy induced by chronic  $\alpha$ -adrenergic stimulation (Tabbi-Anneni et al., 2003). The mechanism described in this recent paper may explain the results of the present study. The involvement of phosphatidylinositol triphosphate in the recycling of AR in cardiac cells was recently suggested (Sathyamangla et al., 2003). This PI is produced from phosphatidylinositol bisphosphate at the membrane level by PI<sub>3</sub>-kinase. The effect of trimetazidine on PI synthesis would allow the restoration of PIP<sub>2</sub>. During adrenergic over-stimulation, phospholipase C chronic activation decreases membrane PIP<sub>2</sub>, which is its natural substrate but also the substrate of PI3-kinase. The more phospholipase C is active, the less PIP<sub>2</sub> is available for PI<sub>3</sub>-kinase. Since trimetazidine was shown to accelerate the synthesis of PI and hence of PIP<sub>2</sub> (which is in equilibrium with the other PI forms through the PI futile cycle), increasing PIP<sub>2</sub> homeostasis by trimetazidine may lead to improvement of the functional recycling of AR and prevent their down-regulation.

In conclusion, this study demonstrated that a treatment with trimetazidine results in a delayed transition from cardiac hypertrophy to heart failure. The mechanism of this action could be related to the properties of the molecule in increasing phospholipid biosynthesis. The preservation of membrane homeostasis capacity could thus appear as a significant action in the prevention of heart failure resulting from pressure overload, associated with the preservation of a satisfactory membrane homeostasis during the evolution of the disease. Moreover, the membrane target could be useful in the treatment of established heart failure as well, but this topic will require further investigations.

#### Acknowledgments

We are indebted to the Société Française de Pharmacologie for the grant provided to I.T.A., as a part of her Ph.D. thesis, and to Dr. A. Carayon (Hopital de la Pitié, Paris) for plasma BNP determinations.

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