Effect of the Alpha-Adrenergic Blocker, Doxazosin, on Endothelial Function and Insulin Action

C. Hamish Courtney, David R. McCance, A. Brew Atkinson, Jonathan Bassett, Cieran N. Ennis, Brian Sheridan, and Patrick M. Bell

Essential hypertension is associated with impairment of both endothelial function and insulin action, and this has provided rationale for the use of antihypertensive agents that are at least neutral, if not beneficial, in these areas. This study examines the effect of the alpha-adrenergic blocker, doxazosin, on endothelial function and insulin action. Sixteen patients with essential hypertension were recruited with 13 (3 men/10 women; median age, 55 years; range, 38 to 65 years) completing the study. A double-blind, placebo-controlled crossover study design was used. After a 6-week placebo run-in, there were two 12-week treatment periods of either placebo or doxazosin, separated by a 6-week wash out period. Subjects were studied at the end of each treatment period with endothelial function assessed by forearm plethysmography and insulin action by the hyperinsulinemic clamp technique. Blood pressure was significantly lowered by doxazosin (doxazosin 144 ±3/86 ± 2 mm Hg; placebo 159 ± 3/96 ± 1 mm Hg, $P < .005$ for both systolic and diastolic pressure; mean ± SEM). Baseline forearm blood flow (FBF) was unchanged (doxazosin 4.9 ± 0.9; placebo 4.0 ± 0.7 mL · 100 mL^{-1} · min^{-1}, $P > .05$), however, FBF responses (area under dose response curve, percentage change in infused:control arm ratio) to acetylcholine (endothelium-dependent vasodilation) were improved by doxazosin (doxazosin 58.6 ± 11.7 standard units [SU]; placebo 22.1 ± 7.0 SU, $P = .03$) with responses to sodium nitroprusside (endothelium-independent vasodilation) unchanged (doxazosin 40.3 ± 5.5 SU; placebo 46.3 ± 8.1 SU, $P > .05$). Exogenous glucose infusion rates to maintain euglycemia during hyperinsulinemia were not significantly different (doxazosin 30.4 ± 0.9; placebo 32.3 ± 1.0 μmol · kg^{-1} min^{-1}, $P > .05$). Suppression of postabsorptive endogenous glucose production by insulin was also unchanged by treatment (doxazosin 65.6% ± 7.5% suppression; placebo 68.3% ± 11.2% suppression, $P > .05$). Doxazosin has a neutral effect on both peripheral and hepatic insulin action, but improves endothelium-dependent vasodilation. These results indicate that doxazosin can be used safely in patients with insulin resistance, while its positive effect on endothelial function may lessen the subsequent incidence of atherosclerosis.

ESSENTIAL HYPERTENSION is associated with impairment of both endothelium-dependent vasodilation and insulin action. Both have been suggested to play a role in the pathogenesis of hypertension, endothelial dysfunction as a consequence of its alteration in vascular tone, and insulin resistance by mechanisms including sympathetic nervous system activation and renal sodium retention.[1-4] Additionally both are implicated in the pathogenesis of atherosclerosis, endothelial dysfunction by its effects on vascular smooth muscle proliferation, and platelet adhesion and insulin resistance by virtue of its association with other conditions with high cardiovascular risk, such as glucose intolerance and dyslipidemia.[5,6] Consequently, there is interest in the effect of antihypertensive therapy on endothelial function and insulin action, as adverse effects of these agents may offset potential benefits of blood pressure reduction.[7]

Alpha-adrenergic receptor blocking agents, such as doxazosin, are effective antihypertensive drugs used in the management of essential hypertension.[8] Studies in subjects with essential hypertension have suggested a beneficial effect of doxazosin on insulin action,[9-11] but these studies have been largely uncontrolled with no double-blind, placebo-controlled trial examining the effect of doxazosin on both peripheral and hepatic insulin action in subjects with essential hypertension. No direct study of the effect of doxazosin on endothelial function in humans has been reported.

The present study was designed to determine the effect of doxazosin on endothelial function and peripheral and hepatic insulin sensitivity in subjects with essential hypertension. To investigate this, we performed a double-blind, randomized, placebo-controlled crossover study using forearm blood flow (FBF) responses to intrabrachial artery infusions of vasoactive agents to assess endothelial function and the hyperinsulinemic clamp technique combined with isotope dilution methodology to assess insulin action.

RESEARCH DESIGN AND METHODS

Patients with essential hypertension were recruited from a general practice in close proximity to Belfast. All were of Caucasian origin. Significant obesity (> 125% ideal body weight), cardiac, hepatic or renal disease, diabetes mellitus, age over 65 years, and necessity for drug treatment that might affect insulin action precluded participation. Subjects with evidence on electrocardiogram (ECG) of left ventricular hypertrophy or albuminuria were also excluded. Patients with secondary hypertension were excluded, as were those with diastolic blood pressure outside the range 95 to 110 mm Hg after a run-in period of 6 weeks. All patients gave written informed consent, and the protocol was approved by the Ethics Committee of the Queen's University of Belfast.

Study Design

A randomized, double-blind crossover design was used. Antihypertensive agents were withdrawn and placebo substituted during a 6-week run-in period. At the end of this period, only patients with a diastolic

From the Regional Centre for Endocrinology and Diabetes, Royal Victoria Hospital, Belfast; and the Montalto Health Clinic, Balynahinch, Co. Down, United Kingdom.

Submitted September 27, 2002; accepted April 22, 2003.

Supported by a Royal Victoria Hospital Research Fellowship (to C.H.C.), Royal Victoria Hospital Metabolic Unit Research Fund, and Pfizer UK (to C.H.C. and P.M.B.).

Address reprint requests to Patrick M. Bell, MD, East Wing Office, Royal Victoria Hospital, Belfast, UK BT12 6BA.

0026-0495/03/5209-0010$30.00/0

doi:10.1016/S0026-0495(03)00190-2

blood pressure between 90 and 110 mm Hg were eligible to continue. These patients were randomized to receive either placebo or doxazosin for a 12-week period. Doxazosin was commenced at 1 mg daily for 3 days, 2 mg daily for the next 4 days, and 4 mg daily thereafter. The dose was increased in 4-mg increments to a maximum dose of 16 mg over the following 4 weeks aiming for a target blood pressure of 135/85 mm Hg. The placebo and active tablets were identical in taste and appearance, and study medication was inspected every 2 weeks to assess compliance. Patients were seen by the same investigator throughout the trial. Blood pressure was measured in the morning with the patient recumbent. Arterial blood pressure was measured to the nearest 2 mm Hg using a Hawksley random zero sphygmomanometer (Hawskley & Sons, Lancing, Sussex, UK) with the arm supported at heart level. The same arm was used on each occasion. Diastolic blood pressure was taken at the point of disappearance of the Korotkoff sound (phase V). Diastolic blood pressure greater than 110 mm Hg on more than 2 occasions or greater than 120 mm Hg on more than 1 occasion required the introduction of an additional antihypertensive agent. In this study, the metabolically neutral calcium channel blocker, amlodipine, was added to the study medication. Patients were instructed to maintain consistent dietary and exercise habits throughout the study. Other potential confounding factors, such as smoking status, also remained constant throughout the study.

Assessment of Endothelial Function

Endothelial function was assessed from FBF responses to brachial artery infusion, a technique already established in our unit.[12] Studies were performed in the morning in a quiet, temperature-controlled environment (22°C to 24°C, within ± 0.5°C during each study). Each subject fasted from 10 PM the previous evening, abstaining from alcohol or caffeine-containing beverages and took their study medication on the morning before the study.

Subjects lay supine with both forearms supported above the level of the right atrium. A 27-gauge unmounted needle (Cooper's Needle Works, Birmingham, UK) sealed with dental wax to an 18-gauge epidural catheter (Portex, Hythe, Kent, UK) was inserted into the brachial artery of the nondominant side (usually the left), under sterile conditions and after local anesthesia with 1% lidocaine hydrochloride (Antigen Pharmaceuticals, Roscrea, Ireland). The infusion of saline or drug solutions throughout the study was at a constant rate of 1 mL per minute using syringe pumps (IVAC P2000; IVAC, Wlmed, Hampshire, UK).

FBF was measured in both arms simultaneously by venous occlusion plethysmography using mercury-in-silastic strain gauges placed at the widest part of the forearms. The strain gauges were electrically calibrated[13] to measure the percentage change in forearm volume. These were connected to a plethysmograph (Vasculab SPG16; Medasonics, Mountain View, CA) that, in turn, was connected to a computerized chart recording system (Maclab; AD Instruments, Castle Hill, NSW, Australia).

The hands were excluded from the circulation by the inflation of wrist cuffs to 200 mm Hg for 1 minute before and during each measurement period. Upper arm cuffs were inflated to 40 mm Hg by a rapid cuff inflator (Model E-20; Hokanson, Bellevue, WA) to occlude venous outflow. Blood flow was recorded for 10 of every 15 seconds during the last 90 seconds of each infusion period.

Resting control FBF values were obtained following a period of at least 30 minutes after needle placement to allow stabilization of blood flow. Subsequently, subjects received 4 incremental doses of acetylcholine (Miochol, Iolab, Bracknell, Berks, UK) at 25, 53, 100, and 200 nmol · min^{-1} (3.7, 7.4, 14.7, 29.4 g/min^{-1}) for 4 minutes each and 4 doses of sodium nitroprusside (Nipride; Roche, Welwyn Garden City, Herts, UK) at 2.5, 5, 10, and 20 nmol · min^{-1} (0.75, 1.5, 3, and 6 μg · min^{-1}) also for 4 minutes each to produce cumulative dose-response curves. The agents were infused in random order and separated by a 20- to 30-minute rest period to allow FBF to return to baseline values between each infusion. FBF was expressed as mL · 100 mL^{-1} forearm volume min^{-1}, as established by Whitney.[14] The average of 5 recordings at each infusion step was calculated for both the infused and noninfused (control) arms. Control FBFs were also recorded to allow for the effects of unavoidable external and systemic factors, which should affect blood flow in both arms similarly.[15]

Blood pressure and heart rate were recorded in the noninfused arm before each of the 4 infusion periods using an automatic oscillometric digital blood pressure monitor (Omron HEM-705CP, Tokyo, Japan). One subject did not attend for repeat assessment of endothelial function, therefore, results are reported for 12 subjects.

Assessment of Insulin Action

Insulin action was assessed at the end of the two 12-week treatment periods using the euglycemic glucose clamp technique.[16] On the morning of the clamp, study medication was taken at 7 AM, and the patients were admitted at 7:45 AM after a 12-hour overnight fast.

An antecubital vein was cannulated (18 gauge; Venflon Viggo, Helsingborg, Sweden) and used for all infusions. A dorsal hand vein on the opposite side was cannulated retrogradely (21 gauge, Venflon Viggo) and the hand placed in a temperature-controlled plexiglass box (Northern Ireland Technology Centre, Automation Division, Queen's University, Belfast, UK), maintained at 55°C to allow intermittent sampling of arterialized venous blood. A primed continuous infusion of high-performance liquid chromatography-purified [3-^{3}H] glucose (New England Nuclear Research Products Division, Dupont, Stevenage, UK) was given during a 2-hour equilibration period (−120 minutes to zero time), after which a 2-hour continuous infusion of insulin (Humulin S; Eli Lilly, Basingstoke, UK) was begun at 1 mU · kg^{-1} min^{-1}. Plasma glucose was maintained at a concentration of 5.1 mmol · L^{-1} by an exogenous glucose infusion (20%). Exogenous glucose was prelabeled with [3-^{3}H] glucose to match predicted basal plasma glucose specific activity as described previously[17] with the modification that the primed continuous tracer infusion was reduced to 50% of the basal rate after 20 minutes and 25% of basal after 40 minutes (to maintain tracer steady state) and was maintained at this rate throughout the remainder of the hyperinsulinemic period.

Analytical Techniques

Arterialized venous blood was used for all analyses. Plasma for measurement of glucose specific activity was deproteinized with barium hydroxide and zinc sulphate by the method of Somogyi.[18] After centrifugation, the supernatant was counted in a liquid scintillation spectrometer (Tri-Carb 2000 CA; Canberra Packard, Pangbourne, UK). Aliquots of tracer infusate and labeled exogenous glucose were spiked into nonradioactive plasma and processed in parallel with plasma samples to allow calculation of [3-^{3}H] glucose infusion rates.

Calculations and Statistical Methods

The FBF measurement in response to drugs was expressed as percentage change from baseline in the ratio of infused to control FBF. These calculated values were used to construct a dose-response curve for each drug. The overall response to each drug was measured as the area under the dose-response curve, calculated by the trapezium rule.[19] Results are expressed as mean ± SEM and given the unit designation, standard units (SU) with the integrated area under the curve results compared using Student's *t* test for paired observations. Between-day mean coefficient of variation for FBF is reported as 10.5%,[20] thus the number of subjects required in each group to give 90% power (at $P < .05$) to detect a 15% change is 10.

The nonsteady state equations of Steele et al[21] as modified by De

Bodo et al[22] were used to determine rates of appearance and disappearance of glucose during the periods −30 minutes to zero time and 90 to 120 minutes, assuming a pool fraction value of 0.65 and an extracellular volume of 190 mL/kg. Rates of infusion of [3-^{3}H] glucose were calculated as the sum of the tracer infused continuously and the tracer in the labeled exogenous glucose infusion. Rates of endogenous (hepatic) glucose production were then calculated by subtraction of the exogenous glucose rates required to maintain euglycemia from the isotopically determined rates of appearance of glucose.

The power of the study, calculated from previous clamp data, gave a 90% chance of detecting a 10% change in insulin action at the 5% level of significance.[23] The values reported are the mean of 3 steady state samples and were compared using the Student's *t* test for paired data. Results are expressed as means ± SEM with $P < .05$ considered statistically significant.

RESULTS

Sixteen patients were recruited for the study. Three patients withdrew from the study, 1 during the run-in phase for reasons unconnected with the study, 1 during the first treatment phase after an episode of vasovagal collapse considered unrelated to medication, and another during the first treatment phase due to a central retinal vein occlusion. This occurred shortly after randomization to doxazosin, and it was unclear if this episode was related to medication or to mild hypertension (160/95 mm Hg) or was simply coincidental. Thirteen patients (3 men/10 women; median age, 55 years; range, 38 to 65 years) completed the full protocol. At the end of the run-in period, mean blood pressure was 164/97 mm Hg, and compliance with study medication was good, with over 95% of the medication being taken by all participants. The median final titrated dose of doxazosin taken was 8 mg (range, 4 to 16 mg). During the first study period while on placebo, 2 subjects had diastolic blood pressure readings greater than 110 mm Hg on more than 2 occasions. Amlodipine, 5 mg daily, was added to their treatment, whereupon their blood pressure became more easily controlled. They both remained on this additional treatment throughout the rest of the study, including the doxazosin arm. Their inclusion did not affect the analysis of the data.

The effect of treatment on blood pressure and biochemical parameters is shown in Table 1. Doxazosin significantly reduced both systolic and diastolic blood pressure after 12 weeks compared with placebo. Weight, glycated hemoglobin, fasting plasma glucose, and serum insulin were not significantly altered by doxazosin. Likewise total cholesterol, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol were unchanged by treatment, although serum triglycerides were significantly lowered by doxazosin.

Table 1. Blood Pressure, Weight, and Biochemical Parameters After 12 Weeks of Treatment With Either Doxazosin or Placebo

	Doxazosin	Placebo
Systolic blood pressure (mm Hg)	144 (3)†	159 (3)
Diastolic blood pressure (mm Hg)	86 (2)†	96 (1)
Weight (kg)	77.4 (3.1)	77.4 (3.0)
Total cholesterol (mmol/L)	5.23 (0.32)	5.50 (0.30)
HDL cholesterol (mmol/L)	1.29 (0.10)	1.22 (0.09)
LDL cholesterol (mmol/L)	3.29 (0.25)	3.57 (0.27)
Triglycerides (mmol/L)	1.20 (0.10)*	1.60 (0.16)
Fasting plasma glucose (mmol/L)	5.1 (0.3)	5.2 (0.3)

NOTE. Results are mean (SEM).
*$P < .05$,
†$P < .005$ for doxazosin *v* placebo.

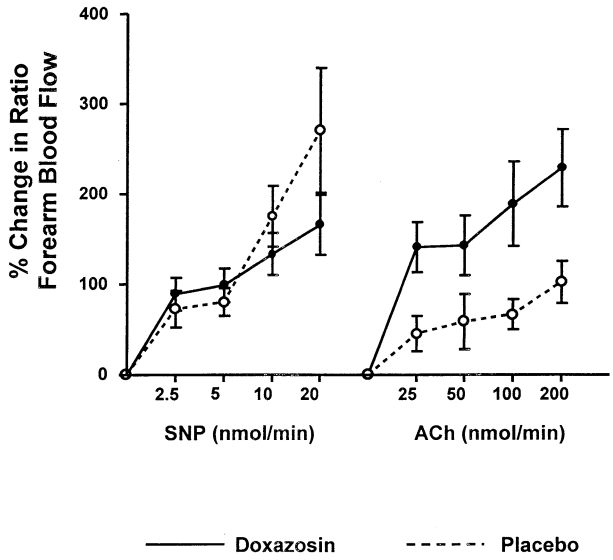

Fig 1. Percentage change over baseline of infused to control arm FBF ratio in response to intra-arterial infusions.

Baseline FBF was unchanged by doxazosin (doxazosin 4.9 ± 0.9 $mL^{-1} \cdot 100\ mL^{-1} \cdot min^{-1}$; placebo 4.0 ± 0.7 $mL^{-1} \cdot 100\ mL^{-1} \cdot min^{-1}$, $P > .05$). Acetylcholine (25 to 200 nmol · min^{-1}) and sodium nitroprusside (2.5 to 20 nmol · min^{-1}) each produced dose-dependent augmentation of basal FBF. However, the vasodilatory response to incremental doses of acetylcholine was enhanced significantly by doxazosin compared with placebo (area under curve of percentage change over baseline of infused:control arm FBF: doxazosin 58.6 ± 11.1 SU; placebo 22.1 ± 7.0 SU, $P = .03$). There was no significant difference between doxazosin and placebo in the vasodilatory response to sodium nitroprusside (area under curve of percentage change over baseline of infused:control arm FBF: doxazosin 40.3 ± 5.5 SU; placebo 46.3 ± 8.1 SU, $P > .05$). Responses are shown in Fig 1. There was no significant correlation between the level of blood pressure reduction caused by doxazosin and the improvement in vascular responsiveness to acetylcholine.

Results from the euglycemic clamp studies are shown in Figs 2 and 3. Plasma glucose concentrations during the clamp studies were similar, and insulin infusion of 1 mU · $kg^{-1} \cdot min^{-1}$ led to comparable steady state plasma insulin concentrations. Exogenous glucose infusion rates required to maintain euglycemia during the last 30 minutes of the glucose clamp (an index of peripheral insulin sensitivity) were not significantly different between doxazosin and placebo (doxazosin 30.4 ± 0.9; placebo 32.3 ± 1.0 μmol · $kg^{-1} \cdot min^{-1}$, $P > .05$). Postabsorptive endogenous glucose production (an index of hepatic insulin sensitivity) was unchanged by doxazosin treatment (doxazosin 11.6 ± 0.7 μmol · $kg^{-1} \cdot min^{-1}$; placebo 11.2 ± 0.5 μmol · $kg^{-1} \cdot min^{-1}$, $P > .05$), and during hyperinsulinemia, endog-

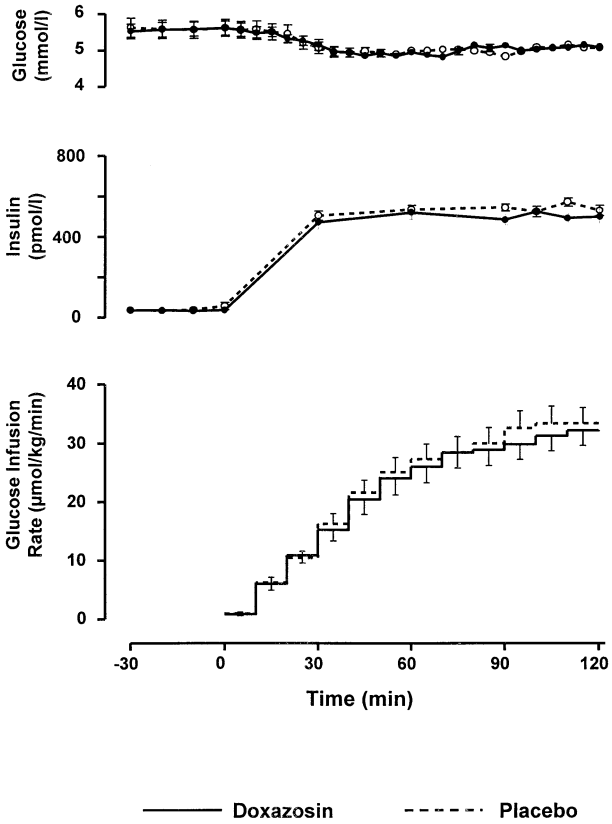

Fig 2. Plasma glucose, serum insulin, and glucose infusion rates before and during euglycemic clamps.

enous glucose production was suppressed to a similar extent after doxazosin and placebo (doxazosin 65.6% ± 7.5% suppression; placebo 68.3% ± 11.2% suppression, $P > .05$).

Postabsorptive concentrations of serum nonesterified fatty acids (NEFA), β- hydroxybutyrate, and glycerol were not changed by treatment and suppressed to a similar extent with hyperinsulinemia.

DISCUSSION

There is increasing awareness of the potential role played by both endothelial dysfunction and insulin resistance in essential hypertension and cardiovascular disease and, consequently, attention has focused on the effects of antihypertensive agents in this area.[24-27]

The endothelium plays an important role in the modulation of vascular tone principally via the release of the vasodilator nitric oxide (NO)[28] with impairment of NO-mediated vasodilation demonstrated in essential hypertension,[1,2] although it remains unclear whether this is a cause or effect relationship. Impaired action of NO may contribute to the maintenance of hypertension and is also implicated in the development of the atherosclerotic plaque and thrombus formation, as NO inhibits platelet aggregation,[29] vascular smooth muscle proliferation[30] and migration,[31] and monocyte adhesion.[32]

Several early studies reported no benefit of antihypertensive agents on endothelial function, although more recent studies have reported a beneficial effect.[24,25] No previous study has used FBF measurements to assess the effect of doxazosin on endothelial function in humans, although indirect evidence of improvement with a reduction in levels of von Willebrand factor has been reported.[33]

We demonstrate a specific beneficial effect of doxazosin on endothelium-dependent vasodilation. This may be due to a direct effect on NO levels both by increasing nitric oxide synthase (NOS) activity,[34] thus potentially enhancing NO synthesis and also by reducing NO degradation.[35] Conversely, endothelial function may have been enhanced by the reduction in blood pressure per se rather than a specific action of doxazosin. However, we observed no correlation between the level of blood pressure reduction and the improvement in endothelium-dependent vasodilation, and previous studies with other agents have reported no improvement in endothelial function despite lowering of blood pressure.[36]

Insulin resistance with its associated hyperinsulinemia has been implicated in the pathogenesis of essential hypertension with several lines of evidence supporting this view, such as the effect of insulin on activation of the sympathetic nervous system, promotion of urinary sodium retention, and proliferation of vascular smooth muscle cells.[37-39] Insulin resistance may also have deleterious effects on lipid and glucose metabolism, such that cardiovascular risk is potentially further increased.[40] Therefore, it is logical that the optimal antihypertensive agent is at least neutral, if not beneficial, in terms of insulin action. Conventional dose thiazide diuretics and β blockers have been shown to impair insulin action, which may explain their diabetogenic propensity.[18,41] Conversely, calcium channel antagonists and angiotensin-converting enzyme (ACE) inhibitors are probably neutral overall in terms of insulin action.[27,42]

A beneficial effect of doxazosin on insulin action has been suggested from several studies in subjects with essential hypertension, although these have been primarily uncontrolled studies with several using suboptimal means of assessing insulin action.[9-11] In our study using a randomized, double-blind, crossover study design and assessing insulin action using the well-validated euglycemic clamp technique, we have shown that doxazosin has a neutral effect on peripheral and hepatic insulin action in non-obese subjects with essential hypertension in association with a significant lowering of blood pressure. As

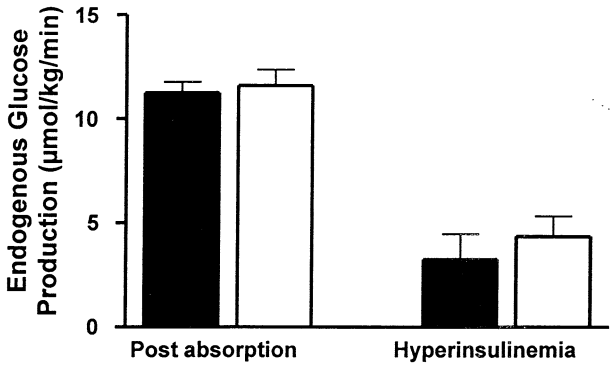

Fig 3. Endogenous glucose production rates before and during euglycemic clamps. (■) Doxazocin; (□) placebo.

a combination of antihypertensive agents is often needed to achieve current blood pressure targets,[43] alpha-adrenergic blockers can be added to the antihypertensive regimen of insulin-resistant patients with confidence that insulin resistance will not be exacerbated. The use of doxazosin in subjects with hypertension and type 2 diabetes has been reported to improve glucose metabolism, despite some of these studies also being limited by methodologic considerations.[44,45] Therefore, it is possible that in a more insulin-resistant population, a positive effect of doxazosin on insulin action may be detected. Insulin-mediated glucose disposal has been suggested to be influenced by endothelial function, as endothelium-derived NO is thought to have a role in the vasodilatory effect of insulin in skeletal muscle.[46] Our findings do not support such an influence, because despite an improvement in endothelial function, we did not demonstrate any change in insulin-mediated glucose disposal. Improvement in endothelium-dependent vasodilation, however, does not necessarily imply an improvement in insulin-mediated blood flow, as temporal dissociation has been reported previously between insulin and acetylcholine-mediated vasodilation.[47] Additionally the role of insulin-mediated blood flow as a determinant of insulin-mediated glucose disposal in skeletal muscle remains unclear.[48,49]

In conclusion, we have demonstrated a beneficial effect of the alpha-adrenergic blocker, doxazosin, on endothelium-dependent vasodilation in subjects with essential hypertension, potentially conferring benefit in terms of cardiovascular risk. We also have shown doxazosin to have a neutral effect on peripheral and hepatic insulin sensitivity, thus indicating its suitability for use in insulin-resistant patients.

ACKNOWLEDGMENT

We thank the Pharmacy Department, Royal Victoria Hospital for patient randomization and drug dispensing.

REFERENCES

1. Panza JA, Quyyumi AA, Brush JE, et al: Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. N Engl J Med 323:22-27, 1990

2. Linder L, Kiowski W, Buhler FR, et al: Indirect evidence for release of endothelium-derived relaxing factor in human forearm circulation in vivo. Blunted response in essential hypertension. Circulation 81:1762-1767, 1990

3. Shen DC, Shieh SM, Fuh MT, et al: Resistance to insulin-stimulated-glucose uptake in patients with hypertension. J Clin Endocrinol Metab 66:580-583, 1988

4. Ferrannini E, Buzzigoli G, Bonadonna R, et al: Insulin resistance in essential hypertension. N Engl J Med 317:350-357, 1987

5. DeFronzo RA, Ferrannini E: Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidaemia, and atherosclerotic cardiovascular disease. Diabetes Care 14:173-194, 1991

6. Cooke J, Tsao P: Is nitric oxide an endogenous antiatherogenic molecule? Arterioscler Thromb Vasc Biol 14:653-655, 1994

7. Weinberger MH: Antihypertensive therapy and lipids. Evidence, mechanisms and implications. Arch Intern Med 145:1103-1105, 1985

8. Neaton JD, Grimm RH Jr, Prineas RJ, et al: Treatment of mild hypertension study. Final results. JAMA 270:713-724, 1993

9. Giorda C, Appendino M: Effects of doxazosin, a selective α 1-inhibitor, on plasma insulin and blood glucose response to a glucose tolerance test in essential hypertension. Metabolism 42:1440-1442, 1993

10. Andersson P-E, Johansson J, Berne C, et al: Effect of selective α1 and β1-adreno-receptor blockade on lipoprotein and carbohydrate metabolism in hypertensive subjects, with special emphasis on insulin sensitivity. J Hum Hypertens 8:219-226, 1994

11. Kageyama S, Yamamoto J, Mimura A, et al: Doxazosin improves insulin sensitivity in hypertensive patients. Clin Ther 15:829-837, 1993

12. McAllister AS, Atkinson AB, Johnston GD, et al: Relationship of endothelial function to birth weight in humans. Diabetes Care 22:2061-2066, 1999

13. Hokanson DE, Sumner DS, Strandness DEJ: An electrically calibrated plethysmograph for direct measurement of limb blood flow. IEEE Trans Biomed Eng 22:25-29, 1975

14. Whitney RJ: The measurement of volume changes in human limbs. J Physiol (Lond) 121:1-27, 1953

15. Greenfield ADM, Patterson GC: Reactions of the blood vessels of the human forearm to increases in transmural pressure. J Physiol (Lond) 125:508-524, 1954

16. Neely RDG, Rooney DP, Atkinson AB, et al: Underestimation of glucose turnover estimated using [6^{3}H]glucose tracer in non-steady state. The role of a tritiated tracer impurity. Diabetologia 33:681-687, 1990

17. Harper R, Ennis C, Heaney AP, et al: A comparison of the effects of low- and conventional-dose thiazide diuretic on insulin action in hypertensive patients with NIDDM. Diabetologia 38:853-859, 1995

18. Somogyi M: Determination of blood sugar. J Biol Chem 160: 69-73, 1945

19. Matthews JNS, Altman DG, Campbell MJ, et al: Analysis of serial measurements in medical research. BMJ 300:230-235, 1990

20. Roberts DH, Tsao Y, Breckenridge M: The reproducibility of limb blood flow measurements in human volunteers at rest and after exercise by using mercury-in-silastic strain gauge plethysmography under standardized conditions. Clin Sci 70:635-638, 1986

21. Steele R, Wall JS, De Bodo RC, et al: Measurement of size and turnover rate of body glucose pool by the isotope dilution method. Am J Physiol 187:15-25, 1956

22. De Bodo RC, Steele R, Altszuler N, et al: On the hormonal regulation of carbohydrate metabolism: Studies with C14 glucose. Recent Prog Horm Res 19:445-488, 1963

23. Rooney DP, Neely RDG, Ennis CN, et al: Insulin action and hepatic glucose cycling in essential hypertension. Metabolism 41:317-324, 1992

24. Taddei S, Virdis A, Ghiadoni L, et al: Effects of angiotensin converting enzyme inhibition on endothelium-dependent vasodilatation in essential hypertensive patients. J Hypertens 116:447-456, 1998

25. Taddei S, Virdis A, Ghiadoni L, et al: Lacidipine restores endothelium-dependent vasodilation in essential hypertensive patients. Hypertension 30:1606-1612, 1997

26. Lithell HOL: Effect of antihypertensive drugs on insulin, glucose, and lipid metabolism. Diabetes Care 14:203-209, 1991

27. Hunter SJ, Harper R, Ennis CN, et al: Effects of combination therapy with an angiotensin converting enzyme inhibitor and thiazide diuretic on insulin action in essential hypertension. J Hypertens 16: 103-109, 1998

28. Palmer RMJ, Ferrige AG, Moncada S: Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 327:524-526, 1987

29. Radomski MW, Palmer RM, Moncada S: Endogenous nitric

oxide inhibits human platelet adhesion to vascular endothelium. Lancet 2:1057-1068, 1987

30. Garg UC, Hassid A: Nitric oxide-generating vasodilators and 8-bromo-cyclic guanosine monophosphate inhibit mitogenesis and proliferation of cultured rat vascular smooth muscle cells. J Clin Invest 83:1774-1777, 1989

31. Gorog P, Kovacs IB: Inhibition of vascular smooth muscle cell migration by intact endothelium is nitric oxide-mediated: Interference by oxidised low density lipoproteins. J Vasc Res 35:165-169, 1998

32. Kubes P, Suzuki M, Granger DN: Nitric oxide: An endogenous modulator of leukocyte adhesion. Proc Natl Acad Sci USA 88:4651-4655, 1991

33. Seljeflot I, Arnesen H, Andersen P, et al: Effects of doxazosin and atenolol on circulating endothelin-1 and von Willebrand factor in hypertensive middle-aged men. J Cardiovasc Pharmacol 34:584-588, 1999

34. Tojo A, Kobayashi N, Kimura K, et al: Effects of antihypertensive drugs on nitric oxide synthase activity in rat kidney. Kidney Int 49:S138-S140, 1996

35. Chait A, Gilmore M, Kawamura M: Inhibition of low density lipoprotein oxidation in vitro by the 6- and 7-hydroxy-metabolites of doxazosin, an α 1-adrenergic antihypertensive agent. Am J Hypertens 7:159-167, 1994

36. Tzemos N, Lim PO, MacDonald TM: Nebivolol reverses endothelial dysfunction in essential hypertension. Circulation 104:511-514, 2001

37. Rooney DP, Edgar JDM, Sheridan B, et al: The effects of low dose insulin infusions on the renin angiotensin and sympathetic nervous systems in normal man. Eur J Clin Invest 21:430-435, 1991

38. De Fronzo RA, Cooke CR, Andres R, et al: The effect of insulin on renal handling of sodium, potassium, calcium and phosphate in man. J Clin Invest 55:845-855, 1975

39. Stout RW: Insulin and atheroma: 20 year perspective. Diabetes Care 13:631-654, 1990

40. Fuller JH, Shipley MJ, Rose G, et al: Coronary heart disease risk and impaired glucose tolerance. Lancet 1:1373-1376, 1980

41. Lithell H, Pollare T, Vessby B: Metabolic effects of pindolol and propranolol in a double-blind cross-over study in hypertensive patients. Blood Press 1:92-101, 1992

42. de Courten M, Ferrari P, Schneider M, et al: Lack of effect of long-term amlodipine on insulin sensitivity and plasma insulin in obese patients with essential hypertension. Eur J Clin Pharmacol 44:457-462, 1993

43. UKPDS Study Group: Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 38. BMJ 317:703-713, 1998

44. Giorda C, Appendino M, Mason MG, et al: Alpha 1-blocker doxazosin improves peripheral insulin sensitivity in diabetic hypertensive patients. Metabolism 44:673-676, 1995

45. Giordano M, Matsuda M, Sanders L, et al: Effects of angiotensin-converting enzyme inhibitors, Ca^{2+} channel antagonists and α-adrenergic blockers on glucose and lipid metabolism in NIDDM patients with hypertension. Diabetes 44:665-671, 1995

46. Baron AD, Steinberg HO, Chaker H, et al: Insulin-mediated skeletal muscle vasodilation contributes to both insulin sensitivity and responsiveness in lean humans. J Clin Invest 96:786-792, 1995

47. Utriainen T, Makimattila S, Virkamaki A, et al: Dissociation between insulin sensitivity of glucose uptake and endothelial function in normal subjects. Diabetologia 39:1477-1482, 1996

48. Utriainen T, Malmstrom R, Makimattila S, et al: Methodological aspects, dose-response characteristics and causes of interindividual variation in insulin stimulation of limb blood flow in normal subjects. Diabetologia 38:555-564, 1995

49. Hunter SJ, Harper R, Ennis CN, et al: Skeletal muscle blood flow is not a determinant of insulin resistance in essential hypertension. J Hypertens 15:73-77, 1997