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Effects of the insurmountable angiotensin AT_1 receptor antagonist candesartan and the surmountable antagonist losartan on ischemia/reperfusion injury in rat hearts

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Abstract

Two angiotensin AT_1 receptor antagonists with different receptor binding characteristics, candesartan (insurmountable antagonism) and losartan (surmountable antagonism), were compared as regards their effects on angiotensin II-induced vasoconstriction and on myocardial ischemia/reperfusion injury. In isolated rat hearts perfused under constant flow, it was found that at equipotent concentrations candesartan (10 nM) and losartan (3 μM) almost completely inhibited the angiotensin II-induced increase in coronary perfusion pressure. However, if a washout period was introduced before the angiotensin II challenge, the effect of losartan quickly vanished, while that of candesartan remained. In hearts subjected to 25 min of global ischemia and 45 min of reperfusion, pre-treatment with candesartan (10 nM) or losartan (3 μM) immediately prior to ischemia improved the recovery of left ventricular developed pressure as compared to the effect of vehicle (69 ± 3.2 and 64 ± 2.3 vs. 44 ± 6.2%, respectively; mean ± S.E.M, $P < 0.05$). When ischemia was initiated following 30 min of washout after drug administration, the recovery of left ventricular developed pressure was higher in the candesartan group (73 ± 3.2%, $P < 0.05$), but not in the losartan group (63 ± 2.8%), than in the vehicle group (58 ± 4.8%). The cumulative creatine kinase release during the first 30 min of reperfusion in the washout experiments was lower in the candesartan group (28.5 ± 2.30 U, $P < 0.05$), but not in the losartan (40.8 ± 6.73 U) group, than in the vehicle group (48.1 ± 4.35 U). No significant difference between groups in left ventricular end-diastolic pressure and coronary perfusion pressure was found. The present results demonstrate that angiotensin AT_1 receptor antagonists at equipotent concentrations could differ in their cardioprotective effects in hearts subjected to ischemia/reperfusion. It is suggested that the insurmountable AT_1 receptor characteristics of candesartan could provide more persistent cardioprotection than the surmountable receptor characteristics of losartan.

Keywords: Angiotensin II; Angiotensin AT_1 receptor; Ischemia; Reperfusion; Myocardium; Vasoconstriction

1. Introduction

Angiotensin II is the primary mediator of the renin–angiotensin system and plays an important role in cardiovascular homeostasis (Ferrario and Flack, 1996; Bermann et al., 1997). It is not only produced in the systemic circulation in the blood compartment but also locally in many organs, including the heart, through a regional autocrine or paracrine renin–angiotensin system (De Graeff et al., 1994; Arakawa, 1996). Systemic renin–angiotensin system is activated in animals and patients with acute myocardial infarction (Ertl et al., 1982; McAlpine and Cobbe, 1988), and it has become evident that the heart is both a target organ and an important site of formation of angiotensin II, the production of which increases during ischemia (Noda et al., 1993; Arakawa, 1996). Furthermore, the expression of the functional receptor (AT_1), which mediates most effects of angiotensin, is increased in ischemic hearts (Yang et al., 1997). Pharmacological and pathophysiological levels of exogenous angiotensin II have been shown to produce myocardial necrosis (Kabour et al., 1994; Senzaki et al., 1998) and to exaggerate the myocardial dysfunction induced by ischemia and reperfusion (Yoshiyama et al., 1994a). The role of endogenous angiotensin II in the development of myocardial injury following ischemia and reperfusion remains, however, to be determined.

Angiotensin-converting enzyme inhibitors, by reducing the formation of angiotensin II from angiotensin I, have been used widely in patients with hypertension and heart

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failure, and in post-infarction patients (De Graeff et al., 1994; Brown and Vaughan, 1998). However, angiotensin-converting enzyme inhibitors are rather unspecific angiotensin II inhibitors, since they not only reduce the formation of angiotensin II but also inhibit the degradation of bradykinin (De Graeff et al., 1994; Brown and Vaughan, 1998), which has a myocardioprotective capacity (Linz et al., 1996). Furthermore, apart from the angiotensin-converting enzyme-dependent pathway, there are other angiotensin II-forming pathways in myocardial tissue (De Graeff et al., 1994; Arakawa, 1996). Thus, a more specific inhibitor of angiotensin II-mediated effects would be preferable in studies of angiotensin II-mediated myocardial damage. Two subtypes of angiotensin receptors, AT_1 and AT_2, have been identified. The cardiovascular actions of angiotensin II are mainly mediated via activation of the AT_1 receptor, whereas the function of the AT_2 receptor remains ''obscure'' (Ferrario and Flack, 1996; Bermann et al., 1997). Several specific non-peptide AT_1 receptor antagonists have been developed, including losartan, irbesartan and candesartan.

Losartan was the first orally active AT_1 receptor antagonist developed and is therefore the most intensively investigated compound of this class so far. Losartan, or its active metabolite 2-*n*-butyl-4-chloro-1-[(2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl)methyl]imidazole-5-carboxylic acid (EXP 3174), has been reported to reduce infarct size in pigs (Schwarz et al., 1997) but not in rabbits (Hartman et al., 1993) or dogs (Richard et al., 1993). It has also been reported to improve (Werrmann and Cohen, 1996; Yang et al., 1997) or have no effect on (Ford et al., 1996) ischemia/reperfusion-induced myocardial injury in isolated rat hearts. The reason for these conflicting results is not readily available. Compared to losartan, the newer AT_1 receptor antagonist candesartan has a greater affinity for AT_1 receptors, having a slow dissociation rate resulting in a longer-lasting insurmountable antagonism (Kinugawa et al., 1997). Candesartan has proven beneficial in reducing ischemia/reperfusion-induced myocardial damage in pigs (Jalowy et al., 1998; Shimizu et al., 1998) as well as in rats (Yoshiyama et al., 1994b). There are, to the best of our knowledge, no conflicting results published regarding the efficacy of candesartan in limiting infarct size. In the present study, we investigated the hypothesis that the angiotensin AT_1 antagonist candesartan, by its receptor-binding properties, would be superior to a surmountable AT_1 antagonist such as losartan as regards effects on ischemia/reperfusion-induced myocardial injury.

2. Materials and methods

2.1. Heart preparation

Male Sprague–Dawley rats (300–400 g) were anesthetized with a mixture of fluanisonum (2.5 mg/kg), fentanylum (0.1 mg/kg) and midazolam (1.25 mg/kg) i.m. After injection of heparin (1000 IU/kg i.v.), the heart was excised and placed in cold (4°C) Krebs–Henseleit buffer solution of the following composition (in mM): NaCl 118, KCl 4.7, $CaCl_2$ 1.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25.2 and glucose 11.1. The hearts were mounted on a column according to the non-recirculating Langendorff perfusion technique and perfused with buffer, bubbled with 95% O_2 and 5% CO_2 at 37.0°C, at a constant flow rate by means of a roller pump. A fluid-filled latex balloon connected to a pressure transducer (MicroSwitch, Freeport, IL, USA) was inserted into the left ventricular cavity via the left atrium to measure the left ventricular isovolumic pressure. The volume of the balloon was adjusted to obtain a left ventricular end-diastolic pressure of 5 mm Hg during the equilibration period. An ultrasonic flow probe (Transonic, Ithaca, NY, USA) connected to a Transonic flowmeter (model 206) was put on the circuit proximal to the aortic cannula for measurement of coronary flow. The left ventricular end-diastolic pressure and coronary flow were recorded on a polygraph (Model 7D, Grass Instrument, Quincy, MA, USA). Two side arms in the perfusion system just proximal to the aorta were used for drug administration and for measurement of coronary perfusion pressure. The perfusion rate of each heart was set during the stabilization period to yield a basal coronary perfusion pressure of 65 mm Hg, which was maintained throughout the study.

2.2. Experimental protocols

2.2.1. Study 1: inhibition of angiotensin II-induced vasoconstriction

Following 30 min of equilibration, the hearts were infused with angiotensin II (0.1 μM) for 3 min in the presence or absence of losartan (0.03–3 μM) or candesartan (0.03–10 nM). Administration of each concentration of each antagonist started 30 min before the angiotensin II infusions. Each heart received no more than two concentrations of one of the antagonists. The increase in coronary perfusion pressure was used as a parameter of the degree of coronary vasoconstriction.

2.2.2. Study 2: duration of angiotensin AT_1 receptor antagonistic effect after a washout period

Based on the results from the concentration–response study, equipotent concentrations of losartan (3 μM) and candesartan (10 nM) were chosen and used in the following studies. The hearts received these concentrations for 90 min and were then perfused with drug-free Krebs–Henseleit buffer for 45 min, which served as a washout period. The vasoconstrictive effect of angiotensin II (0.1 μM, 3-min infusion) was measured at 10, 30, and 45 min during the washout period. Ninety minutes of pre-treatment was chosen based on the observation that this period gives maxi-

mum inhibition of angiotensin II-induced responses in isolated vessels (unpublished observation in our laboratory).

2.2.3. Study 3: ischemia / reperfusion injury

2.2.3.1. Pre-treatment without subsequent washout (Fig. 1). Following stabilization, three groups of hearts randomly received an infusion of either vehicle, losartan (3 μM) or candesartan (10 nM) for 30 min (Fig. 1). The hearts were then immediately subjected to 25 min of global ischemia followed by 45 min of reperfusion. Functional recovery was observed at the end of the 45-min reperfusion period.

2.2.3.2. Pre-treatment with subsequent washout (Fig. 1). Another three groups of hearts were subjected to 25 min of global ischemia followed by 45 min of reperfusion as above. Prior to the initiation of global ischemia, vehicle, losartan (3 μM) or candesartan (10 nM) was administered for 90 min followed by a 30-min washout period with perfusion with drug-free Krebs–Henseleit buffer.

Functional recovery was observed at the end of the 45-min reperfusion period. Aliquots of coronary effluent were collected in pre-cooled test tubes before ischemia and at 1, 2, 3, 4, 5, 10, 20, and 30 min of reperfusion for determination of creatine kinase and noradrenaline.

2.3. Assay for creatine kinase and noradrenaline

Creatine kinase activity was analyzed in samples (4°C) in a Cobas Bio Centrifugal Analyzer (Hoffman-La Roche, Switzerland) within 48 h using a commercially available kit (Boehringer-Mannheim, Germany). The noradrenaline concentration was determined in samples of coronary effluent after storage at −70°C by high performance liquid chromatography with electrochemical detection as previously described (Carlsson and Abrahamsson, 1989).

2.4. Calculations and statistics

The release of creatine kinase was calculated as the cumulative efflux during the first 30 min of reperfusion, and the release of noradrenaline as the cumulative efflux during the initial 5 min of reperfusion. All the results are presented as means ± S.E.M. Differences between groups were compared with the Kruskal–Wallis analysis of variance (ANOVA) followed by the Wilcoxon–Mann–Whitney *U*-test. Comparison of repeated measurements within a group was performed with the Friedman's ANOVA followed by Wilcoxon–Mann–Whitney *U*-test. A *P*-value of less than 0.05 was considered to be significant.

2.5. Drugs

Angiotensin II (Sigma, St. Louis, MO, USA) was dissolved in distilled water. Candesartan and losartan (Astra

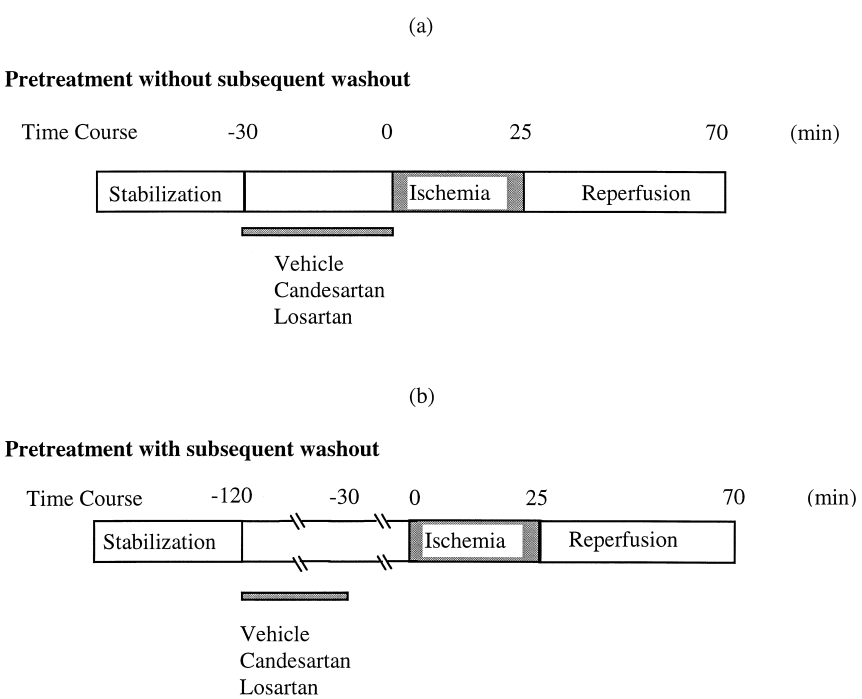

Fig. 1. Schematic outline of the protocol for study 3. Rats were subjected to 25 min of ischemia followed by 45 min of reperfusion. Ischemia was (a) initiated immediately after 30 min of pre-treatment with candesartan or losartan (pre-treatment without subsequent washout), or (b) initiated after a 30-min washout period following 90 min of pre-treatment with candesartan or losartan (pre-treatment with subsequent washout).

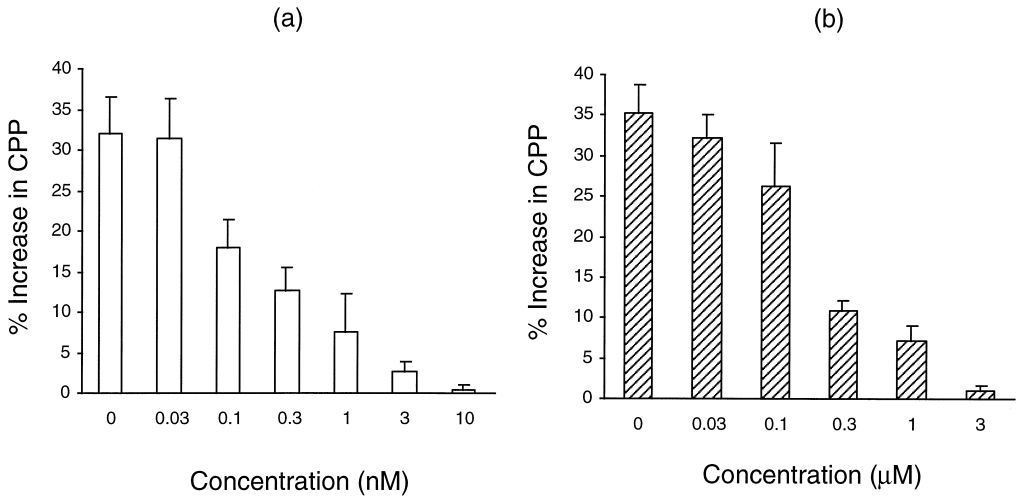

Fig. 2. Concentration-dependent inhibitory effect of (a) candesartan and (b) losartan on the increase in coronary perfusion pressure (CPP) induced by angiotensin II (0.1 μM) in non-ischemic buffer-perfused hearts. Administration of each concentration of each antagonist started 30 min before the Angiotensin II infusion. Data are presented as means ± S.E.M of three to five experiments for each concentration.

Hässle, Mölndal, Sweden) were dissolved in 0.1 N Na_2CO_3 and saline. All drugs were finally diluted in the Krebs–Henseleit solution.

3. Results

3.1. Study 1: inhibition of angiotensin II-induced vasoconstriction

Under basal conditions, infusion of 0.1 μM angiotensin II elicited an increase in coronary perfusion pressure of approximately 30–35% (Fig. 2). Thirty minutes of pretreatment with losartan and candesartan inhibited the angiotensin II-induced increase in coronary perfusion pressure in a concentration-dependent manner. Candesartan showed a higher angiotensin inhibitory potency than losartan. Candesartan at 10 nM almost completely inhibited the increase in coronary perfusion pressure caused by angiotensin II. The corresponding concentration required to achieve a similar degree of antagonism using losartan (Fig. 2) was 300-fold higher (3 μM). These two concentrations were regarded as equipotent and used in the subsequent study.

3.2. Study 2: duration of angiotensin AT_1 receptor antagonistic effect after a washout period

The results presented in Figs. 2 and 3 show that 30 and 90 min of treatment with losartan (3 μM) and candesartan (10 nM) produced an almost complete inhibition of angiotensin II-induced vasoconstriction. When the hearts were subjected to a washout period, it was found that 10 min of washout remarkably reduced the inhibitory effect of losartan, while 30 min of washout totally abolished it. In contrast, the inhibitory effect of candesartan was only modestly affected after 10 and 30 min of washout (Fig. 3).

3.3. Study 3: ischemia / reperfusion injury

3.3.1. Pre-treatment without subsequent washout

Thirty minutes of infusion of candesartan, losartan or vehicle did not affect any of the hemodynamic variables measured, and no differences in cardiac performance were detected between groups prior to ischemia (Table 1). During early reperfusion following 25 min of ischemia, all hearts in the three groups developed ventricular fibrillation. Most hearts reverted to normal rhythm spontaneously within 30 min of reperfusion. However, two hearts in the vehicle group, two in the candesartan group, and five in

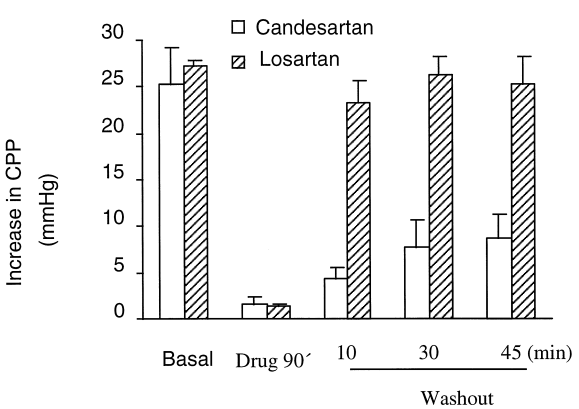

Fig. 3. Increase in coronary perfusion pressure (CPP) induced by angiotensin II (0.1 μM) under basal conditions, after 90 min of infusion of candesartan or losartan, at 10, 30 and 45 min after the termination of drug treatment in non-ischemic buffer-perfused hearts. Data are presented as means ± S.E.M ($n = 3$).

Table 1
Hemodynamic parameters before ischemia in hearts given either vehicle, candesartan or losartan for 30 min immediately prior to the induction of ischemia. Data are presented as mean ± S.E.M. HR: heart rate, LVDP: left ventricular developed pressure, LVEDP: left ventricular end-diastolic pressure, CPP: coronary perfusion pressure, LV dP/dt max: maximal first derivative of left ventricular pressure

Group	Basal					Pre-ischemia				
	HR (beat/min)	LVDP (mm Hg)	LVEDP (mm Hg)	CPP (mm Hg)	LV dP/dt max (mm Hg/s)	HR (beat/min)	LVDP (mm Hg)	LVEDP (mm Hg)	CPP (mm Hg)	LV dP/dt max (mm Hg/s)
Vehicle	278 ± 13	112 ± 4	7 ± 0.7	65 ± 0.6	3859 ± 93	292 ± 13	105 ± 3	6 ± 0.6	65 ± 0.4	3876 ± 125
Candesartan	299 ± 14	105 ± 4	8 ± 0.7	65 ± 0.4	3604 ± 187	297 ± 14	101 ± 3	7 ± 0.8	65 ± 1.2	3674 ± 163
Losartan	272 ± 9	119 ± 4	6 ± 0.3	65 ± 1.0	3880 ± 154	273 ± 8	111 ± 3	5 ± 0.3	63 ± 1.3	3870 ± 146

the losartan group did not resume sinus rhythm by the end of 30 min of reperfusion and were therefore excluded from the study. At the end of reperfusion, the recovery of myocardial contractile function was approximately 45 ± 6.2% of the pre-ischemic value and left ventricular end-diastolic pressure was 67 ± 5.6 mm Hg in the vehicle-treated hearts. Compared to vehicle, pre-treatment with candesartan for 30 min enhanced the recovery of left ventricular developed pressure (Fig. 4a) and dP/dt max (76 ± 3.4 vs. 60 ± 5.1%, $P < 0.05$) at the end of reperfusion. The left ventricular end-diastolic pressure tended to be lower, but not significantly, in the candesartan group than in the vehicle group at the end of reperfusion (Fig. 4b). Losartan produced a similar effect to candesartan as regards the recovery of left ventricular developed pressure and left ventricular end-diastolic pressure (Fig. 4a and b), and no significant difference was detected between the losartan and candesartan groups. There was no significant difference in coronary perfusion pressure by the end of reperfusion between groups (98 ± 2.7, 91 ± 1.6, and 98 ± 3.0 mm Hg for vehicle, candesartan and losartan group, respectively). The recovery of heart rate was similar in all groups (102 ± 2.0, 98 ± 2.0, and 100 ± 2.8%) for vehicle, candesartan and losartan group, respectively; n.s.).

3.3.2. Pre-treatment with subsequent washout

Basal hemodynamic parameters did not differ between groups. During the 90-min of drug infusion, coronary perfusion pressure increased gradually in all groups, while no changes were observed in heart rate, left ventricular developed pressure, left ventricular end-diastolic pressure or dP/dt max (Table 2). There were no significant differences in any of these hemodynamic parameters between groups at the initiation of global ischemia. As in the first series of experiments without washout, all the hearts had ventricular fibrillation during early reperfusion. Two hearts in each group were excluded from further observation because of the persistence of ventricular fibrillation or tachycardia at the end of 30 min of reperfusion. At the end of the reperfusion period, functional recovery did not differ between the vehicle and losartan groups. However, the recovery of left ventricular developed pressure was higher in the candesartan group than in the vehicle group (Fig. 5a). There was no significant difference in left ventricular end-diastolic pressure (Fig. 5b) or in coronary perfusion pressure (103 ± 8.8, 93 ± 3.8, and 89 ± 1.8 mm Hg for vehicle, candesartan and losartan group, respectively) between the groups by the end of reperfusion. Recovery of heart rate was similar in all three groups.

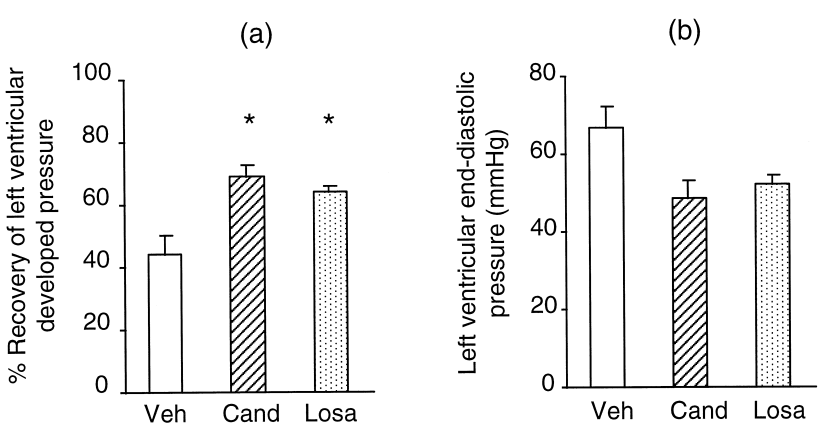

Fig. 4. Recovery of left ventricular developed pressure (a) and left ventricular end-diastolic pressure (b) at the end of 45 min of reperfusion following 25 min of ischemia in hearts receiving either vehicle ($n = 7$), candesartan (Cand; 10 nM; $n = 6$) or losartan (Losa; 3 μM; $n = 7$) for 30 min immediately prior to ischemia. Data are presented as means ± S.E.M. $^*P < 0.05$ vs. vehicle group.

Table 2
Hemodynamic parameters in hearts given either vehicle, candesartan or losartan for 90 min followed by 30 min washout prior to the induction of ischemia. Data are presented as mean ± S.E.M. Veh: vehicle, Cand: candesartan, Losa: losartan. HR: heart rate, LVDP: left ventricular developed pressure, LVEDP: left ventricular end-diastolic pressure, CPP: coronary perfusion pressure, LV dP/dt max: maximal first derivative of left ventricular pressure

Group	Basal					90-min Treatment					Pre-ischemia				
	HR (beat/min)	LVDP (mmHg)	LVEDP (mmHg)	CPP (mmHg)	LV dP/dt max (mmHg/s)	HR (beat/min)	LVDP (mmHg)	LVEDP (mmHg)	CPP (mmHg)	LV dP/dt max (mmHg/s)	HR (beat/min)	LVDP (mmHg)	LVEDP (mmHg)	CPP (mmHg)	LV dP/dt max (mmHg/s)
Veh	299 ± 9	115 ± 6	8 ± 0.6	63 ± 0.7	3269 ± 145	307 ± 13	110 ± 7	9 ± 1.4	77 ± 2.9[a]	3261 ± 178	305 ± 14	110 ± 7	10 ± 1.8	79 ± 3.2[a]	3270 ± 187
Cand	303 ± 13	110 ± 3	7 ± 0.6	63 ± 0.6	3367 ± 94	311 ± 10	108 ± 3	9 ± 0.5	71 ± 1.3[a]	3452 ± 123	306 ± 10	106 ± 2	9 ± 0.7	71 ± 1.0[a]	3413 ± 126
Losa	285 ± 9	118 ± 4	9 ± 0.6	64 ± 0.7	3505 ± 98	287 ± 10	110 ± 4	9 ± 0.9	72 ± 1.3[a]	3394 ± 118	297 ± 9	108 ± 4	9 ± 1.0	75 ± 3.6[a]	3359 ± 148

[a] $P < 0.01$ vs. basal values.

Ischemia/reperfusion resulted in a substantial efflux of creatine kinase during early reperfusion. The cumulative creatine kinase release during the first 30 min of reperfusion was lower in the candesartan group than in the vehicle group or the losartan group (Fig. 6). There was no difference in the creatine kinase release between the losartan group and the vehicle group.

The noradrenaline level in the coronary effluent was undetectable before ischemia. On reperfusion following ischemia, a marked release of noradrenaline was observed in all groups. Compared to the vehicle group, the cumulative release of noradrenaline during the first 5 min of reperfusion was slightly, although not significantly, lower in the candesartan and the losartan groups (data not shown).

4. Discussion

The present results demonstrate that both losartan and candesartan improve post-ischemic myocardial functional recovery, indicating that angiotensin II, via activation of angiotensin AT_1 receptors, plays an important role in the development of myocardial ischemia/reperfusion injury. However, there is a difference between the drugs, since after washout of the drugs with drug-free buffer perfusion only candesartan inhibited the angiotensin II-induced vasoconstriction and showed a persistent cardioprotective effect.

In the present study, as expected, angiotensin II evoked marked coronary vasoconstrictions as indicated by an increase in coronary perfusion pressure. Both losartan and candesartan concentration-dependently inhibited the increase in coronary perfusion pressure by angiotensin II, supporting the notion that the constriction of coronary resistance vessels in our isolated rat heart preparation is induced by activation of angiotensin AT_1 receptors. Candesartan is much more potent than losartan. At 10 nM, candesartan almost completely inhibited the maximal vasoconstrictive response to a 10-fold higher concentration of angiotensin II, while the corresponding concentration for losartan was 3 μM. Furthermore, the inhibitory effect of candesartan was more persistent than that of losartan, since the inhibition of the angiotensin II-induced coronary vasoconstriction by losartan quickly vanished after a short period of washout with drug-free buffer, while the inhibition by candesartan was maintained. These results are in agreement with earlier characterizations of the receptor kinetics of candesartan and losartan. Candesartan, compared to losartan, has been described to dissociate slowly from AT_1 receptors in cellular membrane preparations (Kinugawa et al., 1997) and to cause a more persistent inhibition of the angiotensin II-mediated vascular contractile response (Shibouta et al., 1993; Morsing et al., 1999). Losartan has been shown to cause a parallel rightward shift in the concentration–effect curves for angiotensin II in various vascular preparations, while candesartan causes a

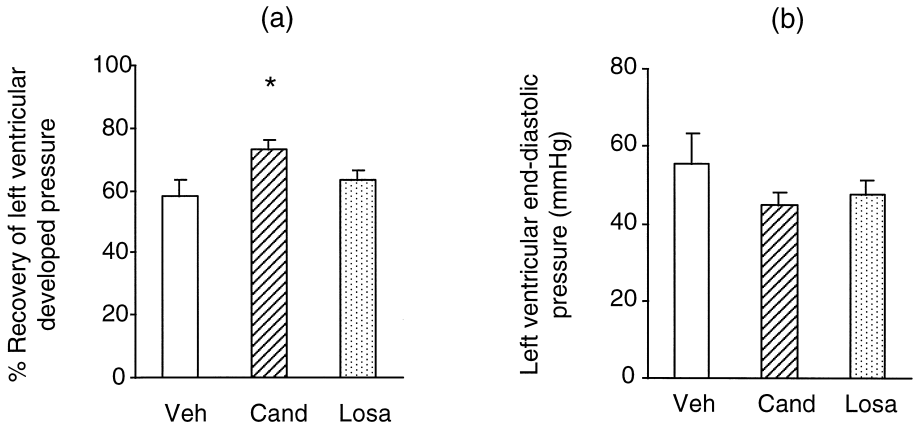

Fig. 5. Recovery of left ventricular developed pressure (a) and left ventricular end-diastolic pressure (b) at the end of 45 min of reperfusion following 25 min of ischemia in hearts pre-treated with vehicle, candesartan (10 nM) or losartan (3 μM) for 90 min followed by 30 min of perfusion with drug-free buffer before the initiation of ischemia. Data are presented as means ± S.E.M ($n = 7$ in each group). $^*P < 0.05$ vs. vehicle group.

marked reduction in the maximum contractile response to angiotensin II (Kinugawa et al., 1997). Thus, candesartan and losartan differ in their angiotensin AT_1 receptor-binding characteristics, causing insurmountable or surmountable antagonism, respectively. In the present study, we postulate that candesartan, due to its slow dissociation from the angiotensin AT_1 receptor, remains at the receptor site to such an extent that not only angiotensin II-induced vasoconstriction is inhibited but also ischemia/reperfusion injury mediated via angiotensin AT_1 receptors is reduced.

Many studies have demonstrated that angiotensin-converting enzyme inhibitors improve myocardial function and limit infarct size following myocardial ischemia and reperfusion (Ertl et al., 1982; Martorana et al., 1990; De Graeff et al., 1994). This effect of angiotensin-converting enzyme inhibition has partly been attributed to reduced degradation of kinins (Martorana et al., 1990; De Graeff et al., 1994), whereas controversy exists concerning the role of reduced angiotensin II formation (Ford et al., 1996; Werrmann and Cohen, 1996; Yang et al., 1997). In the present study, 30 min of pre-treatment with losartan and candesartan immediately before the 25 min of ischemia improved post-ischemic functional recovery, supporting the notion that local cardiac angiotensin II is involved in the development of myocardial ischemia/reperfusion injury, and that the blockade of the angiotensin AT_1 receptor is important for reducing the damage. This is in accordance with earlier studies which showed that angiotensin AT_1 receptor blockade with losartan improved functional recovery following 20 min (Werrmann and Cohen, 1996) and 25 min (Yang et al., 1997) of ischemia in isolated rat hearts, but it is in conflict with the results of a recent study in which losartan was reported to worsen the myocardial dysfunction in hearts subjected to 30 min of ischemia (Ford et al., 1996). There is no obvious explanation for these conflicting findings, except for differences in the length of ischemic period and in the dose and administration time of the compound.

From the present study, it is obvious that a 30-min washout period abolished the protective effect exerted by losartan, while the protection remained in the hearts pre-treated with candesartan. This observation confirms the long-lasting antagonism of the angiotensin AT_1 receptor by candesartan. The improved post-ischemic functional recovery by candesartan was accompanied by attenuated creatine kinase release during reperfusion, suggesting that candesartan also reduced myocardial necrosis induced by ischemia/reperfusion. Taken together, these results indicate that candesartan is able to produce a more persistent beneficial effect than losartan in the setting of myocardial ischemia and reperfusion.

Neither candesartan nor losartan affected cardiac performance or coronary resistance before ischemia, indicating that angiotensin II is not important in maintaining basal cardiac function and coronary circulation in the isolated rat heart, and that the beneficial effect of angiotensin AT_1

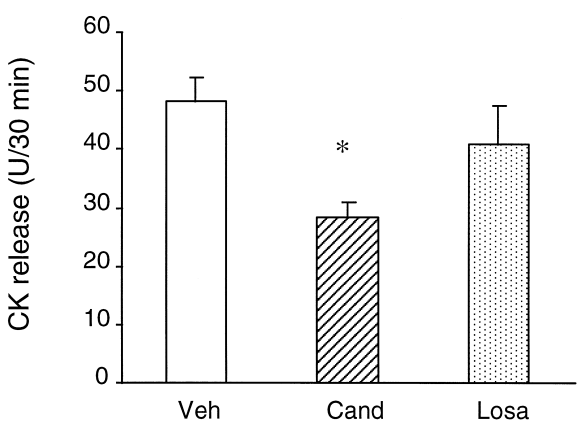

Fig. 6. The cumulative release of creatine kinase (CK) during the first 30 min of reperfusion following 25 min of global ischemia. Hearts received candesartan (10 nM) or losartan (3 μM) for 90 min followed by 30 min of perfusion with drug-free buffer before the initiation of ischemia. Data are presented as means ± S.E.M ($n = 7$ in each group). $^*P < 0.05$ vs. vehicle group.

receptor blockade on ischemia/reperfusion injury is not due to depressed pre-ischemic contractility. There was a similar degree of post-ischemic coronary vasoconstriction in all three groups studied, suggesting that the cardioprotective effect of angiotensin AT_1 receptor blockade is not due to facilitated post-ischemic coronary circulation. Although angiotensin AT_1 receptor blockade has been reported to increase coronary blood flow during ischemia in dogs (Kitakaze et al., 1995), the present use of the zero-flow global ischemia model excluded any contributing role of altered collateral circulation during ischemia in the beneficial effects of candesartan and losartan.

In addition to being a potent vasoconstrictor, angiotensin II exerts important cardiac actions, including positive inotropism and promotion of myocardial hypertrophy (Ferrario and Flack, 1996; Bermann et al., 1997). It has recently been reported that pathophysiological levels of angiotensin II induce myocardial necrosis (Ferrario and Flack, 1996; Bermann et al., 1997). In the isolated rat heart, exogenous angiotensin II, in amounts that do not influence basal cardiac function, accelerated myocardial ischemia/reperfusion injury, supporting its direct cardiotoxic effect (Yoshiyama et al., 1994a,b). The cardiac renin–angiotensin system is activated during acute myocardial ischemia (Ertl et al., 1982; McAlpine and Cobbe, 1988). Augmented angiotensin II formation (Noda et al., 1993; Arakawa, 1996) and angiotensin AT_1 receptor expression (Yang et al., 1997) have been documented in ischemic hearts. Binding of angiotensin II to the angiotensin AT_1 receptor activates phospholipase C, causing hydrolysis of inositol phosphates and subsequent release of intracellular calcium (Ferrario and Flack, 1996; Bermann et al., 1997). These factors increase the susceptibility of the myocardium to damage following ischemia and reperfusion. Blocking of this process may favorably affect the resistance of the myocardium to damage.

Angiotensin II is also an important regulator of noradrenaline release from sympathetic nerve terminals, stimulating local cardiac and vascular sympathetic activity (Ferrario and Flack, 1996; Bermann et al., 1997). In the present study, the noradrenaline release induced by ischemia was only insignificantly lower in hearts receiving candesartan than in hearts given vehicle. The similar degree of insignificant attenuation of noradrenaline release by candesartan and losartan, together with the significant effect of candesartan and the lack of effect of losartan on myocardial functional recovery and creatine kinase release in the washout protocol, suggests that attenuated noradrenaline release is not an important factor in the observed beneficial effect of candesartan.

Both angiotensin AT_1 and AT_2 receptors have been demonstrated in human hearts as well in rat hearts (Feolde et al., 1993). Blockade of the angiotensin AT_1 receptor could cause local angiotensin II to bind to angiotensin AT_2 receptors in endothelial cells, leading to enhanced synthesis and release of the cardioprotective substance bradykinin, as suggested by Wiemer et al. (1993). The existence of such a link between angiotensin AT_1 and AT_2 receptors has been supported by recent findings in transgenic mice. Overexpression of cardiac angiotensin AT_2 receptors attenuated the AT_1 receptor-mediated pressor effect (Masaki et al., 1998), while AT_2 receptor knock-out increased sensitivity to the pressor action of angiotensin II (Hein et al., 1995). Indeed, a recent observation in anesthetized pigs demonstrated that (Jalowy et al., 1998) the infarct-limiting effect of candesartan was abolished with the angiotensin AT_2 receptor antagonist PD123319 and the bradykinin B_2 receptor antagonist HOE140, suggesting that the cardioprotective effect of candesartan involves the activation of angiotensin AT_2 receptors and bradykinin release. However, whether these protective mechanisms of candesartan are important for reducing reperfusion-induced damage in the isolated heart remains to be determined. In the present study, we tried to measure the release of bradykinin from the heart by radioimmunoassay. Unfortunately, the variation within and among individual hearts was so great that no conclusion could be drawn about the effect of candesartan on bradykinin release.

In conclusion, angiotensin AT_1 receptor blockade exerts a cardioprotective effect following myocardial ischemia and reperfusion. Owing to the long duration of its antagonistic effect, candesartan is able to produce a more persistent beneficial effect than losartan.

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