Role of Kinins and Nitric Oxide in the Antihypertrophic Effect of Ramipril

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Abstract

We examined the effect of non-antihypertensive doses of the angiotensin-converting enzyme inhibitor ramipril, kinins, and/or nitric oxide on left ventricular hypertrophy in rats with aortic coarctation. We investigated the effect of either HOE 140, a specific B2 receptor antagonist, or Nω-nitro-L-arginine methyl ester (L-NAME), a nitric oxide synthase inhibitor, on the antihypertrophic effect of ramipril in rats with aortic coarctation-induced hypertension. Ramipril at non-antihypertensive doses (10 μg/kg per day) failed to alter left ventricular hypertrophy significantly, although a small decrease was obtained. Given at a dose of 1 mg/kg per day for 6 weeks, ramipril prevented increased blood pressure and left ventricular hypertrophy after aortic coarctation. Neither of these effects was blocked by simultaneous administration of HOE 140 (500 μg/kg per day). In rats with aortic coarctation treated with L-NAME, blood pressure increased further but left ventricular weight did not. Ramipril (1 mg/kg per day) significantly reduced left ventricular hypertrophy, although blood pressure was still higher than in rats given water alone. The slope of the correlation between left ventricular weight and blood pressure in rats that received L-NAME was significantly lower than in rats that did not (0.52 versus 1.29; P=0.008). This suggests that for each 1 mm Hg that the blood pressure increased, the increase in left ventricular weight was less in the L-NAME groups. Thus, only antihypertensive doses of ramipril possessed antihypertrophic activity. Kinins did not participate in the chronic antihypertensive and antihypertrophic effects of ramipril. In hypertension induced or aggravated by chronic nitric oxide synthase, L-NAME partially impaired development of left ventricular hypertrophy for reasons that are unclear. (Hypertension. 1994;23[part 2]:865-868.)

Key Words: hypertension • left ventricular hypertrophy • kinins • angiotensin • nitric oxide • angiotensin-converting enzyme • aortic coarctation

The increase in mean blood pressure (MBP) produced by aortic coarctation results in left ventricular hypertrophy (LVH). Both increased blood pressure (BP) and LVH are blocked by an antihypertensive dose of angiotensin-converting enzyme (ACE) inhibitors.1,2 Linz et al3,4 reported that this cardioprotective effect of ACE inhibitors is mediated by kinins, since it was blocked by the kinin B2 receptor antagonist HOE 140. Furthermore, these investigators reported that doses of the ACE inhibitor ramipril that did not decrease BP were still able to abolish the LVH effect of hypertension. This antihypertrophic effect was completely blocked by a B2 kinin antagonist. However, this interesting finding has not yet been confirmed by others. In addition, Farhy et al5 in our laboratory have reported that the protective effect of the ACE inhibitor on neointima formation was partly suppressed by the kinin antagonist HOE 140 or by blockade of NO synthesis with an L-arginine analogue. These findings suggest that the cardiovascular protective effect of ACE inhibitors is partially mediated by inhibition of kinin hydrolysis. Furthermore, the work of Farhy et al suggests that the effect of kinins on neointima formation is mediated by NO acting as an endogenous vasodilator. However, it has not yet been established whether NO plays a similar role against cardiac hypertrophy in vivo. Thus, we studied (1) whether non-antihypertensive doses of ACE inhibitors have cardiac antihypertrophic effects, (2) whether kinins participate in the antihypertrophic effect of the ACE inhibitor ramipril, and (3) whether blockade of NO synthase with Nω-nitro-L-arginine methyl ester (L-NAME) reverses the cardioprotective effect of the ACE inhibitor.

Methods

Male Sprague-Dawley rats (Charles River) weighing 260 to 285 g were housed in an air-conditioned room with a 12-hour light/dark cycle; they were given standard laboratory rat chow (0.4% sodium) and water (alone or mixed with drugs) as follows: 30, 40, and 50 mL per day during the first, second, and subsequent weeks of treatment, respectively. Volume was adjusted weekly to make sure the animals drank all the water offered and consequently ingested all the drug. After 5 days of adjustment to the new environment, rats were divided into nine groups as follows: (1) controls, sham-operated (n=28); (2) aortic coarctation (n=21); (3) aortic coarctation+ramipril, 10 μg/kg per day (n=20); (4) aortic coarctation+ramipril, 1 mg/kg per day (n=13); (5) aortic coarctation+ramipril, 50 μg/kg per day+HOE 140, 500 μg/kg per day (n=5); (6) aortic coarctation+ramipril, 1 mg/kg per day+HOE 140, 500 μg/kg per day (n=11); (7) controls+L-NAME, 100 mg/kg per day (n=12); (8) aortic coarctation+L-NAME, 100 mg/kg per day (n=9); and (9) aortic coarctation+L-NAME, 100 mg/kg per day+ramipril, 1 mg/kg per day (n=16). All surgical interventions were carried out under pentobarbital sodium anesthesia (50 mg/kg IP) and aseptic conditions. The study was approved by the Henry Ford Hospital Care of Experimental Animals Committee.

For aortic coarctation, the abdominal aorta was exposed above the left renal artery and a silk thread passed beneath it. A cannula (outer diameter, 0.749 mm) was placed alongside the aorta, and both were tied; the cannula was then removed, leaving an aortic lumen determined by the diameter of the cannula. Control rats were subjected to the same procedure

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but without aortic coarctation. At the end of surgery, miniosmotic pumps were implanted subcutaneously in the neck to deliver HOE 140 (Hoechst) or saline. Ramipril (Hoechst) or L-NAME (Sigma) was started the day after the operation and continued for 6 weeks via the drinking water. During the first 5 days after the operation, the animals were given tetracycline (1.0 g/350 mL) in their drinking water. Six weeks after surgery, rats were anesthetized with ether, and a polyethylene catheter (PE-50, Clay Adams) filled with heparinized saline was inserted into the thoracic aorta via the left carotid artery; the distal end was tunneled under the skin and brought out at the back of the neck. In some rats, an additional PE-10 fused to a PE-50 catheter was inserted into the left femoral artery for bradykinin challenge. Twenty-four hours later, the conscious rat was placed in a plastic restrainer in a quiet environment, and MBP was recorded on a four-channel recorder (Gould Brush 220) every 1 minute for 90 minutes by a pressure transducer connected to a pressure processor (model MP15, Micron). After MBP was measured, the animals were anesthetized with 50 mg/kg pentobarbital sodium; the heart was excised, cleaned of blood with saline, and gently blotted to dryness, and left ventricular weight (LVW) was determined, including the septum. LVW was normalized for 100 g body wt.

Statistical analysis was performed using Student's two-sample t test to compare MBP and LVW of specified groups. If two groups exhibited unequal variance, Welch's t test was performed. Linear regression analysis was performed on two groups of data: (1) all groups without L-NAME and (2) all groups treated with L-NAME. In addition, the two regression models (slopes) were compared.

Results

As expected, aortic coarctation induced hypertension and LVH. A low dose of ramipril (10 μg/kg per day) did not prevent increased blood pressure (BP) or significantly reduce LVH, whereas a high dose of ramipril (1 mg/kg per day) prevented both increased BP and LVH (Fig 1). The antihypertensive and/or antihypertrophic effects of the ACE inhibitor were not reversed by HOE 140 (500 μg/kg per day), which completely blocked the hypotensive effect of bradykinin (100 ng) administered into the abdominal aorta via the left femoral artery. In untreated rats, the change in BP was -23.0±4.4 mm Hg (n=8), whereas in those treated with ramipril (1 mg/kg per day)+HOE 140, it was 0 mm Hg (n=6).

In normal rats, long-term NO synthase blockade with L-NAME (100 mg/kg per day) was associated with a significant increase in BP (153.7±7.3 mm Hg) compared with rats given plain water (130.6±2.7 mm Hg; P<.01), accompanied by a slight but significant increase in LVW (224.5±8.8 versus 193.4±3.2 mg/100 g body wt; P<.005) (Fig 2). Although BP was not significantly different in rats given L-NAME (153.7±7.3 mm Hg) compared with rats with aortic coarctation given water (157.6±4.0 mm Hg), LVW was about 13% lower in the L-NAME group (224.5±11.3 versus 258.7±12.7 mg/100 g) but did not reach statistical significance (P=.07) (Fig 2). BP among rats with aortic coarctation given L-NAME (179.9±7.9 mm Hg) was higher than in those given water (157.6±4.0 mm Hg; P<.01); however, LVW was not significantly increased (241.4±9.3 versus 258.7±12.7 mg/100 g body wt) or even slightly reduced (Fig 2). A high dose of ramipril (1 mg/kg per day) in rats with aortic coarctation given L-NAME caused a small decrease in BP (172.0±4.0 mm Hg) and significantly reduced LVW (221.5±5.5 mg/100 g body wt; P<.005) compared with those given L-NAME but not ramipril.
be attributed to a decrease in BP and angiotensin II (Ang II) generation, as has been demonstrated in rats with spontaneous hypertension. In aortic coarctation–induced hypertension, the adaptive increase in cardiac mass is characterized as concentric hypertrophy, in which wall thickness increases without chamber enlargement. LVH correlates with BP, suggesting that it is at least partially due to increased afterload; however, a direct effect of the vasopressor hormone Ang II cannot be excluded. Ang II seems to be a stimulus for protein synthesis and thus increased cardiac mass.

In addition, our study confirms that long-term oral administration of L-NAME raises BP,10-12 which is an important determinant of LVH in most rat models of experimental hypertension.13-16 Although BP in rats treated with L-NAME and those with aortic coarctation were still similar, the increase in LVW was less; nevertheless, some degree of cardiac hypertrophy was observed. Thus, these data only partially confirm the observation of Arnal et al.17 that hypertension due to blockade of NO synthesis does not cause cardiac hypertrophy. We also found that although oral administration of L-NAME increased BP further in rats with aortic coarctation, LVW instead decreased slightly. Although we do not know why L-NAME–induced hypertension did not induce severe LVH, it is possible that L-NAME, which acts as a competitive substrate for NO synthase, may also compete with ribosome enzymes involved in the incorporation of L-arginine into proteins. This effect combined with a decrease in coronary blood flow due to the chronic inhibition of NO release may reduce metabolism in cardiocytes, especially the protein synthesis necessary for the adaptive state of the heart during hypertension. It is possible that the endothelium influences cardiocyte function through release of NO and intracellular elevation of cGMP in cardiocytes. Indeed, cGMP has been shown to induce a negative inotropic effect in ferret papillary muscle.18 Thus, complete inhibition of NO synthase with L-NAME may induce a positive inotropic effect by reducing NO and cGMP production. This positive inotropic effect may be another functional adaptation that could replace the structural adaptation of the heart to increased afterload. However, these are only speculations and need to be confirmed.

In conclusion, we have shown that only an antihypertensive dose of ramipril is able to abolish LVH. We also found that a kinin antagonist failed to prevent the antihypertensive and antihypertrophic effects of the ACE inhibitor ramipril. Blockade of NO synthase increased BP in aortic coarctation and slightly decreased LVW, although the reason for this remains to be determined.

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References


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