Effect of Enalapril on the Inotropic Response to Isoproterenol in Renal Hypertensive Rats
Hernán Gómez Llambi, Alejandro Mazzadi, María Fontán, and Carlos M. Taquini

It is not clear whether regression of cardiac hypertrophy normalizes cardiac contractility. We studied the effect of enalapril treatment on the contractile response to β-adrenergic stimulation with isoproterenol in renal hypertension. Male Wistar rats (n=28) were divided into a clipped group (n=14) and control group (n=14). Three weeks after surgery, half of the animals from each group received for 21 days either enalapril (2.5 mg/kg) twice a day or vehicle by gastric intubation. Arterial pressure and body weight were measured twice a week. At the end of the experimental period, the hearts were excised, the ventricles were weighed, and the left ventricular papillary muscle was mounted in a bath. Myocardial contractility was characterized by the maximal developed tension, the maximal rate of rise of tension (+T), and the maximal velocity of relaxation (-T), which were measured at basal conditions and after cumulative doses of isoproterenol (10⁻¹¹ to 10⁻⁴ M). The ratio of ventricular weight to body weight increased in hypertensive rats. Enalapril induced a decrease in arterial pressure and in the cardiac mass in both treated groups (p<0.05). The basal values of maximal developed tension, +T, and -T were similar in the four groups. The increment in +T and -T in response to isoproterenol (10⁻⁴ M) was depressed in the hypertensive animals and in both treated groups (p<0.05). There was no significant difference in the +T/-T ratio or in the ED₅₀ among the groups. In conclusion, regression of cardiac hypertrophy after treatment with enalapril is not followed by normalization of the inotropic response to β-adrenergic stimulation, probably through a direct effect of the drug on the β-receptor response. (Hypertension 1992;19[suppl II]:II-125-II-128)

In vivo and in vitro studies have demonstrated that cardiac hypertrophy secondary to experimental hypertension in rats is followed by an attenuation in the contractile response to isoproterenol stimulation.1-3 This blunted response has been attributed to changes in excitation-contraction coupling,3 even though the β-receptor number has been reported to be unchanged, increased, or decreased.3-5

On the other hand, regression of cardiac hypertrophy after normalization of blood pressure by nephrectomy or captopril treatment restores left ventricular contractile response.5 Antihypertensive drugs can also induce regression of cardiac hypertrophy, followed by an improvement in cardiac function.6,7 In this sense, treatment with α-methyl-dopa improves the cardiac function curve in hypertensive rats; however, the left ventricular ability to maintain cardiac output remains diminished when the afterload is increased.8 Accordingly, it has not been clearly established whether some drugs are acting on the regulatory mechanisms of the myocardium or the functional improvement is solely the response to a decreased afterload.

Reversal of cardiac hypertrophy in the two-kidney, one clip rat model by nephrectomy of the clipped kidney or by captopril administration also normalizes the β-receptor number.5 In contrast, in the same model similar regression of cardiac mass after long-term treatment with enalapril failed to normalize the β-receptor concentration. Moreover, an increase in the β-receptor number was observed in treated control rats, raising the possibility that the drug modulates the sympathetic drive to the heart.10

We have previously reported that when enalaprilic acid is added to the bath, the contractile response of the papillary muscle to isoproterenol stimulation is blunted. This diminished response could not be attributed to angiotensin II inhibition.11 The present investigation was designed to elucidate the effect of long-term treatment with enalapril in two-kidney, one clip hypertensive rats on the cardiac contractile response to β-adrenergic stimulation after regression of cardiac hypertrophy.

Methods
Experiments were performed on male Wistar rats (n=28) weighing 270 g. All rats had access to a
standard dry meal and water ad libitum. The animals were divided into two groups: a K group (n=14), in which a silver clip (0.22 mm i.d.) was placed around the left renal artery, and a C group (control, n=14). Twenty-one days after surgery, seven rats from each group received either enalapril (2.5 mg/kg) (KE and CE groups) or vehicle (C and K groups) by gastric intubation twice a day during 3 more weeks. Body weight and arterial pressure (tail-cuff method) were measured twice a week. At the end of the sixth week, 1 hour after enalapril or vehicle administration, the rats were anesthetized with sodium pentobarbital (40 mg/kg i.p.), and the heart was rapidly removed and placed in an oxygenated Ringer’s solution in which a papillary muscle from the left ventricle was dissected. The papillary muscle was mounted vertically in a chamber containing Ringer’s solution of the following composition (mM): NaCl 128.3, KCl 4.7, CaCl2 1.35, NaHCO3 20.23, NaH2PO4 0.35, MgSO4 1.05, and glucose 11. When isoproterenol was used, the solution also contained EDTA (0.045 mM) and ascorbic acid (0.11 mM). The solution was equilibrated with a gas mixture of 5% CO2-95% O2 and pH and temperature were kept constant at 7.4 and 29°C, respectively.

Isometric mechanograms were recorded on a Model RS 11 A (Beckman Instruments Inc., Schiller Park, Ill.) with a Statham force transducer and 9853 coupler (Gould-Statham, Oxnard, Calif.), and first derivative of developed tension was obtained by a 9879 dP/dt coupler (Gould-Statham). Rectangular pulses of 10-msec duration and of an amplitude 20% higher than the threshold of each preparation were delivered from a stimulator (Grass Instrument Co., Quincy, Mass.).

Contraction frequency was kept constant at 12 beats per minute. The papillary muscles were allowed to stabilize for 1 hour after mounting and then were stretched until they reached the length at which maximum developed tension occurred.3 Once the papillary muscles were mounted, the large vessels and the atria were excised from the heart, and the ventricles were weighed. In all the groups, after the stabilization period, a cumulative dose-response curve to isoproterenol (hydrochloride, Sigma Chemical Co., St. Louis, Mo.) from 10^-11 to 10^-4 M was performed. Fast record of developed tension and its first derivative with respect to time were obtained (T). Myocardial contractility was characterized by maximal developed tension and maximal rate of rise of tension (+T). The mentioned parameters, the maximal velocity of relaxation (-T), and the rest tension were measured at basal conditions and after each cumulative dose of isoproterenol. The +T/-T ratio was used to characterize the relaxant effect of isoproterenol.12 At the end of each experiment, the muscle length at which the experiment was performed was measured with a caliper. The muscle was then blotted dry and weighed. The cross-sectional area of the muscle was calculated, assuming that the muscle was a cylinder with a specific gravity of 1.

Data are expressed as mean±SEM. Statistical analysis was performed by analysis of variance and the Newman-Keuls test, as appropriate. A value of p<0.05 was taken as the level of significance.

Results

There was no significant difference in body weight in the four groups, and all animals showed a similar rate of growth during the experimental period (C, 346±6 g; CE, 355±4 g; K, 366±1 g; KE, 360±10 g). The clipped animals became hypertensive in the first week after surgery, with a progressive increase in blood pressure up to 172±5 mm Hg at the end of the third week. During enalapril treatment, blood pressure fell in the hypertensive rats (KE, 115±5 mm Hg) and in the control animals (CE, 95±1 mm Hg). Ventricular mass (ventricular weight/body weight ratio) increased in the hypertensive rats (K, 3.10±0.13 mg/g) when compared with control (C, 2.31±0.04 mg/g). Enalapril treatment regressed cardiac hypertrophy significantly in the KE group (2.30±0.08 mg/g) and reduced cardiac mass in the CE group (2.00±0.03 mg/g) (p<0.05).

There was no significant difference in the basal values of maximal developed tension, +T, and -T in the four groups. The increment in +T and -T, expressed as a percentage of increment with respect to basal values due to cumulative doses of isoproterenol, was significantly attenuated in the K group when compared with the C group (p<0.05). Regression of hypertrophy with enalapril failed to normalize this response in the KE group. Furthermore, in the CE group there was a decrease in contractile and relaxation response to isoproterenol when compared with the C group.

On the other hand, there was no difference among the groups in the relaxant effect of isoproterenol measured by the +T/-T ratio at a concentration of 10^-4 M (C, 1.01±0.06; CE, 1.10±0.07; K, 1.01±0.06; KE, 1.19±0.10). The log ED50 (50% of effective dose) for the inotropic effect of isoproterenol was similar in the different groups (C, -6.60±0.17; CE, -6.05±0.07; K, -6.38±0.20; KE, -6.32±0.10). The cross-sectional area from the papillary muscles was similar in all the groups (C, 0.72±0.06 mm^2; CE, 0.62±0.09 mm^2; K, 0.90±0.08 mm^2; KE, 0.80±0.07 mm^2).

Results are summarized in Figures 1 and 2 and Table 1.

Discussion

Previous observations have demonstrated that normalization of blood pressure by surgical maneuvers or by medical treatment is followed by regression of cardiac hypertrophy.5,7,13 The present results confirm that long-term treatment with enalapril normalizes arterial pressure and induces a reduction in relative ventricular weight in hypertensive animals as well as in treated control rats. Although the decrease in blood pressure could account for the reduction in ventricular mass in both treated groups, the inhibi-
Cardiac hypertrophy secondary to experimental hypertension is followed by an impaired inotropic response to β-adrenergic stimulation in the whole heart and in the isolated papillary muscle. This attenuated response has been ascribed to a post-receptor alteration. On the other hand, regression of left ventricular hypertrophy after normalization of blood pressure by uninephrectomy or captopril administration restored the contractile response in hypertensive rats. In contrast, regression of left ventricular hypertrophy after long-term treatment with enalapril failed to normalize the β-receptor number and, even more, increased the receptor density in treated control rats. Different durations of treatment as well as possible nonequivalent potencies of both drugs could explain this contradictory result. The effect of enalapril on the β-receptors raises the possibility that lack of angiotensin II formation may upregulate the β-receptor concentration through modulation of the sympathetic drive to the heart.

All these data suggest that long-term treatment with enalapril interferes with the β-receptor response and that, in treated animals, mechanisms other than just the number of β-receptors may be involved with the inotropic response to β stimulation. The inhibitory effect of enalapril could not be attributed to a competitive action at the adrenergic recep-
In summary, in renal hypertensive rats, regression of cardiac hypertrophy after long-term treatment with enalapril; K, hypertensive group; KE, hypertensive group pretreated with enalapril.

Arterial pressure shown for the end of experimental period (sixth week). Basal values for maximal rate of rise of tension (+T) and maximal velocity of relaxation (−T) are before isoproterenol; stimulated values to 10^{-4} M isoproterenol expressed as percent of increment with respect to basal values (% +T, % −T). VW/BW, ventricular weight/body weight ratio; C, control group; CE, control group pretreated with enalapril; KE, hypertensive group pretreated with enalapril.


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