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Dose-response studies with idrapril in the rat heart during acute myocardial ischaemia and reperfusion

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Abstract

We assessed the effects of idrapril, a novel angiotensin-converting enzyme inhibitor, and captopril in the isolated rat heart after ischaemia and reperfusion and measured angiotensin-converting enzyme activity in myocardial tissue. Hearts were perfused and subjected to global ischaemia and reperfusion. Idrapril (0.1, 1, 10, and 50 μg/ml), captopril (80 μg/ml) or vehicle were given before ischaemia and throughout reperfusion. Post-ischaemic recovery of coronary flow was significantly decreased with 50 μg/ml of idrapril (43 ± 9% compared to 64 ± 3% in controls) whereas heart rate was unaffected. Recovery of developed pressure and activity of cardiac angiotensin-converting enzyme were significantly reduced by idrapril in a dose-dependent manner. This study suggests that protection or lack of protection by idrapril on recovery of contractile function seems to depend on the degree of inhibition of tissue angiotensin-converting enzyme activity in the setting of acute heart ischaemic insult. Our results suggest that while a certain degree of inhibition of angiotensin-converting enzyme in the heart is beneficial, marked tissue inhibition may be deleterious.

Keywords: Ischemia; Reperfusion; Angiotensin-converting enzyme; Idrapril; Captopril; Heart; (Rat)

1. Introduction

The renin-angiotensin system is involved in the homeostasis of the cardiovascular system. Its clinical relevance is demonstrated by the use of angiotensin-converting enzyme inhibitors in the setting of myocardial infarction and heart failure. Each component of the renin-angiotensin system (renin, angiotensinogen, angiotensin II receptors, angiotensin-converting enzyme mRNA) is present in the heart (Baker et al., 1992; Yamada et al., 1991). Local activation of angiotensinogen leading to intracardiac conversion of angiotensin I and II also occurs in the isolated perfused rat heart (Lindpaintner et al., 1990).

Several experimental studies have shown that angiotensin-converting enzyme inhibitors protect the ischaemic myocardium (Van Gilst et al., 1986; Grover et al., 1991; Linz et al., 1986; Ferrari et al., 1992). Captopril has been reported to reduce metabolic injury (Van Gilst et al., 1988; Ferrari et al., 1992; Cushman et al., 1989), the occurrence or duration of ventricular arrhythmias (Rochette et al., 1987; Fleetwood et al., 1991; Arad et al., 1992; Van Gilst et al., 1986) and the post-ischaemic recovery of contractility (Grover et al., 1991; Ferrari et al., 1992).

Idrapril is the prototype of a completely new chemical class of angiotensin-converting enzyme inhibitors, the hydroxamic non-amino acid derivatives (Turbanti et al., 1993). The novelty of this structure lies mainly in the first application of the peptoid principle to a known group of drugs, with the substitution of proline, typical of angiotensin-converting enzyme inhibitors, with a cyclohexane dicarboxylic acid. Idrapril inhibits plasma and lung angiotensin-converting enzyme activity and reduces blood pressure in a dose-dependent manner (Subissi et al., 1992).

The aim of the present study was three-fold: (i) to assess the dose-response effect of idrapril on the consequences of ischaemia and reperfusion in the isolated perfused rat heart, (ii) to compare these effects with those of a standard concentration of captopril, and (iii) to define the

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relationship between inhibition of tissue angiotensin-converting enzyme and post-ischaemic recovery of function and other indices of injury.

2. Materials and methods

2.1. Animals

Adult male Sprague Dawley rats were obtained from Charles River (Calco, Italy). Rats and litters were housed at room temperature of 21–24°C with 55 ± 10% humidity. Rats were fed standard animal chow and maintained on a 12 : 12 h light : dark schedule with the light on from 07:00 a.m.

Procedures involving animals and their care have been conducted in conformity with the institutional guide-lines in compliance with National and International Laws and Policies (EEC Council Directive 86/609, OJ L 358,1, December 12, 1987; NIH Guide for the Care and Use of Laboratory Animals, NIH Publication No. 86-23, revised 1986).

2.2. Surgical preparation and perfusion technique

Rats were anaesthetised with diethyl ether and 500 IU sodium heparin was administered into a peripheral vein. Thirty seconds later, hearts were excised and placed in cold (4°C) perfusion buffer until contraction had ceased (approximately 15 s). Each heart was then cannulated through the aorta.

2.2.1. Perfusion procedures

Perfusion was carried out in the Langendorff mode at a constant pressure (Langendorff, 1895). The apparatus consisted of a glass reservoir with a sintered glass filter at its base, from which gassed perfusion fluid flowed to a bubble trap through a glass extension unit. The heart was enclosed in a glass chamber to maintain a controlled environment. To facilitate this, the top of the heart chamber was closed using plastic film (Parafilm M, Jencons). All components of the perfusion apparatus were surrounded by a water jacket system through which water was circulated from a thermocirculator (Techne C-400). This kept the heart temperature at 37°C, assessed by a thermoelectrode (Comark type 2001) placed in the left ventricle in initial control studies. Perfusion pressure was set at 80–90 cm H_2O. These values were selected to correspond with the in vivo mean arterial blood pressure in rats (Litchfield, 1958).

Hearts were perfused with a bicarbonate buffer solution containing (in mM): NaCl 118.5, $NaHCO_3$ 25.0, KCl 4.8, $MgSO_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 1.4 and glucose 11.0. The perfusion fluid was filtered (5 μm pore size) before use and was continuously gassed with 95% oxygen plus 5% carbon dioxide (pH 7.4 at 37°C).

A compliant but non-elastic balloon, constructed from plastic wrapping film (cling-film), to match the size of the left ventricle, was introduced into the left ventricle through the mitral valve. The balloon was filled with saline and attached to a pressure transducer through a saline-filled rigid cannula. Using a syringe attached to a side arm of the transducer, the volume of the balloon was adjusted to give a left ventricular end-diastolic pressure of 0–10 mm Hg. After 30-min control perfusion with bicarbonate buffer, values for cardiac function were recorded (see section 2.5). Hearts which did not satisfy pre-defined inclusion criteria (see paragraph 2.4.) were then excluded.

2.3. Experimental protocol

Hearts were randomly assigned to one of six groups and at least one control heart was perfused every day in parallel with those assigned to the drug groups.

After 30 min of control perfusion, hearts were perfused with normal bicarbonate buffer and infused with either distilled water (vehicle) or an aqueous solution of idrapril or captopril by a Harvard pump (Mod 975) through a side arm of the aortic cannula. The drugs were infused by a pump since captopril is unstable in oxygenated bicarbonate buffer (Grover et al., 1991). Infusion rate was set between 0.59 ml/min and 1.1 ml/min according to the coronary flow and the effective concentrations of idrapril delivered were 0.1 μg/ml (4×10^{-7} M), 1 μg/ml (4×10^{-6} M), 10 μg/ml (4×10^{-5} M), and 50 μg/ml (2×10^{-4} M) whereas that of captopril was 80 μg/ml (4×10^{-4} M). Coronary flow of 13 ml/min, which was the average value during basal perfusion, required an infusion rate of 0.82 ml/min.

Values for cardiac function were recorded before and after 2, 5 and 10 min of infusion to detect any cardiac effect of drugs. Each heart was then subjected to 45 min of normothermic global ischaemia. Reperfusion was carried out with normal bicarbonate buffer and the heart was infused with the same concentrations of idrapril or captopril as before ischaemia. The duration of global ischaemia was selected in order to achieve approximately 50% recovery of contractile function. This was expected on the basis of pilot studies. A recovery of 50% in controls allows scope for the detection of deterioration or protection of contractile function.

2.4. Exclusion criteria

Pre-defined exclusion criteria were applied before the start of the study and after 30 min of control perfusion. The minimum acceptable values for coronary flow, heart rate and left ventricular developed pressure in the isolated rat heart were 8 ml/min, 220 beats/min, and 90 mm Hg, respectively. Any heart that was excluded was immediately replaced.

The presence of a faulty aortic valve, as evidenced by a high coronary flow (at least 50% higher than that of our

Table 1
Basal values of cardiac function 10 min before and after drug or vehicle infusion (means ± S.E.)

Group	Rat (*n*)	Dose (μg/ml)	BW (g)	VWW (mg)	HW/BW (mg/g)	CF (ml/min)		HR (beats/min)		LVDP (mm Hg)	
						Before	After	Before	After	Before	After
Control	20	–	305 ± 7	792 ± 25	2.6 ± 0.1	13.3 ± 0.5	13.3 ± 0.4	278 ± 6	270 ± 6	116 ± 2	114 ± 2
Idrapril	8	0.1	294 ± 6	809 ± 32	2.8 ± 0.1	12.8 ± 0.5	12.3 ± 1.1	294 ± 13	274 ± 12	108 ± 3	104 ± 4
Idrapril	9	1	297 ± 7	788 ± 24	2.7 ± 0.1	13.9 ± 0.5	14.5 ± 0.5	290 ± 5	288 ± 7	114 ± 4	111 ± 3
Idrapril	8	10	319 ± 15	842 ± 27	2.7 ± 0.1	12.6 ± 0.8	13.4 ± 0.8	286 ± 11	272 ± 9	108 ± 3	111 ± 3
Idrapril	8	50	301 ± 11	820 ± 40	2.7 ± 0.1	15.1 ± 1.5	15.0 ± 1.4	300 ± 12	283 ± 11	114 ± 5	123 ± 4
Captopril	6	80	308 ± 5	825 ± 26	2.7 ± 0.1	12.7 ± 0.5	14.3 ± 0.9	294 ± 7	278 ± 7	114 ± 3	110 ± 2

BW = body weight, VWW = ventricular wet weight, CF = coronary flow, HR = heart rate, LVDP = left ventricular developed pressure.

historical controls) was an additional exclusion criterion but no heart had to be excluded for this reason.

2.5. Variables measured

Left ventricular systolic and end-diastolic pressures were obtained from high-speed recordings (25 mm/s) of the pressure trace and left ventricular developed pressure was calculated as the difference between these two (Battaglia Rangoni, 8 channels, KO 380, Bologna, Italy). Heart rate was also derived from the pressure trace and coronary flow was measured by direct timed collection of coronary effluent. Incidence, magnitude, time-to-onset, and time-to-peak of ischaemia-induced contracture were recorded. Coronary flow, heart rate, and left ventricular developed pressure were measured throughout the perfusion period. Left ventricular end-diastolic pressures were measured throughout reperfusion.

2.6. Myocardial angiotensin-converting enzyme activity

At the end of the reperfusion period, hearts were weighed, frozen and stored at −20°C until analysis. The enzyme was assayed on the homogenised heart according to Neels et al. (1982). Angiotensin-converting enzyme activity was measured on hearts given vehicle and 1, 10 and 50 μg/ml idrapril. Hearts given captopril were not assayed because captopril interferes with the assay.

2.7. Expression of variables

Results are reported as means ± S.E. Control pre-ischaemic variables are expressed as absolute values. Post-ischaemic recovery of function is expressed as a percentage of the values recorded before ischaemia and those after reperfusion. Indices of ischaemia- and reperfusion-induced injury such as magnitudes of ischaemic contracture, its time-to-onset and time-to-peak, and left ventricular end-diastolic pressure, are expressed as absolute values (mm Hg and min).

2.8. Statistical analysis and expression of results

One-way analysis of variance (ANOVA) was used for multiple comparisons when a significant F value was obtained; comparison of means was followed by Dunnett's test. Student's t-test for paired data was used to compare cardiac function before and after the drugs. The relation between post-ischaemic recovery of cardiac function and myocardial angiotensin-converting enzyme activity was compared by linear regression analysis. Differences are considered significant at $P \leq 0.05$.

3. Results

3.1. Basal characteristics and function

There were no significant differences in basal characteristics and function of control hearts and any of the five treated groups (Table 1). Mean body weight and ventricular wet weight in control hearts were 305 ± 7 g and 792 ± 25 mg, respectively. Pre-ischaemic values for coronary flow, heart rate and left ventricular developed pressure before and after infusion of vehicle were 13 ± 0.5 and 13 ± 0.4 ml/min, 278 ± 6 and 270 ± 6 beats/min, 116 ±

Table 2
Post-ischaemic recovery of coronary flow and heart rate, and other indices of ischaemia-induced injury (means ± S.E.)

Group	(*n*)	Dose (μg/ml)	CF (%)	HR (%)	LVEDP (mm Hg)	IC (mm Hg)	TTO (min)	TTP (min)
Control	20	–	64 ± 3	90 ± 3 (*n* = 19)	49 ± 4	51 ± 2	12 ± 1	22 ± 1
Idrapril	8	0.1	60 ± 12	77 ± 16	59 ± 6	56 ± 2	10 ± 2	18 ± 1
Idrapril	9	1	63 ± 3	87 ± 3	44 ± 5	48 ± 3	12 ± 1	23 ± 1
Idrapril	8	10	58 ± 3	87 ± 6 (*n* = 7)	58 ± 7	57 ± 3	9 ± 1	22 ± 2
Idrapril	8	50	43 ± 9 [a]	67 ± 14 (*n* = 7)	58 ± 5	61 ± 6	8 ± 1 [a]	16 ± 1 [b]
Captopril	6	80	64 ± 3	90 ± 4 (*n* = 5)	48 ± 5	47 ± 6	11 ± 1	22 ± 2

CF = coronary flow, HR = heart rate, LVEDP = left ventricular end-diastolic pressure, IC = ischaemic contracture, TTO and TTP = time-to-onset and time-to-peak of ischaemic contracture, respectively. [a] $P = 0.05$ and [b] $P < 0.01$ vs. control (ANOVA + Dunnett's test).

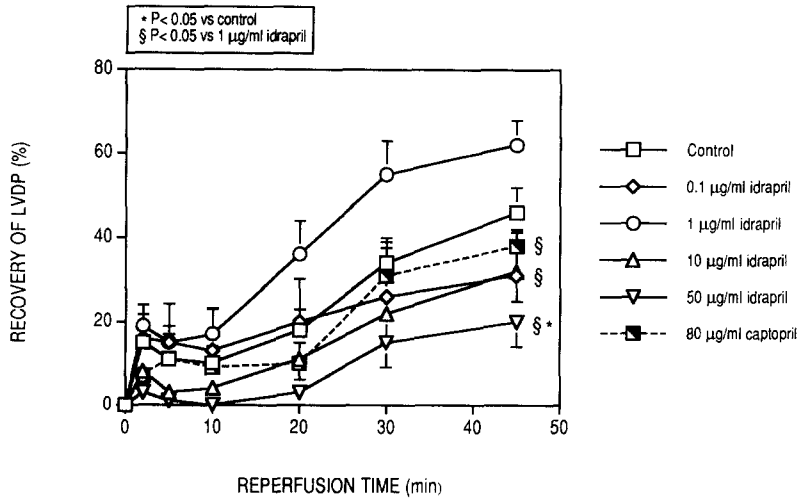

Fig. 1. Time course of left ventricular developed pressure (LVDP) recovery (percentage of pre-ischaemic values) during reperfusion in control hearts ($n = 20$) and in hearts receiving idrapril ($n = 8$–9) or captopril ($n = 6$). Means ± S.E.

2 and 114 ± 2 mm Hg, respectively. Cardiac function did not change after 10 min infusion of idrapril and captopril.

3.2. Post-ischaemic recovery of function and other indices of ischaemia-induced injury

Post-ischaemic recovery of coronary flow and heart rate are shown in Table 2. The time course of left ventricular developed pressure during reperfusion is shown in Fig. 1.

Recoveries of coronary flow and left ventricular developed pressure were significantly reduced by the highest concentration of idrapril (50 μg/ml) while heart rate recovered to the same extent in each group. Contractility of hearts infused with 1 μg/ml idrapril increased throughout the reperfusion period compared with other groups, although the difference from controls was not significant (Fig. 1).

During reperfusion left ventricular end-diastolic pressure increased to a similar extent in controls and treated hearts (Table 2). Ischaemia-induced contracture occurred in all hearts.

Time-to-onset and time-to-peak of ischaemic contracture were significantly reduced in hearts given the highest concentration of idrapril (50 μg/ml) compared to other groups ($P = 0.05$ and $P < 0.01$ versus controls) (Table 2). The magnitude of ischaemia-induced contracture was similar in all groups (Table 2).

3.3. Myocardial angiotensin-converting enzyme activity

Idrapril infused for 10 min prior to ischaemia and during 45 min of reperfusion lowered myocardial angiotensin-converting enzyme activity in a dose-dependent manner (Fig. 2A). The cardiac activity was significantly inhibited by 10 μg/ml ($P < 0.01$) and 50 μg/ml ($P < 0.01$) idrapril. Left ventricular developed pressure (Fig. 2B) refers to hearts ($n = 49$) whose angiotensin-converting enzyme activity was measured, as opposed to the left ventricular developed pressure data reported in Fig. 1, which are the average of all hearts in the study ($n = 59$).

3.4. Correlations between indices

We examined the relationship of recovery of coronary flow and left ventricular developed pressure with myocar-

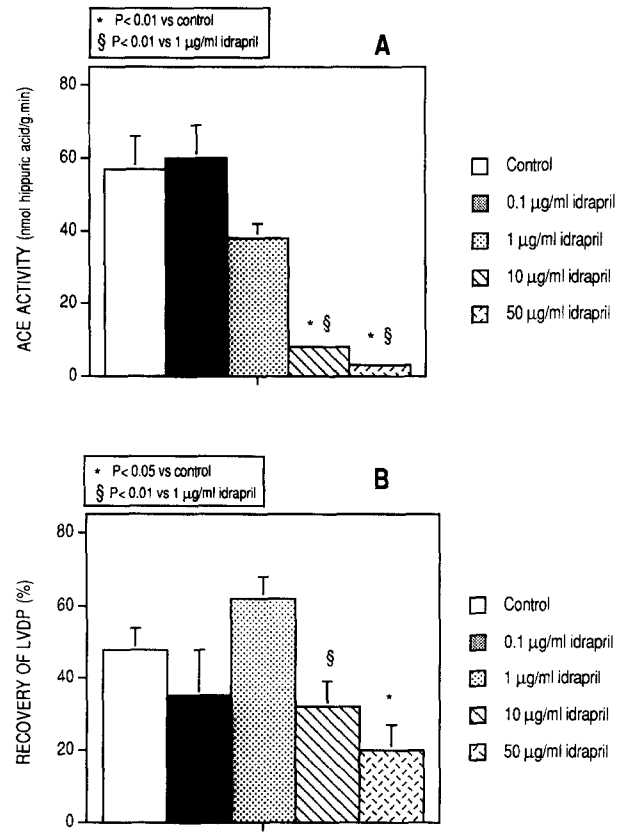

Fig. 2. Myocardial activity of angiotensin-converting enzyme (ACE) (A) and left ventricular developed pressure (LVDP) recovery (percentage of pre-ischaemic values) (B) in control hearts and in hearts receiving idrapril. Means ± S.E.

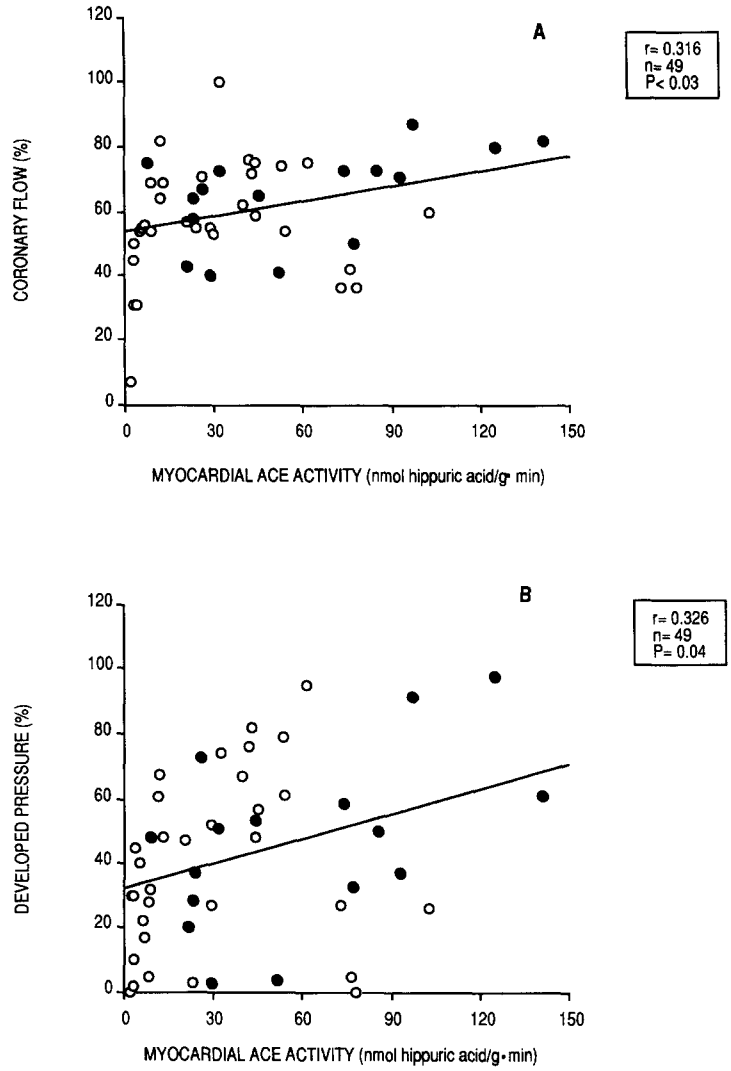

Fig. 3. Myocardial activity of angiotensin-converting enzyme versus coronary flow (A) and left ventricular developed pressure recovery (B) (percentage of pre-ischaemic values) in control hearts (filled dots) and in hearts receiving idrapril (empty dots).

dial angiotensin-converting enzyme activity. A linear correlation was found between myocardial angiotensin-converting enzyme activity and post-ischaemic recovery of coronary flow ($P < 0.03$) and left ventricular developed pressure ($P = 0.04$) (Fig. 3).

No correlation was seen between ischaemia-induced contracture or left ventricular end-diastolic pressure and angiotensin-converting enzyme inhibition.

4. Discussion

Several clinical studies have shown that inhibition of the renin-angiotensin system, which is activated in cardiac diseases, is beneficial and improves a compromised cardiac function (Dzau, 1990; Pfeffer et al., 1988, 1992). Angiotensin-converting enzyme inhibitors are now part of the standard therapy in patients with myocardial infarction (ISIS-4 Collaborative Group, 1995; Gruppo Italiano per lo studio della sopravvivenza nell'infarto miocardico GISSI-3, 1994) and heart failure (Pfeffer et al., 1992; The SOLVD Investigators, 1992; AIRE and Study Investigators, 1993).

The present study illustrates the effects of idrapril, a novel angiotensin-converting enzyme inhibitor, on the post-ischaemic recovery of cardiac function in the isolated rat heart. The concentrations of idrapril (ranging between 0.1 and 50 μg/ml) used in the present study, inhibit angiotensin-converting enzyme activity in rat plasma by 95–100% (Criscuoli et al., 1993) and are quantitatively equivalent to 80 μg/ml of captopril used in this study. This concentration inhibits rat plasma angiotensin-converting enzyme activity by 100% (Endoh et al., 1989) and was used by other investigators in similar studies (Van Gilst et al., 1986; Arad et al., 1992; Huizer et al., 1992).

The effects of idrapril on post-ischaemic recovery of contractility depended on the concentrations infused: the

dose-response curve was bell-shaped. Recovery of left ventricular developed pressure was best with 1 μg/ml idrapril, whereas higher concentrations did not afford protection compared with controls. The recovery of left ventricular developed pressure was significantly reduced by 50 μg/ml of idrapril; this concentration led to almost complete inhibition of the intracardiac angiotensin-converting enzyme activity.

Our results show that maximal angiotensin-converting enzyme inhibition during global ischaemia and reperfusion may impair the recovery of left ventricular developed pressure, and in fact there is an inverse relationship between these two variables. Marked inhibition of myocardial angiotensin-converting enzyme activity and the consequent decrease of angiotensin II production during acute ischaemia may not be advisable for the heart, for example, in the setting of cardiac surgery. Angiotensin II exerts a positive inotropic effect which may be essential to maintain contractility which is impaired by the ischaemic- and reperfusion-induced insult at the end of cardiopulmonary by-pass.

The low post-ischaemic recovery of coronary flow in hearts given the highest concentration of idrapril (50 μg/ml) is hard to explain since the drug at each concentration did not alter coronary perfusion when infused during the basal period. One possibility is that there is a negative interaction between myocardial inhibition of the angiotensin-converting enzyme activity and ischaemia/reperfusion-induced injury in the endothelium of the coronary bed.

The time-to-onset of ischaemic contracture was significantly reduced in the group of hearts receiving the highest dose (50 μg/ml) of idrapril, suggesting that either greater depletion of ATP or an increase in calcium overload occurred earlier in this group of hearts than in controls or any other trial group. Severe depletion of ATP and overload of calcium are believed to be involved in the injury associated with ischaemia and reperfusion and govern the onset and the magnitude of ischaemia-induced contracture, respectively. However, since left ventricular end-diastolic pressure (diastolic stiffness) in hearts receiving 50 μg/ml idrapril was similar to that of controls and the other trial groups, we can exclude that calcium influx increased in these hearts.

Our objective was to examine whether idrapril is capable of protecting against a specific dysfunction (contractile dysfunction) associated with ischaemia and reperfusion. To achieve this we require two things. First, we need a model where contractile function may be conveniently evaluated. The isolated heart with global ischaemia is preferred because global contractile dysfunction is more easily measurable than regional. Second, we need a model in which moderate (i.e., modulatable) contractile dysfunction occurs. The duration of ischaemia we chose (45 min) achieves this. With longer ischaemia, irreversible injury may arise. With briefer ischaemia (stunning) no injury may occur. We need a level of dysfunction that may be alleviated or exacerbated by a drug and which can be detected statistically. The present model achieves this.

We used multiple functional indices to assess the extent of tissue injury in the ischaemic heart, such as systolic (contractility) and diastolic (relaxation) function, heart rate, coronary flow, and ischaemia-associated contracture. We preferred such indices to metabolic ones such as enzyme leakage or depletion of high energy phosphates, for the reason that they are, in our opinion, the ultimate arbiter of cardiac injury. Cases have been reported where angiotensin-converting enzyme inhibitors did significantly reduce the metabolic injury but did not improve cardiac function (contractility) in the setting of acute myocardial ischaemia (Cushman et al., 1989; Arad et al., 1992).

Many mechanisms that are affected by the renin-angiotensin system in the intact animal play no role in the isolated rat heart, perfused with bicarbonate buffer. Global rather than regional ischaemia is another reason for differences in results. Isolating the myocardial protective properties from the effect on collateral flow, by applying global instead of regional ischaemia, may explain the lack of protection in our experiments. Likewise, availability of collateral vessels may account for differences in vivo (Jeremic et al., 1996).

The fact that captopril and idrapril failed to improve the recovery of cardiac contractile function in our study is in contrast with a large majority of investigations with angiotensin-converting enzyme inhibitors. However other authors too have found that angiotensin-converting enzyme inhibitors such as captopril (Huizer et al., 1992; Cushman et al., 1989; Arad et al., 1992), enalapril (Cushman et al., 1989; Fleetwood et al., 1991) and lisinopril did not improve myocardial function after ischaemia (Werrmann and Cohen, 1994). Captopril exerted deleterious cardiac effects during acute myocardial ischaemia in the dog, despite a significant reduction of myocardial metabolic demand (Alam et al., 1992; Daniel et al., 1984): there was some increase of ischaemia with captopril during the early phase of coronary occlusion (Alam et al., 1992). In a model of coronary occlusion and reperfusion angiotensin-converting enzyme inhibitors may have different or even opposite effects on the various stages of the evolving process of myocardial injury. Westlin and Mullane (1988) showed that captopril conferred myocardial protection by scavenging free radicals during reperfusion but did not modify injury sustained during the period of ischaemia (Westlin and Mullane, 1988). They postulated a temporal variability of the influence of angiotensin-converting enzyme inhibition during the evolution of myocardial ischaemia and injury. Considering that we examined the early stage of ischaemia, our findings do not negate the beneficial effects of angiotensin-converting enzyme inhibition after myocardial necrosis or preclude potential benefits during ischaemia under different haemodynamic conditions.

In conclusion, our results suggest that protection or lack

of protection by idrapril on recovery of contractile function seems to depend on the degree of inhibition of tissue angiotensin-converting enzyme activity in the setting of acute heart ischaemic insult, and highlight the need for dose-response studies in order to examine the entire spectrum of action of new angiotensin-converting enzyme inhibitors. It appears that while a certain degree of inhibition of angiotensin-converting enzyme in the heart is beneficial, marked tissue inhibition may be deleterious under certain conditions.

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