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Coronary Vascular Actions of the Converting Enzyme Inhibitor, Enalapril[1] (41790)

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Abstract. Enalapril, a potent angiotensin converting enzyme inhibitor, effectively blocked the constrictor actions of angiotensin I in isolated perfused cat coronary arteries. Enalapril, at concentrations of 25 to 100 μg/ml, inhibited angiotensin I by 65 to 80%. Moreover, enalapril at 100 to 200 μg/ml, markedly antagonized the coronary vasoconstrictor effects of angiotensin II. At 150 μg/ml, enalapril blocked the angiotensin II response by 80 ± 5%, and at 200 μg/ml, it was blocked by 95 ± 4%. Enalaprilic acid at 0.5 to 1.0 μg/ml also blocked the angiotensin II response by 94 ± 5%. Captopril, up to 250 μg/ml, failed to significantly antagonize angiotensin II, although it readily blocked angiotensin I in this preparation. The duration of this angiotensin II blockade lasted about 60–90 min. This angiotensin II antagonism may help explain the beneficial effects of enalapril in situations such as acute myocardial ischemia.

Angiotensin II has been shown to increase myocardial contractility under a wide variety of conditions (1, 2). Since angiotensin II is also a potent coronary vasoconstrictor, this humoral mediator increases myocardial oxygen demand as well as restricts myocardial oxygen supply. Both effects would contribute to the exacerbation of myocardial ischemia. In this regard, infusion of angiotensin II for several hours has been shown to induce cardiac lesions characterized by petechial hemorrhages and microvascular effusion (3, 4). It is not therefore surprising that angiotensin converting enzyme inhibitors have been shown to protect against the extension of cardiac injury during the early stages of myocardial ischemia (5, 6). Enalapril, a newly developed angiotensin converting enzyme inhibitor, dramatically protected the ischemic myocardium by reducing myocardial oxygen demand in cats during myocardial ischemia (6). The major purpose of this study was to determine whether enalapril exerts direct coronary vasodilator effects or modulates the coronary vasoconstrictor effects of the renin–angiotensin system.

Methods. *Vascular preparation.* Adult cats were anesthetized with pentobarbital sodium (30 mg/kg) intravenously and a midline thoracotomy was prepared. The heart was rapidly removed and submerged in a dissecting dish containing Krebs–Henseleit (K–H) bicarbonate buffer at pH 7.3 and room temperature. The K–H solution was vigorously bubbled with 95% O_2 + 5% CO_2. Epicardial coronary arteries were cannulated with a 22- to 26-gauge blunt hypodermic needle and the branches tied. The vessels were removed and most of the myocardial tissue was surgically removed. The cannulated coronary arteries were placed in a perfusion apparatus described by Ogletree *et al.* (7). Isolated coronary arteries were perfused with K–H solution under constant flow at rates of 20 to 40 ml/min with a Harvard Apparatus peristaltic pump. The arteries (usually two from each heart) were placed in 10-ml perfusion chambers in which the oxygenated (95% O_2 + 5% CO_2) K–H solution was recirculated, so that the volume in each chamber remained constant.

Downstream from the pump, a Lucite T-connector connected to a Statham P-23 pressure transducer was placed in the perfusion line for the measurement of perfusion pressure. Perfusion pressure was stable at about 45–60 mm Hg prior to addition of drugs. An increase in perfusion pressure represents a vasoconstriction in the isolated constant-flow, perfused artery, and a decrease in perfusion pressure corresponds to a vasodilation. Each vessel was allowed to equilibrate at the constant flow perfusion for 60 to 90 min prior to testing for vasoactivity. Volumes of 10 to 50 μl of drugs were used so as not to significantly alter the volume of the bathing medium.

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Test substances. Angiotensins I and II were obtained as the free peptide from Sigma Chemical Company (St. Louis, Mo.) and prepared in distilled water prior to use. Enalapril (MK-421) and enalaprilic acid (MK-422) were obtained from the Merck Institute for Therapeutic Research (West Point, Pa.) and prepared fresh daily in K–H solution. Captopril (SQ 14,225) was obtained from the Squibb Institute for Medical Research (Princeton, N.J.) and prepared fresh in K–H solution. The synthetic endoperoxide, 9,11-methano-epoxy PGH_2 (U-46619) was obtained from The Upjohn Company (Kalamazoo, Mich.), dissolved in 100% ethanol, and stored at −25°C until just prior to use.

Results. Enalapril was added to the coronary arterial perfusate at concentrations of 1 to 200 μg/ml without any detectable direct effect on coronary vasoactivity. However, enalapril at concentrations of 25 to 100 μg/ml, effectively inhibited the coronary vasoconstrictor effects of angiotensin I (500 ng/ml), by inhibiting conversion to angiotensin II. Figure 1 illustrates typical examples of these effects (upper panels). Presumably, the modest slow constrictor effect of angiotensin I after enalapril administration is due to a small direct vasoconstrictor effect of angiotensin I. At concentrations of 100 to 200 μg/ml, enalapril dramatically antagonized the coronary constrictor effects of angiotensin II (lower panels). This effect was not due to a nonspecific blockade of the vascular smooth muscle to all constrictors, since enalapril did not affect the coronary constrictor effects of U-46619, a potent coronary vasoconstrictor.

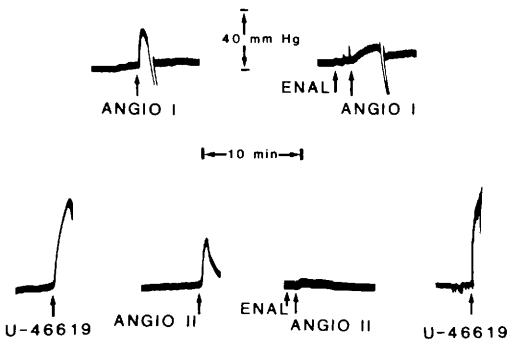

FIG. 1. Representative recordings of perfusion pressure in isolated perfused cat coronary arteries. Calibration markings are 40 mm Hg and 10 min. The upper panels show the response to 500 ng/ml of angiotensin I in the absence (left side) and presence (50 μg/ml) of enalapril (right side). The bottom panels depict the vascular responses to 15 n*M* 9,11-methanoepoxy PGH_2 (U-46619) and 500 ng/ml of angiotensin II in the absence (left side) and presence (right side) of 150 μg/ml of enalapril.

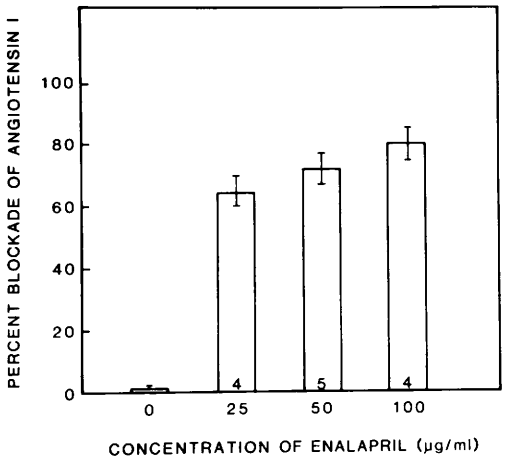

FIG. 2. Bar graph depicting constrictor responses of cat coronary arteries to angiotensin I (500 ng/ml) with varying concentrations of enalapril. Bar heights are mean % blockade of the angiotensin I response, brackets indicate ± SEM, numbers at bottom of bars indicate numbers of arteries studied. Significant blockade of the constrictor response of angiotensin I occurred at 25, 50, and 100 μg/ml. There was no statistical difference among the three enalapril responses ($P < 0.01$).

Figure 2 summarizes the angiotensin converting enzyme action of enalapril on perfused coronary arteries. Angiotensin I at 500 ng/ml produced a constriction of 23 ± 2 mm Hg in 25 vessels (mean ± SEM). At concentrations of 1 to 10 μg/ml, very little blockade of the constrictor effects of 500 ng/ml of angiotensin I were observed (i.e., less than a 20% attenuation). However, at 25, 50, and 100 μg/ml a substantial blockade (i.e., >65% attenuation) occurred. The modest residual unblocked effect of angiotensin I (i.e., 20 to 35%) is presumably a direct effect of angiotensin I, and is not blocked by higher concentrations of enalapril.

In addition to being an effective converting enzyme inhibitor in the coronary vasculature, enalapril acted as an angiotensin II antagonist. Figure 3 summarizes the responses of coronary arteries to 500 ng/ml of angiotensin II. Angiotensin II at 500 ng/ml produced a con-

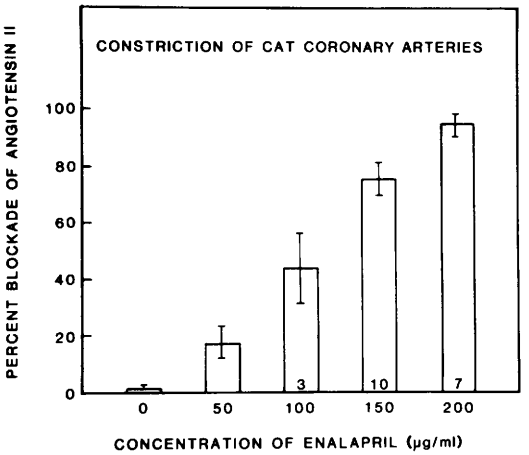

FIG. 3. Bar graph depicting the constrictor responses of cat coronary arteries to angiotensin II (500 ng/ml) to varying concentrations of enalapril. Bar heights are mean percentage blockade of the angiotensin II response, brackets indicate ± SEM, numbers at bottom of bars indicate numbers of arteries studied. A progressive blockade of the angiotensin I response occurred, achieving significance at 100 μg/ml ($P < 0.025$), 150 μg/ml ($P < 0.01$), and 200 μg/ml ($P < 0.001$).

striction of 27 ± 3 mm Hg in 35 coronary arteries (mean ± SEM). At enalapril concentrations of 100 μg/ml or higher, there was a significant ($P < 0.025$ to $P < 0.001$) attenuation of the angiotensin II constrictor effect. Since the response to U-46619, and KCl as well, was unaffected, this blockade of angiotensin II appears to be specific, and not due to a generalized inhibition of vascular smooth muscle constriction, as is observed with calcium channel blockers. Moreover, there was no direct vasodilator effects of enalapril, as occurs with calcium channel blockers. The duration of this blockade of the constrictor effect of angiotensin II was relatively long lasting. Addition of 150–200 μg/ml of enalapril to the perfusion fluid resulted in a virtually complete blockade of the constrictor effect of angiotensin II for one hour or in a few cases 90 min. This was determined by testing angiotensin II every 30 min after a single dose of enalapril. This frequency of application of angiotensin II did not result in tachyphylaxis in experiments without the addition of enalapril. Moreover, enalaprilic acid at concentrations of 0.5 to 1.0 μg/ml totally antagonized the constrictor effects of angiotensin II at 500 ng/ml (Table I). This effect lasted for at least 2 hr. Moreover, no tachyphylaxis to angiotensin occurred in the untreated arteries (Table I).

Not all converting enzyme inhibitors possess this angiotensin II antagonism. Captopril, at concentrations of 100 μg/ml attenuates the constrictor response to angiotensin I by 55 ± 6% ($P < 0.02$) in the perfused cat coronary artery. However, at 250 μg/ml, the response to angiotensin II is decreased only from 35 ± 6 to 30 ± 5 mm Hg (in six vessels), a difference of only 14%. This difference was not statistically significant.

Discussion. The results presented in this report describe an important new action of enalapril, a newly synthesized potent angiotensin converting enzyme inhibitor (8). Enalapril, in contrast to another potent converting enzyme inhibitor, captopril, antagonizes the vasoconstrictor effects of angiotensin II on coronary arteries. This effect is not due to a nonspecific blockade of the coronary vascular smooth muscle since enalapril does not block the action of 9,11-methano-epoxy PGH_2 or KCl. The effect is probably due to a direct antagonism of angiotensin receptors, although it could be due to the: (a) action of other vasoconstrictors released by angiotensin (e.g., thromboxane A_2), or (b) selective inhibition of angiotensin III formation by enalapril. These alternate explanations are unlikely because angiotensin II has been shown to constrict the cat coronary artery directly by activating saralasin-antagonizable receptors (4, 10), and the cat coronary artery is unable to release significant amounts of thromboxane A_2 (7, 11). Moreover, enalapril by blocking

TABLE I. EFFECT OF ENALAPRILIC ACID ON ANGIOTENSIN II-INDUCED CORONARY CONSTRICTION IN PERFUSED CAT CORONARY ARTERIES

Time (hr)	Angiotensin II + K-H solution	Angiotensin II + enalaprilic acid (1 μg/ml)
0[a]	36 ± 4	38 ± 5
1	34 ± 5	3 ± 6
2	35 ± 5	6 ± 5
3	32 ± 5	17 ± 4

Note. All values are mean increases in perfusion pressure ± SEM for four arteries.
[a] Pre-enalaprilic acid.

conversion of angiotensin I to angiotensin II would presumably prevent the formation of angiotensin III at the same time (9).

Since enalapril is converted to enalaprilic acid (8) in biological systems, it was of interest to determine the angiotensin II antagonistic effects of enalaprilic acid. Moreover, enalaprilic acid blocked angiotensin II at concentrations about one-hundredth of that required for enalapril. These results lend more credence to the significance of the angiotensin blockade by enalapril.

The significance of the angiotensin II blockade by enalapril may be of great value in a variety of pathophysiologic actions where the renin–angiotensin system exerts important cardiac or coronary vascular effects. These situations include cardiogenic and other forms of circulatory shock (12, 13), acute myocardial ischemia (5, 6), and perhaps coronary vasospasm resulting from angiotensin-II-induced vascular effects (14) where angiotensin II is known to induce coronary endothelial damage (15). Indeed, enalapril may thus prove to be a valuable agent in the treatment of these circulatory disorders. Our laboratory has already found this to be the case in preventing the extension of ischemic damage in acute myocardial infarction (6).

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