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Improved glucose metabolism following blockade of angiotensin converting enzyme but not angiotensin AT_1 receptors

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Abstract

This study compared the effect of benazepril, an angiotensin converting enzyme inhibitor ([*S*-(*R**,*R**)]-3-[[1-(ethoxycarbonyl)-3-phenylpropyl]amino]-2,3,4,5-tetrahydro-2-oxo) to valsartan, an angiotensin AT_1 receptor antagonist ((*S*)-*N*-valeryl-*N*-[2'-(1*H*-tetrazol-5-yl)biphenyl-4-yl]-methyl-valine) on glucose metabolism and plasma lipid levels in 11- to 12-week-old conscious spontaneously hypertensive rats. Intraperitoneal infusion of benazepril or valsartan at 1, 3 and 10 mg/kg/day produced dose-related reductions in systolic blood pressure after 12 weeks which were not significantly different from each other. During the infusion, valsartan-treated animals gained weight at the same rate as controls, but all infusion rates of benazepril significantly suppressed normal weight gain, despite both compounds having similar antihypertensive effects. At the end of the 12-week treatment period, benazepril (3 and 10 mg/kg/day) significantly increased glucose disposal but did not significantly affect insulin disposal in insulin/glucose tolerance tests. In contrast, none of the infusion rates of valsartan significantly affected glucose or insulin disposal. Finally, compared to controls benazepril and valsartan were without effect on the fasting basal plasma concentrations of glucose, insulin, triglycerides, total cholesterol and K^+. The results demonstrate that angiotensin converting enzyme inhibition and angiotensin AT_1 receptor antagonism have similar antihypertensive effects, but express differences on body weight gain and insulin-stimulated glucose disposal in the conscious spontaneously hypertensive rat.

Keywords: Spontaneously hypertensive rat (SHR); Glucose tolerance; Blood pressure; Angiotensin converting enzyme inhibitor; Angiotensin AT_1 receptor antagonist

1. Introduction

Epidemiological studies have suggested a close association between hypertension and insulin resistance (Ferrannini et al., 1990). Insulin resistance has been postulated not only as a cause of essential hypertension but also as the reason why this disorder is so often associated with metabolic abnormalities such as hyperinsulinemia, glucose intolerance, and disturbances of plasma lipids. The abnormal metabolic profile associated with hypertension, commonly referred to as syndrome X, is believed to play a major role in the development of atherosclerosis (Reaven, 1990). Consequently there is considerable interest in antihypertensive agents that not only lower blood pressure but also improve glucose tolerance and insulin resistance.

Four classes of drugs are currently recommended for the initial therapy of hypertension: diuretics, adrenoceptor antagonists, Ca^{2+} channel antagonists and angiotensin converting enzyme inhibitors. Although all four classes of drugs are effective antihypertensive agents, they have different effects on insulin sensitivity. β-Blockers and thiazide diuretics worsen insulin sensitivity (The National High Blood Pressure Education Program Working Group, 1994; The Working Group on Hypertension in Diabetes, 1987). Ca^{2+} channel antagonists are neutral, while α-adrenoceptor antagonists and angiotensin converting enzyme inhibitors have in some cases been shown to mildly increase insulin sensitivity in hypertensive patients (Pollare et al., 1988; Marre and Fressinaud, 1990).

Angiotensin converting enzyme inhibitors are perceived to have a better tolerability profile than α-adrenoceptor antagonists, β-blockers, Ca^{2+} channel antagonists and diuretics. These factors will contribute

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to an increasingly major share of the market for antihypertensive drugs (Menlo Biomedical Associates, 1993). Correspondingly, there has been considerable interest in the development of other blockers of the renin-angiotensin system such as angiotensin AT_1 receptor antagonists. Valsartan and losartan are examples of angiotensin AT_1 receptor antagonists under development as antihypertensive agents (Criscione et al., 1993; Timmermans et al., 1993). Current evidence suggests that angiotensin AT_1 receptor antagonists are effective antihypertensives and are devoid of the cough that occurs in a significant proportion of patients taking angiotensin converting enzyme inhibitors (Hamada et al., 1993; Strodter et al., 1994). However, while superior over angiotensin converting enzyme inhibitors in one respect it is far from clear whether these agents will possess all of the beneficial actions of this class of antihypertensive agents, in a list which includes improved glucose tolerance and insulin resistance (Levens et al., 1992).

Therefore, in the following studies the effect of the new selective angiotensin AT_1 receptor antagonist, valsartan, has been compared to the effect of the angiotensin converting enzyme inhibitor, benazepril, on insulin/glucose tolerance in the conscious spontaneously hypertensive rat. The spontaneously hypertensive rat was used in these studies as an animal model of essential hypertension with coexistent glucose intolerance, insulin resistance and dyslipidemia (Bursztyn et al., 1992; Reaven and Chang, 1991; Dall' Anglio et al., 1991).

2. Materials and methods

2.1. Animals

These experiments were performed with male, spontaneously hypertensive rats (SHR/NIco; Iffa Crédo, Lyon, France) weighing between 260–280 g. Upon arrival at our facilities, rats were housed in groups of 5 in sawdust-littered, wire-topped plastic cages (35 × 18 × 57 cm). The cages were maintained in a room with a 12 h light/dark cycle (6 a.m.–6 p.m. light) at a temperature of 20–24°C and 55% relative humidity. The animals were allowed access to a normal diet (NAFAG, Gossau, Switzerland) and given tap water to drink. Before use in the following experiments, each animal was acclimatized to the above conditions for at least 7 days to ensure a standard state of Na^+ intake and hydration.

2.2. Experimental protocols

After acclimatization, body weight was determined to the nearest gram and systolic blood pressure was measured by the tail-cuff method (Ugo Basile, Comerio, Italy). Body weight and systolic pressure were always measured between 8 a.m. and 10 a.m. The measurement of body weight and systolic blood pressure was repeated on two further occasions, over the next 7 days, to establish baseline values. To facilitate accurate tracking over time, rats were studied in groups of approximately 12 animals. Shortly after the last baseline measurement, equal numbers of rats within an experimental group were randomly assigned treatment with either the isotonic saline vehicle or a single infusion rate of benazepril or valsartan (either 1, 3 or 10 mg/kg/day). Although treatment of the groups was randomized, the experimental design was such that during the infusion period, each dose of the 2 drugs could be considered to have a parallel control group. Over the course of the experiments body weight and systolic blood pressure were determined at times close to the removal of the mini-pumps. At the end of the treatment period, each group of animals was assigned to one of 2 experimental protocols determining either insulin/glucose tolerance, or plasma lipid concentrations.

2.3. Surgical procedures

Implantation of mini-pumps

Benazepril and valsartan were given by osmotic mini-pumps implanted into the abdominal cavity. Rats were anesthetized with a mixture of 1.5% gaseous halothane (Hoechst-Pharma, Zurich, Switzerland) in oxygen. Once immobile, the rats were placed, in dorsal recumbency, upon a metal operating table and kept at a temperature of 36 ± 0.5°C. After a surgical plane of anesthesia had been reached, defined as the loss of both blink and pedal reflexes, a small opening was made through the ventral midline just above the head of the bladder and an Alzet osmotic mini-pump (model 2ML4; Alza Corp, Palo Alto, CA, USA) containing either benazepril, valsartan or vehicle was inserted into the abdominal cavity. After implantation of the mini-pump, the body wall and skin were sutured closed and the animal returned to his home cage. The mini-pumps delivered benazepril or valsartan at 1, 3 or 10 mg/kg/day or vehicle at 2.5 μl/h for 28 days. Over the 12-week infusion period, the pumps were changed every 28 days.

Implantation of catheters

At the end of the infusion period, rats were anesthetized as described above and catheters were implanted into the right femoral artery and vein. The vessel catheters were 56 cm in length and made of polyethylene tubing (0.58 mm i.d. × 0.96 mm o.d.; Portex, Hythe, Kent, UK). To the end of the catheter inserted into the vessel, a 2.5 cm length of smaller

polyethylene tubing (0.40 mm i.d. × 0.80 mm o.d.) was attached. To minimize vessel damage during the implantation period, a 1 cm piece of Silastic tubing (0.012″ i.d. × 0.025″ o.d.; Dow Corning, Midland, MI, USA) was attached to the tip of the smaller catheter. The vessel catheters were filled with heparinized (10 IU/ml; Liquemine, Roche-Pharma, Basel, Switzerland) isotonic saline, plugged with stainless-steel pins and tunnelled subcutaneously, to exit dorsally from the animals in the interscapular region.

After recovery from surgery, the animals were then placed singly within small plastic cages (19 × 23 × 30 cm). The arterial and venous catheters passed to the outside of the cage through a 31 cm stainless-steel spring attached to the animal with a Silastic collar. The venous catheter remained plugged while the arterial catheter was connected through a single-channel infusion swivel (Instech Labs, Horsham, PA, USA) to a computerized data collection system (Buxco Electronics, Sharon, CT, USA). The arterial catheter was kept patent by the continuous infusion of 17 μl/h heparinized (10 IU/ml) saline. The rats were allowed to recover from surgery for 48 h before the performance of the intravenous insulin/glucose tolerance tests.

2.4. Collection of blood for the measurement of plasma lipids

Because of the quantity of blood that had to be removed from the animals for the measurement of plasma lipid levels, Swiss animal protection legislation required that the rats first be anesthetized.

After an overnight fast, rats were anesthetized as described above. A midline incision was performed to expose the viscera and the abdominal aorta located and cleaned of connective tissue. A 22 gauge needle attached to an ice-cold heparin-coated 10 ml syringe was inserted into the lumen of the aorta and 6 ml of blood quickly withdrawn. The blood was placed into 10 ml polyethylene tubes, centrifuged at 4°C to separate the red cells from the plasma, and stored frozen at −20°C until assay. Plasma lipids were determined as described below.

2.5. Insulin / glucose tolerance test

Insulin/glucose tolerance tests were conducted according to previously published methods (Levy et al., 1984). Rats were fasted overnight and allowed free access to water. With each animal resting quietly, mean arterial pressure was determined as described above. Thereafter, 0.35 ml of arterial blood was quickly withdrawn into an ice-cold 1 ml syringe and placed into a heparin-coated microcentrifuge tube maintained on ice. Each rat then received an intravenous injection of 500 mg/ml dextrose followed immediately by injection of 0.174 IU/kg human insulin (Actrapid, Novo Nordisk Pharma, Keunact, Switzerland) flushed into the animal by a 0.5 ml bolus of isotonic saline. The duration of each injection was less than 10 s. The volume of the dextrose solution injected (0.5–0.6 ml) was adjusted to deliver glucose at a dose of 700 mg/kg. The volume of insulin solution injected (0.174 IU/ml) varied between 0.35 ml and 0.45 ml depending upon the weight of the animal. Blood samples (0.35 ml) were taken at 2, 5, 10, 20, 30 and 40 min after injection of the glucose solution and replaced with an equivalent volume of isotonic saline. At the end of the experiment, the rat was killed with Vetanarcol (Veterinaria, Zurich, Switzerland) and weighed. Plasma was separated from blood cells at 4°C and glucose concentrations immediately measured. The remaining plasma was frozen and maintained at −80°C prior to the measurement of plasma insulin concentrations.

2.6. Biochemical analyses

Plasma glucose concentrations were measured using a Beckman glucose analyzer 2 (Beckman Instruments, Brea, CA, USA). Plasma triglycerides and total cholesterol concentrations were measured using a Ciba-Corning Express 550 autoanalyser (Ciba-Corning Diagnostics, Zurich, Switzerland) according to previously described methods (Allain et al., 1974; Nagele et al., 1984). Plasma K^+ concentrations were determined by flame photometry (AutoCal FP 743, Instrumentation Laboratory, Milan, Italy). Plasma insulin concentrations were measured by the double-antibody dilution method using a commercially available kit for human insulin (INSI5-SB; Medipro, Teufen, Switzerland). Rat insulin cross-reacted 100% with the human insulin antibody supplied with the kit and was used as the standard (Novo-Nordisk).

2.7. Drugs used

The angiotensin converting enzyme inhibitor benazepril ([*S*-(*R**,*R**)]-3-[[1-(ethoxycarbonyl)-3-phenylpropyl]amino]-2,3,4,5-tetrahydro-2-oxo) and the angiotensin AT_1 receptor antagonist valsartan (valeryl-*N*-[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]-methyl-valine) were synthesized by Ciba-Geigy. Benazepril was dissolved in distilled water. Valsartan was dissolved in water alkalinized with NaOH and neutralized with hydrochloric acid (Criscione et al., 1993).

2.8. Data analysis

In the insulin/glucose tolerance tests, log plasma glucose and log plasma insulin concentrations were plotted by computer as a function of time. A straight line exponential function was fitted by the computer

using either the glucose or insulin values obtained at time points 2 through 20 min. The insulin disappearance rates were calculated as $t_{0.5}$ values, defined as the time taken for the plasma peptide levels to decrease by one-half. The $t_{0.5}$ values were calculated from the slope of the insulin disappearance curves. Glucose disposal was calculated as k values, defined as the diminution rate of the blood glucose concentration in percentage per min. k Values were derived from the slopes of the glucose disappearance curves according to the equation $k = 0.0693/t_{0.5}$ (Lundbaek, 1962; Franchrou et al., 1962). Areas under the curves were calculated by trapezoid integration.

2.9. Statistical analysis

Statistical analysis was by analysis of variance followed by the least-significant difference procedure. Where parameters were followed as a function of time, the data were analyzed using multivariate analysis of variance for repeated measures using the significance of the Wilks' lambda in place of the F value (Armitage and Berry, 1987). In all analyses P values < 0.05 were regarded as statistically significant.

3. Results

3.1. Effect of benazepril and valsartan on systolic blood pressure and body weight

Systolic blood pressure

Compared to the respective group of time control animals, the angiotensin I converting enzyme inhibitor benazepril produced a dose-related fall in systolic blood pressure (Fig. 1). The AT_1 receptor antagonist valsartan produced a fall in systolic blood pressure that was at least as great but not significantly different to that of benazepril.

Body weight

The results show that during the treatment period, body weight increased significantly from baseline values in each of the control, benazepril and valsartan treatment groups (Fig. 1). The increase in body weight in animals treated with either 1, 3 or 10 mg/kg/day valsartan was not significantly different from the increase in body weight observed in time control animals. In contrast, the increase in body weight in animals treated with either 1, 3 or 10 mg/kg/day benazepril

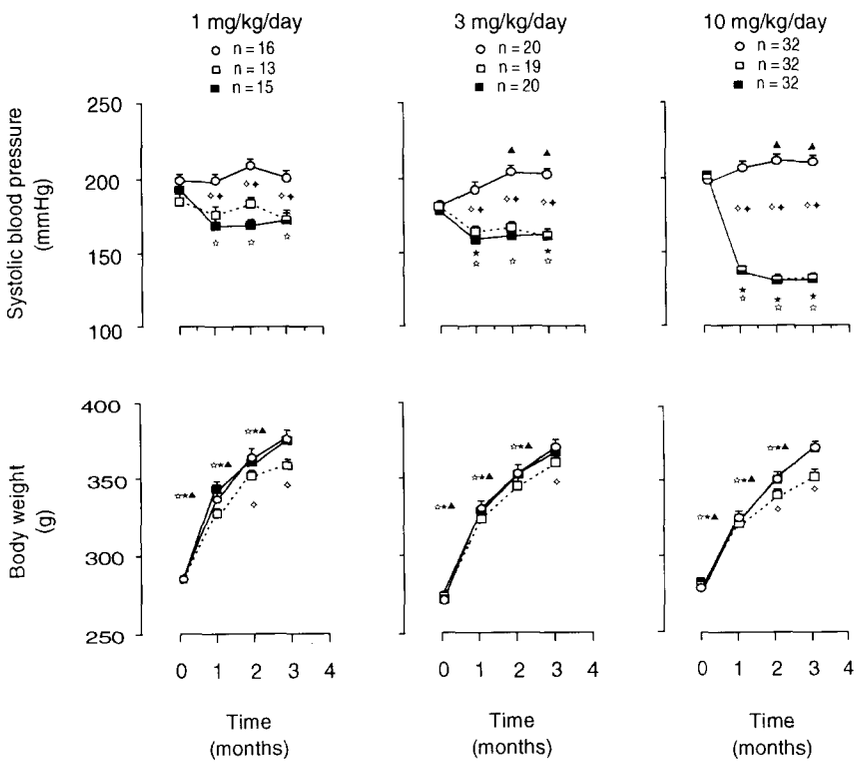

Fig. 1. Benazepril and valsartan on systolic blood pressure and body weight. Results presented are a compilation of all experiments conducted with vehicle (○), benazepril (□) or valsartan (■). In the body weight graphs, the symbols for valsartan are in many cases obscured by the control symbols. Results expressed as mean + S.E. Numbers of animals used at each dose of drug and control are the same for both blood pressure and body weight. Statistics by multivariate analysis of variance for repeated measures, followed by the least-significant difference test. Significant comparisons to time 0 in each vehicle (▲), benazepril (☆) or valsartan (★) treatment group. Significant comparisons at each time point to control group from benazepril (◇) and valsartan (◆) treatment groups.

Table 1
Effect of benazepril and valsartan on mean blood pressure and fasting basal glucose levels measured in the insulin/glucose tolerance tests

	Infusion rate			
	Control	1 mg/kg/day	3 mg/kg/day	10 mg/kg/day
Mean blood pressure (mm Hg)	160 ± 3			
Benazepril		147 ± 3	135 ± 5 [a]	105 ± 4 [a]
Valsartan		143 ± 7 [a]	129 ± 3 [a]	93 ± 4 [a]
Fasting plasma glucose (mmol/l)	5.4 ± 0.1			
Benazepril		5.1 ± 0.2	5.2 ± 0.2	5.7 ± 0.1
Valsartan		5.3 ± 0.2	5.2 ± 0.1	5.6 ± 0.2

Results are expressed as mean ± S.E. The number of experiments is the same as in Fig. 2B. Statistics compare each infusion rate of benazepril and valsartan with control and each other by analysis of variance. [a] Significantly different from control values by the least-significant difference procedure. In the blood glucose measurements no significant differences were found.

was reduced significantly below values obtained in control animals.

At the end of the treatment period, analysis of variance indicated that systolic blood pressure and body weight in the 3 control groups of animals were not significantly different. The control experiments were therefore combined for analysis in the following studies.

3.2. Benazepril and valsartan on components of the insulin / glucose tolerance test

Blood pressure

Mean blood pressure measured from the arterial catheter in the time period immediately prior to conducting the insulin/glucose tolerance tests is shown in Table 1. The results show that both benazepril and valsartan produced dose-related, and significant falls in mean blood pressure. The fall in blood pressure in response to valsartan was slightly more pronounced than produced by benazapril but the effects of the drugs were not significantly different from each other.

Glucose metabolism

Baseline plasma glucose was 5.4 ± 0.1 mmol/l in the control group of animals and was not significantly affected by any of the infusion rates of benazepril or valsartan (Table 1). In the control group of animals, intravenous injection of 700 mg/kg dextrose together with 0.174 IU/kg insulin produced the changes in plasma glucose shown in Fig. 2A. Regression analysis of the disappearance of glucose from the plasma allowed the calculation of k values which were 9.09 ± 0.15%/min in the control group of animals (Fig. 2B). The rate of glucose disappearance from the plasma was not significantly affected by any of the infusion rates of valsartan. In contrast, infusion rates of 3 and 10 mg/kg/min benazepril significantly enhanced glucose disposal both from control values and from the corresponding values obtained in valsartan-infused ani-

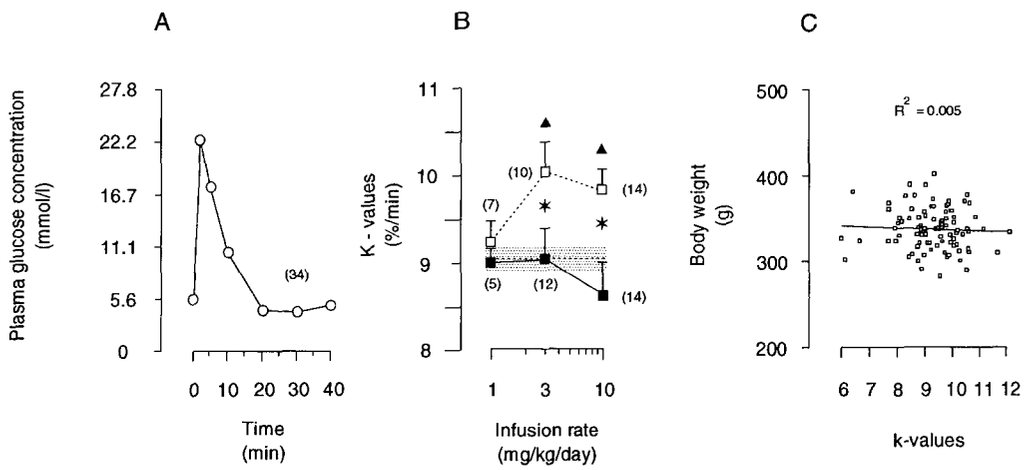

Fig. 2. Benazepril and valsartan on glucose metabolism during the insulin/glucose tolerance test. Results expressed as mean + S.E. The numbers of animals in each treatment group are shown in parentheses. (A) Control (○), in this case the S.E. bars are smaller than the symbols. (B) Benazepril (□), valsartan (■). Horizontal broken line and accompanying shading are the mean ± S.E. of the control group of experiments. Statistics by analysis of variance followed by the least-significant difference test. ▲ Significantly different from control values. * Significant difference between drugs at equivalent infusion rates.

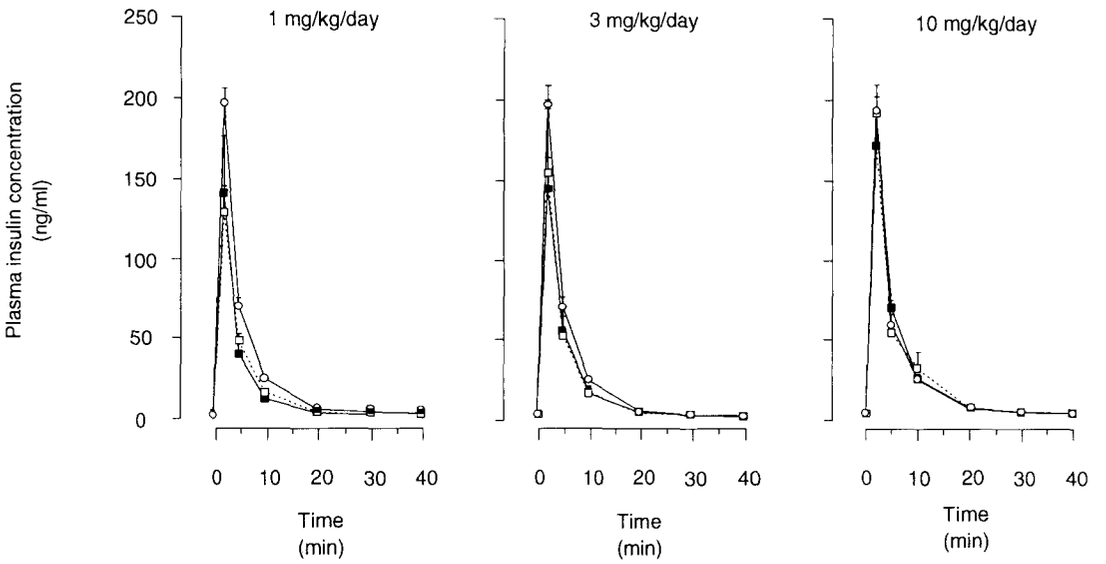

Fig. 3. Benazepril and valsartan on insulin disappearance during the insulin/glucose tolerance test. Results expressed as mean + S.E. The numbers of animals in each treatment group are the same as in Fig. 2B. Control (○), benazepril (□), valsartan (■). Statistics compare the insulin disappearance curves obtained with each infusion rate of benazepril and valsartan with each other and control by multivariate repeated measures analysis of variance. At each infusion rate, there were no significant differences between benazepril and valsartan or control.

mals. The results in Fig. 2C demonstrate a lack of correlation between body weight at the end of the experiment and the k values obtained in all control, benazepril and valsartan experiments.

Insulin disappearance

The changes in plasma insulin concentration obtained as a function of time during each of the insulin/glucose tolerance tests are shown in Fig. 3. Baseline plasma insulin concentration was 2.27 ± 0.21 ng/ml in the control group of animals and was not significantly affected by any of the infusion rates of benazepril or valsartan. At all 3 infusion rates studied, both benazepril and valsartan produced essentially identical insulin disappearance curves, which were not significantly different either from each other or from the insulin disappearance curves obtained in the control group of animals. Furthermore, neither benazepril nor valsartan at any infusion rate affected the calculated $t_{0.5}$ values for insulin disappearance during the time period 2–20 min after insulin/glucose injection (Table 2).

Table 2
Effect of benazepril and valsartan on the rate of insulin disappearance in the insulin/glucose tolerance tests

	Insulin disappearance rate $t_{0.5}$ (min)			
	Control	1 mg/kg/day	3 mg/kg/day	10 mg/kg/day
Benazepril	3.5 ± 0.1	3.4 ± 0.1	3.9 ± 0.2	3.6 ± 0.1
Valsartan		3.1 ± 0.3	3.5 ± 0.1	3.7 ± 0.2

Results are expressed as mean ± S.E. The numbers of animals are the same as in Fig. 2B. Statistics compare each infusion rate of benazepril and valsartan with control and each other by analysis of variance. In this analysis, no significant differences were found.

Plasma K^+

Baseline plasma K^+ concentration was 5.4 ± 0.1 mmol/liter in the control group of animals and was not significantly affected by any of the infusion rates of benazepril or valsartan (results not shown).

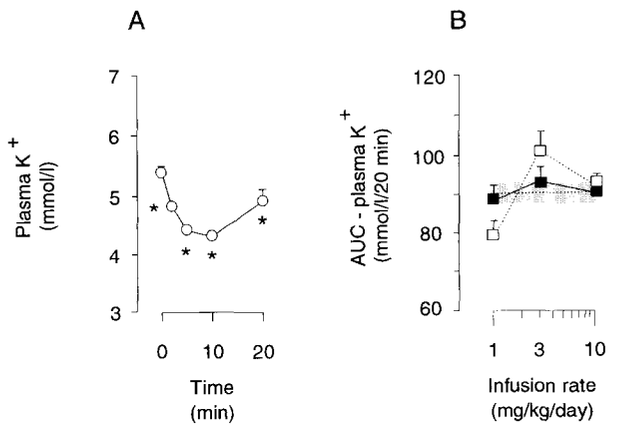

Fig. 4. Benazepril and valsartan on the plasma K^+ concentration during the insulin/glucose tolerance test. Results expressed as mean + S.E. The numbers of experiments in each treatment group are the same as in Fig. 2B. Control (○), benazepril (□), valsartan (■). (A) Statistics compare the decrease in plasma K^+ concentration at each time point with control by multivariate repeated measures analysis of variance followed by the least-significant difference test. * Significantly different from time 0. (B) Statistics compare each dose of drug with each other and control by analysis of variance. In this analysis no significant differences were found.

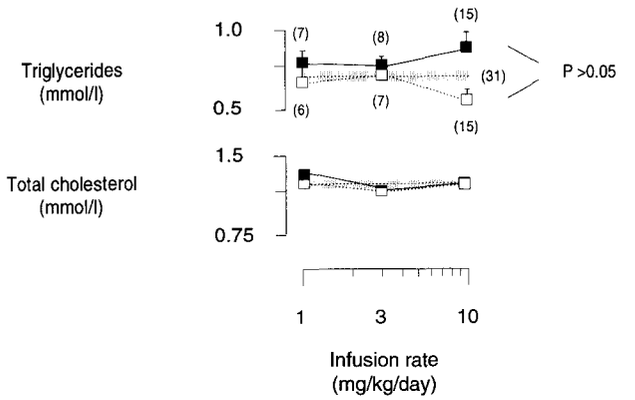

Fig. 5. Benazepril and valsartan on fasting plasma triglyceride and total cholesterol concentrations. Results expressed as mean + S.E. Horizontal line and accompanying dotted line are the mean ± S.E. of the control group of animals. The numbers of experiments are shown in parentheses and are the same for both lipids. Statistics by analysis of variance followed by the least-significant-difference test which compared each dose of drug with each other and with control values.

In the control group of animals, the combined injection of insulin and glucose resulted in an immediate and significant fall in plasma K^+ concentration which peaked after 10 min (Fig. 4A). The area under this curve was calculated, and is shown in Fig. 4B. At all 3 infusion rates studied, neither benazepril nor valsartan significantly altered the area under the plasma K^+/time curve described for control animals (Fig. 4B).

3.3. Effect of benazepril and valsartan on fasting plasma triglyceride and total cholesterol

Compared to control levels, neither benazepril nor valsartan produced a significant change in the fasting plasma concentrations of triglycerides or total cholesterol. However, plasma triglyceride levels in valsartan-treated animals were significantly higher than in benazepril-treated animals following infusion of either drug at 10 mg/kg/day.

4. Discussion

The purpose of this study was to compare the effects of angiotensin converting enzyme inhibition with angiotensin AT_1 receptor antagonism on glucose/insulin tolerance and plasma lipid levels in the conscious spontaneously hypertensive rat

Many studies have shown that chronic inhibition of angiotensin converting enzyme not only lowers blood pressure but also significantly inhibits weight gain in young spontaneously hypertensive rats (Harrap et al., 1990; Thybo et al., 1994). Similar results are shown in the present studies following inhibition of angiotensin converting enzyme with benazepril. The mechanism by which blockade of angiotensin converting enzyme inhibits weight gain in spontaneously hypertensive rats is unclear. Reduced blood pressure is an unlikely explanation since chronic antihypertensive treatment with the non-specific vasodilator, pinacidil, is without effect on body weight gain in young genetically hypertensive rats (Jespersen et al., 1986). In addition, angiotensin converting enzyme inhibitors suppress weight gain not only in spontaneously hypertensive rats but also in young normotensive animals in which changes in blood pressure are small or absent (Harrap et al., 1986; Dyer et al., 1992). It appears that effects on the growth process must be involved since chronic angiotensin converting enzyme inhibition leads to a proportional decrease in body and organ weight in growing animals and does not produce weight loss in adult spontaneously hypertensive animals (Harrap et al., 1990; Oddie et al., 1993).

Surprisingly, the results of the present study show that blockade of angiotensin AT_1 receptors with valsartan did not alter body weight gain in 8- to 10-week-old spontaneously hypertensive rats. Little is known concerning the effect of angiotensin AT_1 receptor blockade on weight gain in spontaneously hypertensive rats. The few studies where this parameter has been measured do not give a clear picture of the effect of angiotensin AT_1 receptor antagonism on weight gain. For example, chronic oral treatment of spontaneously hypertensive rats from the age of 4 weeks with either losartan or enalapril produced equivalent reductions in body weight gain (Morton et al., 1992). However, in other studies, angiotensin AT_1 receptor antagonism has been shown to be either ineffective or less effective than enalapril at retarding body weight gain in spontaneously hypertensive rats (Gillies and Lee, 1995; Friberg et al., 1994). Recently obtained unpublished studies comparing the effects of valsartan and enalapril in 4-week-old New Zealand genetically hypertensive rats have also demonstrated that intraperitoneal infusion of valsartan was less effective than enalapril at slowing weight gain over the following 6-week treatment period (Ledingham, J.M., personal communication). The reason for the discrepancies between studies are not known but may well be related to the age of the animals when treatment was instituted, the duration of treatment, or to the different methods of drug administration.

Insulin-stimulated glucose uptake was quantitated in the present studies using an insulin/glucose tolerance test. In this minimal model, the glucose disappearance rate has been used as an index of insulin sensitivity (Levy et al., 1984). Infusion of benazepril at 3 and 10 mg/kg/day significantly increased glucose disposal relative to control animals. Surprisingly, when compared to control values, infusion of valsartan at 1, 3 or 10

mg/kg/day did not significantly affect glucose disposal. The curves describing plasma insulin concentration as a function of time in response to each infusion rate of benazepril and valsartan were not significantly different from control values and were virtually identical to each other. Furthermore, the $t_{0.5}$ values describing insulin disposal were not significantly influenced by either benazepril or valsartan. It is unlikely, therefore, that changes in insulin clearance can explain the differences in glucose disposal observed between benazepril- and valsartan-treated animals. The data may, however, be explained by a differential effect of each drug on insulin sensitivity.

The above contention is supported by previous observations using the hyperinsulinemic euglycemic clamp which demonstrated that in 4-week-old spontaneously hypertensive rats infusion of enalapril for 3 weeks led to a fall in blood pressure and to an increase in insulin sensitivity (Tomiyama et al., 1994). In contrast, infusion of an equi-antihypertensive dose of the angiotensin AT_1 receptor antagonist losartan failed to increase insulin sensitivity, an observation in complete agreement with those obtained with valsartan in the present studies. The fact that neither valsartan nor losartan acted to improve insulin sensitivity in the spontaneously hypertensive rat appears to reflect a class effect of AT_1 receptor antagonists in this experimental model.

Recently, losartan has been shown to lower blood pressure and to increase insulin sensitivity in human subjects with severe hypertension, a result opposite to those observed in the spontaneously hypertensive rat (Moan et al., 1994; Tomiyama et al., 1994). The explanation for this discrepancy between the effects of AT_1 receptor antagonism on insulin sensitivity between animal and human hypertension is not known.

The reason why increased insulin sensitivity in response to inhibition of angiotensin converting enzyme in spontaneously hypertensive rats is not a response shared by angiotensin AT_1 receptor inhibition remains unclear at the present time. Endogenous kinin production appears to be involved since the increase in insulin sensitivity produced by inhibition of angiotensin converting enzyme in the spontaneously hypertensive rat can be prevented by a kinin receptor antagonist (Tomiyama et al., 1994).

In the present studies, animals treated with 1, 3 and 10 mg/kg/day benazepril lost weight, while those treated with valsartan grew at the same rate as control animals. Although weight loss has been shown to decrease insulin resistance it is unlikely to explain the differential effects of benazepril and valsartan, since insulin-stimulated glucose disposal in response to either compound did not correlate with body weight. Furthermore, following infusion at 1 mg/kg/day, benazepril significantly inhibited body weight gain but did not affect glucose disposal in the insulin/glucose tolerance tests.

The site of the improvement in insulin sensitivity with angiotensin converting enzyme inhibition was not determined in this or the previously mentioned hyperinsulinemic-euglycemic clamp studies (Tomiyama et al., 1994). However, inhibition of angiotensin converting enzyme induces large changes in blood pressure and vascular perfusion which have been used in the past to explain the effect of angiotensin II on insulin sensitivity (Moan et al., 1994; Fliser et al., 1993). Presumably, in the present studies, a component of the increase in insulin sensitivity in response to inhibition of angiotensin converting enzyme could involve vasodilation and increased blood flow to peripheral vascular beds, particularly skeletal muscle. Elevated blood flow would enhance the rate of insulin and glucose delivery and, ultimately, increase glucose disposal. Although both inhibitors of the renin-angiotensin system produced equivalent lowering of blood pressure their effects on tissue vascular perfusion were not studied. Therefore, it is not known if the differential effects of both inhibitors on glucose/insulin sensitivity are secondary to variant effects on tissue blood flow, although this hypothesis amongst others would provide a fruitful area for future research.

Insulin decreases plasma K^+ levels, an effect secondary to the increased cellular uptake and to the decreased renal excretion of this ion (DeFronzo et al., 1980; Santoro et al., 1992). Previous studies have suggested that chronic inhibition of angiotensin converting enzyme results in resistance to the K^+-lowering actions of insulin and thereby to increased plasma K^+ levels. In this hypothesis, increased plasma K^+ causes vasodilation which contributes to the antihypertensive effects of angiotensin converting enzyme inhibitors (Santoro et al., 1992). However, in the present studies significant changes in plasma K^+ in the basal state or in response to insulin were not observed with either benazepril or valsartan. Thus, under the conditions of the present study resistance to the K^+-lowering effects of insulin did not appear to contribute to the antihypertensive actions of benazepril and valsartan in the spontaneously hypertensive rat.

In addition to effects on carbohydrate metabolism, genetic hypertension in rats is associated with an elevation in plasma lipid levels (Reaven and Chang, 1991; Dall' Anglio et al., 1991). Essential hypertension in man is often associated with elevated plasma lipid levels (Hauf-Zachario et al., 1993). Angiotensin converting enzyme inhibitors have been shown either not to affect plasma lipids or to slightly lower lipid levels in hypertensive subjects with or without hyperlipidemia (Moser and Menard, 1993). In contrast, the effects of blockade of angiotensin AT_1 receptors on plasma lipid levels in animals or man remain unreported at this

time. In the present studies, benazepril did not significantly affect plasma lipid levels from control values, a result in accord with previous observations. Compared to control values, blockade of angiotensin AT_1 receptors also did not significantly affect plasma lipid concentrations. Based on these observations, it appears that blockade of the renin-angiotensin system with either an angiotensin converting enzyme inhibitor or an AT_1 receptor antagonist does not act to affect plasma lipids, at least in the genetically hypertensive rat. Although neither inhibitor affected plasma lipid levels from control animals, there may be a difference in the actions of the 2 classes of inhibitors since at the highest infusion rate studied there was a significant difference between lipid levels in the 2 groups of animals.

In summary, inhibition of angiotensin converting enzyme with benazepril lowered blood pressure, slowed body weight gain and improved insulin/glucose tolerance in the 11- to 12-week-old spontaneously hypertensive rat. Blockade of angiotensin AT_1 receptors also lowered blood pressure but did not affect body weight gain or insulin/glucose tolerance. The increased insulin/glucose tolerance in response to benazepril was not explained by the parallel decrease in body weight. Neither inhibitor of the renin-angiotensin system affected plasma lipids from control values in these experiments. Thus, AT_1 receptor antagonism and angiotensin converting enzyme inhibition appear to exert different effects on body weight gain and glucose metabolism in the spontaneously hypertensive rat.

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