Effect of Enalaprilic Acid on Cardiac Contractile Response to β-Adrenergic Stimulation

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Studies in two-kidney–one clip hypertensive rats have demonstrated that long-term treatment with enalapril induced regression of cardiac hypertrophy, but the cardiac contractile response to β-adrenergic stimulation remained depressed. In the present study, we evaluate the contractile response to β-adrenergic stimulation of isolated papillary muscle in normal rats with isoproterenol (10^{-11} M to 10^{-4} M) in the presence of enalaprilic acid (10^{-6} M or 10^{-4} M) or enalaprilic acid (10^{-4} M) and angiotensin II (10^{-4} M). Myocardial contractility was characterized by maximal developed tension and maximal rate of rise of tension (+T), and the relaxant effect of isoproterenol by the ratio of (+T), and the maximal velocity of relaxation (-T)(+T/-T ratio). The rest tension (g/mm²) and the cross-sectional area (mm²) were similar in all the muscles studied. Enalaprilic acid (either 10^{-6} M or 10^{-4} M) in the bath did not induce any change in contractile and relaxation parameters. The increment in +T and -T (expressed as percentage) in response to cumulative doses of isoproterenol (10^{-11} M to 10^{-4} M) was significantly depressed in the presence of enalaprilic acid (10^{-4} M) when compared with control hearts in which only vehicle was added before isoproterenol (p<0.05). The addition of angiotensin II after enalaprilic acid (10^{-4} M) did not normalize the response in +T and -T. Enalaprilic acid diminishes the contractile response of the papillary muscle to β-adrenergic stimulation. The inhibition of the local angiotensin II does not seem to be involved in this result. (Hypertension 1990;15(suppl I):I-51-I-54)

Cardiac hypertrophy secondary to experimental hypertension is followed by an impaired contractile response to β-adrenergic stimulation with isoproterenol.\(^1\)\(^-\)\(^2\) Some investigators have attributed this alteration to a postreceptor alteration. On the other hand, reversal of cardiac hypertrophy after normalization of the blood pressure by uninephrectomy returns the left ventricular contractile response back to normal.\(^3\) Antihypertensive drugs can also induce regression of cardiac hypertrophy followed by an improved cardiac function.\(^4\)\(^-\)\(^5\) It has not been clearly established, however, whether some of these drugs are acting on regulatory mechanisms of the myocardium or the functional improvement is solely the response to the decreased afterload. In this sense, treatment with α-methyl-dopa improves the cardiac function curve of spontaneously hypertensive rats, but the left ventricular ability to maintain the cardiac output is diminished when the afterload is increased.\(^6\)

Long-term treatment of hypertensive rats with enalapril also regresses cardiac hypertrophy, while the β-adrenergic receptors remain elevated\(^6\) and the contractile response to β-adrenergic stimulation remains depressed (H.G. Llambi, C.M. Taquini, A. Mazzadi, A. Gallo, M. Fontan, A.C. Taquini, unpublished observations). Other investigations also indicate that, in the isolated preparation, pre-treatment with converting enzyme inhibitors induces a decrease in the cardiac function to sympathetic stimulation,\(^7\) and in humans, the intracoronary administration of converting enzyme inhibitors induces a decrease in the ejection fraction.\(^8\)

On the other hand, the coexpression of renin and angiotensinogen genes in the heart is strong evidence for the existence of a local endogenous renin-angiotensin system.\(^9\) Also, there are data supporting the existence of a functionally active cardiac angiotensin converting enzyme.\(^10\) Angiotensin II (Ang II), locally generated, could increase cardiac contractility either through a direct physiological action or by the facilitation of sympathetic neurotransmission.\(^11\)\(^-\)\(^13\) Finally, it has been reported...
that in some tissues, β-adrenergic sympathetic stimulation with isoproterenol induces an increase in Ang II release.14

In the present investigation, based on previous information, we have used an isolated left ventricular papillary muscle preparation to analyze the effect of enalaprilic acid on the contractile response to β-adrenergic stimulation with isoproterenol and the role played by local Ang II on any possible alterations.

Methods

Experiments were performed in male Wistar rats (n=25) weighing 330 g. All the rats had access to a standard dry meal and water ad libitum. The rats were anesthetized with sodium pentobarbital (40 mg/kg i.p.), and the heart was rapidly removed and placed in an oxygenated Ringer solution, where a papillary muscle from the left ventricle was dissected. The papillary muscle was mounted vertically in a chamber containing Ringer solution of the following composition (mM): NaCl 128.3, KCl 4.7, CaCl2 1.35, NaHCO3 20.23, NaH2PO4 0.35, MgSO4 1.05, 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 and 95% O2, and the pH and temperature were kept constant at 7.4 and 29° C, respectively. Isometric mechanograms were recorded on a Beckman R511A (Beckman Instrs. Inc, Schiller Park, Illinois) with a Statham force transducer and 9853 coupler (Gould-Statham, Oxnard, California), and the first derivative of developed tension was obtained with 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 Grass stimulator (Grass Instr. Co., Quincy, Massachusetts). Contraction frequency was kept constant at 12 beats/min. 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 (Lmax).2

Four experimental protocols were designed based on the different concentrations and combinations of the drugs used and are as follows: 1) enalaprilic acid 10−6 M (n=6), E6; 2) enalaprilic acid 10−4 M (n=6), E4; 3) enalaprilic acid 10−4 M and angiotensin II 10−8 M (n=6), E4 Ang II; 4) no drugs (vehicle, distilled water), control (n=7) (C). Enalaprilic acid 10−6 M or 10−4 M or Ang II (Sigma Chemical Co., St. Louis, Missouri) 10−6 M were used.

In all the groups, a cumulative dose-response curve to isoproterenol (hydrochloride, Sigma) from 10−11 M to 10−4 M was performed 10 minutes after adding enalaprilic acid, or enalaprilic acid and Ang II, or vehicle.

Fast records of developed tension and its first derivative with respect to time were obtained (T).

Myocardial contractility was characterized by maximal developed tension (dt) and maximal rate of rise of tension (+T). All these parameters, the maximal velocity of relaxation (−T), and the rest tension were measured in basal condition, after addition of enalaprilic acid, enalaprilic acid and Ang II, and after each cumulative dose of isoproterenol. The +T/−T ratio was used to characterize the relaxant effect of isoproterenol.15

At the end of each experiment, the muscle length at which the experiments had been 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.

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

### Results

The cross-sectional area from papillary muscles was similar in the different groups (mm2) (C, 0.78±0.03; E6, 0.78±0.07; E4, 0.73±0.03; E4 Ang II, 0.82±0.07). The rest tension (g/mm2) did not show any difference in the four groups (C, 1.79±0.26; E6, 1.63±0.17; E4, 1.92±0.07; E4 Ang II, 1.7±0.25). Basal contractility values before the addition of enalaprilic acid or Ang II were similar in the different groups. Enalaprilic acid in either dose did not induce any changes in +T, −T, and dt. The increment in +T and −T, expressed as a percentage of the basal values due to cumulative doses of isoproterenol, was lower in the E6 group than in the control (p<0.05). The addition of Ang II (E4 Ang II group) did not normalize the contractile and relax-

| Table 1. Contractile Values After Enalaprilic Acid and After Isoproterenol 10−4 M |
|------------------|-----------|------------------|
| Drug             | Value     | Increment (%)    |
|                  | Basal     | After Drugs      | After Drugs % Increment (10−4 M) |
|                  |           |                  |                                  |
|                  |           |                  |                                  |
| C (n=7)          | dt         | 1.97±0.26        | 25.35±3.65                      |
|                  | +T         | 16.89±1.17       | 75.84±4.8                       |
|                  | −T         | 11.07±1.08       | 158.2±15.00                     |
|                  |            |                  |                                  |
| E6 (n=6)         | dt         | 1.9±0.28         | 17.83±3.39                      |
|                  | +T         | 17.32±2.34       | 60.84±6.43                      |
|                  | −T         | 12.81±1.77       | 123.13±18.16                    |
|                  |            |                  |                                  |
| E4 (n=6)         | dt         | 1.56±0.15        | 17.62±3.21                      |
|                  | +T         | 15.03±1.51       | 40.12±5.56*                     |
|                  | −T         | 13.51±1.35       | 74.86±15.06*                    |
|                  |            |                  |                                  |
| E4 Ang II (n=6)  | dt         | 1.76±0.36        | 15.73±3.23                      |
|                  | +T         | 20.04±3.8        | 37.22±10.1*                     |
|                  | −T         | 19.03±3.42       | 59.07±17.8*                     |

C, control group; E6, enalaprilic acid 10−4 M; E4, enalaprilic acid 10−4 M; E4 Ang II, enalaprilic acid 10−4 M plus angiotensin II 10−8 M; dt, developed tension g/mm2; +T, maximal rate of rise of tension g/mm2/sec; −T, maximal velocity of relaxation g/mm2/sec.

*p<0.05 versus control group.
Changes in $+\tau$ with isoproterenol

### Figure 1

Graph showing percentage of increment in maximal rate of rise of tension ($+\tau$) with isoproterenol ($10^{-11}$ M to $10^{-4}$ M) in the different groups. C, control; $E_6$, group given enalaprilic acid $10^{-6}$ M; $E_4$, group given enalaprilic acid $10^{-4}$ M; $E_4$ Ang II, group given enalaprilic acid $10^{-4}$ M and angiotensin II.

Changes in $-\tau$ with isoproterenol

### Figure 2

Graph showing percentage of increment in maximal velocity of relaxation ($-\tau$) with isoproterenol ($10^{-11}$ M to $10^{-4}$ M) in the different groups. C, control; $E_6$, group given enalaprilic acid $10^{-6}$ M; $E_4$, group given enalaprilic acid $10^{-4}$ M; $E_4$ Ang II, group given enalaprilic acid $10^{-4}$ M and angiotensin II.

Our results demonstrate that enalapril does not modify the basal contractile properties of the rat papillary muscle, but it does decrease the contractile and relaxation responses to $\beta$-adrenergic stimulation with isoproterenol. Furthermore, Ang II did not normalize the blunted response.

In a previous report, we have shown that long-term treatment of two-kidney, one clip (2K1C) hypertensive rats with enalapril-induced regression of cardiac hypertrophy, whereas the number of $\beta$-adrenergic receptors remained elevated, suggesting an up-regulation of these receptors, secondary to a decreased sympathetic drive, probably related to the lack of Ang II formation. Moreover, the fact that the blunted contractile response to $\beta$-adrenergic stimulation observed during cardiac hypertrophy in 2K1C hypertension is normalized when regression of the hypertrophy is obtained with uninephrectomy of the ischemic kidney, and not when it is the consequence of long-term treatment with enalapril (unpublished observations, H.G. Llambi, C.M. Taquini, A. Mazzadi, A. Gallo, M. Fontan, A.C. Taquini), raises the possibility of an alternative interaction between the drug and the adrenergic response. On the other hand, in patients with myocardopathy, characterized by elevated sympathetic tone and increased plasma catecholamines, intracoronary administration of enalapril induces a decrease in ejection fraction, independent of the afterload. A decreased response, in the force of contraction and in heart rate, to norepinephrine infusion and sympathetic nerve stimulation was also reported in the isolated heart preparation from rabbits pretreated with ramipril (HOE 498).

Finally, in the present study, enalaprilic acid decreases the contractile and relaxation response to $\beta$-adrenergic stimulation with isoproterenol without...
inducing any alteration in the basal contractility of the isolated cardiac muscle.

All this information suggests that enalaprilic acid is capable of interfering with the normal inotropic response of the heart to sympathetic stimulation. This effect of the converting enzyme inhibitor could be the result of the inhibition of the local angiotensin converting enzyme or of an interaction between the drug and the agonist at the β-adrenergic receptor.

Renin enzymatic activity was detected in homogenates of whole hearts, and both renin and angiotensinogen messenger RNA were expressed in the arterial and ventricular myocytes. Linz et al measured coronary sinus Ang II formation in the isolated rat heart perfused with angiotensin I (Ang I), and their results showed that Ang I was converted to Ang II. Furthermore, the addition of angiotensin converting enzyme inhibitors prevented this conversion, indicating the existence of a functionally active cardiac angiotensin converting enzyme. The local Ang II can have a positive inotropic effect on the heart by an increase in calcium current, by potentiation of the sympathetic activity, or by both.11-13

On the other hand, local release of Ang II by isoproterenol has been described in isolated perfused mesenteric arteries.14 If a similar β-adrenergic receptor mechanism was also present in the isolated heart preparation, the release of local Ang II could further potentiate the contractile response to isoproterenol. In our experiments, however, the addition of Ang II to the bath after enalaprilic acid failed to restore the normal response to isoproterenol, indicating that the inhibition of Ang II release is not the responsible mechanism.

Enalaprilic acid does not exert a competitive antagonist action at the β-adrenergic receptor because no displacement in the ED50 of the dose-response curve to isoproterenol was observed. Furthermore, the attenuation of the contractile and relaxation response to isoproterenol, without changes in the +T/-T ratio, indirectly indicates the possibility that the action could be searched at the adenylcyclase level.

Enalaprilic acid blunts the contractile and relaxation response to β-adrenergic stimulation with isoproterenol without inducing changes in the basal contractile properties of the heart. The inhibition of the local renin-angiotensin system does not seem to be the mechanism involved in this effect.

References


KEY WORDS  • converting enzyme inhibitors • enalapril • isoproterenol • angiotensin II
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