Effects of Intra-Arterial Infusion of Insulin on Forearm Vasoreactivity in Hypertensive Humans

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We previously demonstrated that intra-arterial infusion of insulin attenuates vasoreactivity to phenylephrine and angiotensin II in the forearm of normotensive humans, which suggests that the pressor effects of insulin via sympathetic activation may be offset in peripheral arteries. In the present study, we investigated the effects of insulin on vasoreactivity in hypertensive humans. Seven male patients with essential hypertension (age, 26 ± 2 yr; blood pressure, $159 \pm 4/103 \pm 4$ mmHg; mean $\pm$ SEM) were studied. We measured forearm blood flow by a strain gauge plethysmograph while infusing phenylephrine (1, 4, 12 nmol/min) and angiotensin II (5, 10, 20 pmol/min) locally into the brachial artery before and during simultaneous intra-arterial infusion of human insulin (0.15 mU/kg/min). Forearm vascular resistance was calculated from directly measured arterial pressure and forearm blood flow. Intra-arterial infusion of insulin raised the local plasma insulin level without changing the blood glucose level, basal forearm blood flow, or forearm vascular resistance. Phenylephrine and angiotensin II increased forearm vascular resistance dose-dependently before and during insulin infusion. In contrast to the previous results in normotensive subjects, locally infused insulin did not attenuate vasoconstrictive responses to phenylephrine and angiotensin II in patients with essential hypertension. These results suggest that the balance between the pressor and depressor effects of insulin might be altered in favor of a pressor effect in patients with hypertension. (*Hypertens Res* 1995; 18: 227-233)

Key Words: insulin, hypertension, phenylephrine, angiotensin II, vasoreactivity, humans, plethysmography

Hypertension is associated with insulin-resistance or hyperinsulinemia (1-7). It is postulated that hyperinsulinemia may contribute to hypertension via several different mechanisms (8-14). Although acute hyperinsulinemia activates sympathetic nerve activity (10, 15-17), it does not elevate blood pressure in normotensive humans (17, 18). To explain this paradox, several investigators reported that insulin has vasodilating effects (19-21), and that insulin decreases vasoreactivity to vasopressor agents (22-25). In particular, we previously demonstrated that intra-arterially infused insulin attenuates the vasoconstricting actions of pressor agents in young normal volunteers (26). Lembo *et al.* (27) also reported that local hyperinsulinemia reduces the vasoconstrictive response to reflex sympathetic activation in normotensive subjects. Thus in normotensive humans, pressor effects of insulin via sympathetic activation are offset in the peripheral resistance vessels.

Hypertensive patients are insulin-resistant in many aspects (4, 28). Furthermore, Baron *et al.* (8) reported that in patients with hypertension insulin-

mediated increases of blood flow in the skeletal muscle were inversely proportional to the basal level of blood pressure, indicating that the vasodilator action of insulin may be decreased in patients with hypertension. Accordingly, we determined the effects of local hyperinsulinemia on basal forearm vascular resistance and on vasoreactivity to intra-arterially infused pressor agents in patients with essential hypertension.

Methods

Subjects

We studied 7 male patients with essential hypertension. They were in their early 20s except for patient W.J. Hypertension was defined as a systolic or diastolic pressure consistently in excess of 140 mmHg or 90 mmHg, respectively, or both, and a diagnosis of hypertension for each patient was made based on several measurements of blood pressure during clinic visits. The patients were hospitalized for evaluation of hypertension. They were in stage I or II of the World Health Organization classification of hyper-

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Table 1. Patient Profiles

Patient	Age (yr)	Ht (cm)	Wt (kg)	BMI (kg/m ²)	BP (mmHg)	Duration (yr)	Response to 75gOGTT					
							Glucose (mmol/l)			IRI (μ U/ml)		Σ IRI (μ U/ml)
							Before	60 min	120 min	Before	120 min	
W.J.	32	173	62	20.7	178/126	15	5.0	12.3	12.8	1.7	112.6	114.3
I.Y.	22	174	69	22.8	152/ 96	4	4.7	9.6	4.2	4.3	9.5	13.8
M.Y.	24	169	62	21.7	170/112	2	4.4	5.7	6.5	4.4	53.0	57.4
H.K.	22	159	74	29.3	146/ 92	1	3.7	6.7	6.0	3.2	107.8	111.0
E.K.	35	167	60	21.5	160/100	9	4.0	8.3	5.7	3.2	25.7	28.9
T.F.	23	160	59	23.0	148/ 90	6	5.2	5.8	5.5	8.7	52.4	61.1
M.T.	22	174	94	31.0	144/ 92	1	5.2	8.7	7.4	9.6	76.7	86.3

Ht, height; Wt, weight; BP, blood pressure; BMI, Body mass index; Duration, duration of hypertension; 75gOGTT, 75-g oral glucose tolerance test; IRI, immunoreactive insulin.

tension. Before the present study, no subject was taking medications that could lower blood pressure, and none showed any evidence of secondary hypertension. Table 1 summarizes their clinical profiles. Their blood pressure was $159 \pm 4/103 \pm 4$ mmHg (means $\pm$ SEM), measured using a mercury sphygmomanometer, on the admission day. Three patients had electrocardiographic or echocardiographic findings suggestive of left ventricular hypertrophy. Two of the seven patients were obese, with a body mass index > 27 kg/m². One patient showed a diabetic pattern in response to a 75-g oral glucose tolerance test (75gOGTT), but his hemoglobin A1c was 5.5% (normal range, 4.0–6.0%). Five of the seven patients showed high levels of plasma insulin (immunoreactive insulin, IRI) in 75gOGTT (Σ IRI, 57.4–114.3 μ U/ml) compared with the Japanese population (29), suggesting that they had insulin-resistance. Omori *et al.* (29) reported that the mean Σ IRI was 26.5 ± 0.1 μ U/ml in the non-hypertensive Japanese male population ($n=791$; age, 55 ± 10 yr), and 33.4 ± 0.1 μ U/ml in the hypertensive Japanese male population ($n=282$; age, 60 ± 10 yr).

The study protocol was approved by the Human Study Committee of the Research Institute of Angiocardiology and Cardiovascular Clinic. Informed consent was obtained from each subject.

General Procedures

The study was performed with the subjects in the supine position after an overnight fast. Room temperature was maintained at 25°C. Under local anesthesia with 2% (W/V) procaine, the left brachial artery was cannulated with a 20-gauge intra-vascular over-the-needle (tetrafluoroethylene) polycatheter (Quick-Cath; Travenol Laboratories, Inc., Baxter Healthcare Corp., Deerfield, Ill.), which was used for drug infusion as well as for recording arterial pressure. Arterial pressure was recorded by connecting the arterial line to a pressure transducer (Viggo-Spectramed, Oxnard, Calif.) with a three-way stop cock. The arterial line was kept open by infusing heparinized saline (0.1 ml/min) when no drug was being infused. To obtain blood samples for the measurement of blood glucose levels and plasma insulin concentrations, an antecubital vein of

the ipsilateral arm was cannulated with the same type of cannula as that used for the artery. Heart rate was obtained by counting the pulse rate for a few minutes on arterial pressure recordings.

Measurements of Forearm Blood Flow

Forearm blood flow was measured with a mercury-in-silastic strain-gauge plethysmograph by a venous occlusion technique (30, 31). The strain gauge was placed approximately 5 cm below the antecubital crease. Forearm blood flow (milliliters per minute per 100 milliliters) was calculated from the rate of increase in forearm volume while venous return from the forearm was prevented by inflating a cuff at the upper arm. The pressure in the venous occlusion cuff at the upper arm was 40 mmHg. Circulation to the hand was arrested by inflating a cuff around the wrist to a suprasystolic pressure (about 200 mmHg). The wrist cuff was inflated before the determination of forearm blood flow and continuously throughout the measurements. Forearm vascular resistance was calculated by dividing the mean blood pressure (diastolic blood pressure plus one-third of the pulse pressure in millimeters of mercury) by the forearm blood flow. These values are expressed as arbitrary units throughout this report. An average of four flow measurements made at 15-second intervals, calculated by two authors independently, was used for analysis.

Forearm Vascular Responses to Drugs

After the placement of cannulas and a strain-gauge plethysmograph, at least 15 minutes were allowed for subjects to become accustomed to the study conditions before the experiments were begun. We examined responses to intra-arterial infusion of phenylephrine ($n=7$) and angiotensin II ($n=7$), administered at graded doses before and during intra-arterial infusion of insulin. First, we examined forearm responses to intra-arterial infusions of phenylephrine (1, 4, 12 nmol/min) and angiotensin II (5, 10, 20 pmol/min) for two minutes at each dose with simultaneous infusion of saline at 0.1 ml/min. Forearm blood flow reached a steady state by one minute after starting infusion of drugs at each infusion rate. Infusion of these two drugs was

alternated, and infusion of the second drug was begun at least 15 minutes after the termination of the first drug, when forearm blood flow had returned to the baseline value. Fifteen minutes after the second drug infusion, intra-arterial insulin infusion (0.15 mU/kg/min) was started and continued. We confirmed previously that infusion of this dose of insulin does not alter the level of either blood glucose or serum insulin in the contralateral antecubital vein (26). After insulin infusion for 20 minutes, baseline variables were measured. While insulin was being infused, intra-arterial infusion of phenylephrine or angiotensin II was performed in the same way as before insulin infusion. Infusions of phenylephrine and angiotensin II were alternated. All drug infusions were finished within 1 hour after insulin administration was started. Forearm blood flow was recorded continuously during drug infusion. The concentration of all drugs was adjusted so that the infusion volume did not exceed 0.6 ml/min. We had confirmed that the volume of infusion itself did not alter forearm blood flow (26). The last 1-minute measurements of forearm blood flow during infusion of each dose of drug were used for subsequent analysis.

Measurement of Plasma Insulin and Blood Glucose Levels

Five milliliters of blood was drawn for measurement of plasma insulin level and blood glucose level from the venous cannula of the ipsilateral arm during saline infusion and during intra-arterial infusion of insulin before simultaneous infusion with phenylephrine or angiotensin II. Blood was centrifuged immediately and stored in a freezer. Plasma insulin level was measured by radioimmunoassay at a commercial laboratory (SRL, Tokyo, Japan). Blood glucose level was measured by the glucose oxidase method.

Preparation of Drugs

Insulin (Insulin Actrapid Human; Novo Nordisk A/S, Dagsvaerd, Denmark) was diluted in saline containing 1% serum albumin and a 10-ml sample of stock solution containing 0.1 U/ml of insulin was made by the pharmaceutical department of our hospital. Angiotensin II (Hypertensin CIBA; Ciba-Geigy, Basel, Switzerland) was dissolved in saline at a concentration of 100 nmol/l and 10-ml stock solutions were made as well. Phenylephrine (Neosynsin Kowa; Kowa, Nagoya, Japan) was diluted in saline at a concentration of 10 μ mol/l immediately before use.

Statistical Analysis

One-way analysis of variance (ANOVA) was used to compare baseline hemodynamic values, plasma insulin, and blood glucose levels before and during insulin infusion. The changes in forearm blood flow and forearm vascular resistance in response to phenylephrine and angiotensin II were examined by one-way ANOVA. The changes in forearm blood flow and forearm vascular resistance in response to phenylephrine and angiotensin II infusion during in-

Fig. 1. Bar graphs show blood glucose levels (left panel) and serum concentrations of immunoreactive insulin (IRI) (right panel) in the ipsilateral antecubital vein before and during insulin infusion (0.15 mU/kg/min). Although the levels of IRI increased significantly during insulin infusion, blood glucose levels did not change. Means $\pm$ SEM.

sulin infusion were compared with those during saline infusion (control) by two-way ANOVA. The percent changes in forearm blood flow and forearm vascular resistance in response to vasoconstrictors were also calculated. All values are expressed as means $\pm$ SEM. Duncan's multiple comparison test was applied when ANOVA showed significant differences, and a value of $p < 0.05$ was considered to indicate statistical significance.

Results

Effects of Insulin on Baseline Hemodynamics

Insulin infusion at 0.15 mU/kg/min raised the insulin level of the venous effluents of the ipsilateral arm significantly ($p < 0.01$) without changes in glucose levels in the venous effluents (Fig. 1). Insulin infusion had no overall effect on arterial pressure, baseline forearm blood flow, and forearm vascular resistance. However, in two patients, insulin infusion increased forearm blood flow (Table 2). Heart rate was not altered by insulin infusion (data not shown).

Effects of Insulin on Vasoconstriction Induced by Phenylephrine and Angiotensin II

Figure 2 shows representative plethysmographic recordings of forearm blood flow in a subject in response to intra-arterial infusion of angiotensin II at graded doses before and during simultaneous infusion of insulin at a rate of 0.15 mU/kg/min. Angiotensin II infusion decreased forearm blood flow dose-dependently before and during insulin infusion. Table 3 shows hemodynamic changes in response to phenylephrine and angiotensin II. Forearm blood flow decreased and vascular resistance increased in response to these vasoconstrictors during insulin infusion as well as during saline infusion. These changes were similar, and there was no significant difference between before and during insulin infusion. These drugs did not alter arterial pressure. Figure 3 shows the changes in forearm vascular resistance evoked by phenylephrine and angiotensin

Table 2. Baseline Forearm Hemodynamics Before and During Insulin Infusion

Patients	Before Phenylephrine						Before Angiotensin II					
	FBF		BP		FVR		FBF		BP		FVR	
	Before	During	Before	During	Before	During	Before	During	Before	During	Before	During
W.J	5.44	5.31	178/126	178/128	26.3	27.2	4.83	5.46	180/126	176/125	29.8	26.0
I.Y	3.06	7.44	152/ 96	148/ 98	37.5	15.4	2.41	3.27	144/ 96	142/ 98	46.5	34.5
M.Y	3.07	2.50	166/110	164/110	41.9	51.2	3.45	2.79	170/112	162/110	38.0	45.6
H.K	3.96	3.55	148/ 92	138/ 90	28.0	29.8	3.68	5.30	146/ 92	142/ 96	29.8	21.0
E.K	2.20	3.11	160/100	172/110	54.4	42.1	1.96	1.83	170/108	162/106	65.7	68.2
T.F	2.58	2.33	162/ 98	156/100	46.2	50.9	2.83	3.15	164/106	158/102	44.4	38.3
M.T	5.76	7.20	136/ 78	138/ 86	16.9	14.3	6.53	7.13	148/ 80	138/ 82	15.7	14.1

FBF, forearm blood flow (ml/min/100 ml); BP, blood pressure (mmHg); FVR, forearm vascular resistance (units).

Table 3. Forearm Hemodynamics Before and During Insulin Infusion

		Phenylephrine (nmol/min)				Angiotensin II (pmol/min)			
		Control	1	4	12	Control	5	10	20
		Before	During	Before	During	Before	During	Before	During
MBP	Before	119±5	118±5	120±5	119±5	122±5	121±5	120±6	121±6
	During	121±6	120±6	122±6	123±6	120±5	121±6	121±6	123±5
FBF	Before	3.7±0.5	3.3±0.4	3.2±0.4	2.5±0.3	3.7±0.6	2.8±0.3	2.7±0.3	2.4±0.2
	During	4.5±0.8	3.7±0.7	3.5±0.6	2.7±0.3	4.1±0.7	3.6±0.6	3.3±0.5	2.9±0.3
FVR	Before	35.9±4.9	38.5±4.3	41.5±5.4	51.3±6.2	38.6±6.0	45.9±5.6	49.0±6.5	53.4±5.7
	During	33.0±5.9	38.2±5.9	39.7±5.3	50.3±6.9	35.4±6.8	40.0±7.7	42.4±7.0	47.1±6.7

MBP, mean blood pressure (mmHg); FBF, forearm blood flow (ml/min/100 ml); FVR, forearm vascular resistance (units).

Fig. 2. These figures show representative plethysmographic recordings of forearm blood flow in a subject (W.J.) in response to intra-arterial infusion of angiotensin II at graded doses before (upper figures) and during (lower figures) simultaneous infusion of insulin at a rate of 0.15 mU/kg per minute. Angiotensin II decreased forearm blood flow dose-dependently during insulin infusion, as well as in the control state during saline infusion. FBF, forearm blood flow.

II. In this study, the baseline values of forearm blood flow and forearm vascular resistance slightly varied in each subject. The duration of insulin infusion was only 20 min before an infusion of the first vasoconstrictor (phenylephrine or angiotensin II); therefore, insulin may have dilated forearm vessels before infusion of the second vasoconstrictor. To eliminate the effects of the variations in baseline values, we also analyzed normalized values, regarding the control value as 100%. The percent changes

Fig. 3. Line graphs show changes in forearm vascular resistance evoked by intra-arterial infusion of graded doses of phenylephrine (left panel) and of angiotensin II (right panel). Both phenylephrine and angiotensin II increased forearm vascular resistance dose-dependently during intra-arterial infusion of insulin, as well as before insulin infusion. Cont, control. Means±SEM.

in forearm vascular resistance evoked by both phenylephrine and angiotensin II were similar before and during insulin infusion (Fig. 4).

Discussion

The major finding of the present study was that hyperinsulinemia did not attenuate vasoconstrictive responses to phenylephrine and angiotensin II in

Fig. 4. Line graphs show percent changes in forearm vascular resistance evoked by intra-arterial infusion of graded doses of phenylephrine (left panel) and of angiotensin II (right panel). Both phenylephrine and angiotensin II increased forearm vascular resistance similarly and dose-dependently during intra-arterial infusion of insulin and before infusion of insulin. Control value was 100%. FVR, forearm vascular resistance; Cont, control. Means $\pm$ SEM.

hypertensive humans, which differs from our previous results in normal subjects (26). It is still not clear whether insulin resistance or hyperinsulinemia causes hypertension (32, 33); however, the results of this study may provide one explanation as to how hyperinsulinemia contributes to hypertension. Although the vasoconstriction due to hyperinsulinemia-induced sympathetic activation is offset by the peripheral effects of insulin on vasoreactivity in normotensive humans (20, 21), the peripheral anti-vasoconstrictive effects of insulin may be lost in hypertensive patients, resulting in vasoconstriction in response to pressor stimuli.

Effects of Insulin on Resting Forearm Vascular Resistance

Because systemic insulin infusion may alter forearm vascular resistance via sympathetic activation, we infused insulin locally into the brachial artery. This infusion of insulin caused physiological hyperinsulinemia, approximately 120 μ U/ml, in the ipsilateral antecubital veins. This level of local hyperinsulinemia slightly increased the baseline forearm blood flow, but the increase was not statistically significant. Although we did not measure systemic insulin levels in this study, the insulin level in the contralateral arm was 11.0 ± 0.8 μ U/ml, measured using the same methodology, in our previous study (26). Furthermore, neither blood pressure nor heart rate was altered during insulin infusion in the present study. Thus, systemic effects of insulin were excluded.

The present finding of minimal effects of insulin on baseline forearm vascular resistance in hypertensive patients is similar to that in normotensive humans. We and others have shown in normotensive humans that local hyperinsulinemia induced by intra-arterially infused insulin does not alter or slightly increases peripheral blood flow (5, 20, 26, 34–36). In hypertensive humans, Lembo *et al.* (15)

and Neahring *et al.* (37) reported that local hyperinsulinemia induced by intra-arterial infusion of insulin increased forearm blood flow. We failed to observe significant changes in forearm blood flow in response to intra-arterially infused insulin in the present study. We and previous investigators (15, 37) raised the local plasma insulin to comparable levels (approximately 60–120 μ U/ml); therefore, the difference between the present results and those of previous studies was not caused by different insulin levels. One possible cause for the different results is that the duration of insulin infusion was shorter in the present study (20 min) than in the previous studies (>60 min) (37). Another possibility is that our patients were much younger (mean, 26 yr) than those of the two previous studies (mean, 36 and 39 yr).

Effects of Insulin on Vascular Reactivity to Pressor Agents in Hypertensive Subjects

Recently, we (26) and Lembo *et al.* (27) reported that local hyperinsulinemia induced by intra-arterial infusion of insulin blunted the responses to vasoconstrictive stimuli in young normotensive subjects. In this study, we determined effects of intra-arterially infused insulin on vasoreactivity in young patients with hypertension. In contrast to normotensive humans, intra-arterially infused insulin did not attenuate vasoconstrictive responses to pressor agents in the patients with hypertension. Because the control values of forearm vascular resistance varied in each subject, we analyzed not only absolute values but also normalized values, regarding the control value to be 100%. The percent changes in forearm vascular resistance evoked by both phenylephrine and angiotensin II were similar before and during insulin infusion.

Several possibilities were considered to explain the different effects of insulin between normotensive and hypertensive subjects. First, the difference was not due to the difference in the plasma insulin level, because in both studies insulin levels rose similarly to approximately 120–130 μ U/ml. Second, the difference is not likely due to structural changes of vessels. Although we did not directly estimate structural changes, the maximum values of forearm vascular resistance evoked by phenylephrine and angiotensin II without insulin infusion were similar in the normotensive subjects in our previous study (26) and the hypertensive patients in the present study. Third, the difference may have resulted from endothelial dysfunction in patients with hypertension. A recent report suggests that insulin may release nitric oxide from the endothelium in humans (38). Because of endothelial dysfunction in patients with hypertension (39–42), the effects of insulin on the release of nitric oxide may be impaired, resulting in loss of the anti-vasoconstrictive activity of insulin. Fourth, the presence of circulating ouabain-like substance may be implicated in the etiology of essential hypertension in humans (43, 44). Insulin may cause vasodilation by activating the Na^+ - K^+ ATPase activity of vascular smooth muscle (25, 43, 44). Increased circulating levels of ouabain-

like substance may inhibit the action of insulin in patients with essential hypertension. This possibility should be explored. Finally, many of our hypertensive patients were insulin-resistant. Although we did not study insulin-resistance by the euglycemic clamp method, five of our seven patients showed high levels of plasma insulin in 75gOGTT. It was reported that hypertensive patients have resistance to insulin-induced increases in skeletal muscle blood flow (8), indicating that effects of insulin on vascular resistance are attenuated. Although the underlying mechanism responsible for this resistance remains to be elucidated (8), the same mechanism may be responsible for the resistance to the insulin-induced attenuation of vasoreactivity. Thus, an insulin level of 120 μ U/ml was effective in attenuating vasoreactivity in normotensive healthy subjects in our previous study (26), but may have not been sufficient to produce this effect in patients with hypertension and insulin-resistance. If we had infused larger doses of insulin, we may have observed anti-vasoconstricting effects of insulin in patients with hypertension. The mechanisms responsible for attenuating the effects of insulin on vasoreactivity cannot be explained solely on the basis of insulin-resistance, because two hypertensive patients without insulin-resistance showed similar results to those in the insulin-resistant patients.

Clinical Implications

It is controversial whether hyperinsulinemia contributes to hypertension (32, 33). Acute systemic hyperinsulinemia activates sympathetic nerve activity but does not raise blood pressure in normotensive humans by its anti-pressor effects on the peripheral blood vessels (18). Our study suggests that hyperinsulinemia may contribute to hypertension in patients with hypertension because the anti-pressor effects of insulin are lost.

Anderson *et al.* (45) and Lembo *et al.* (15) reported that systemic hyperinsulinemia produced by an euglycemic glucose clamp method did not raise arterial pressure in hypertensive subjects. However, plasma insulin concentrations in their studies (73 ± 7 and 56 ± 7 μ U/ml, respectively) were relatively low. It is possible that higher concentrations of plasma insulin may have raised arterial pressure in their studies.

If loss of the anti-vasoconstrictive effects of insulin is due to insulin resistance, hyperinsulinemia may contribute to the development of hypertension in subjects who have insulin resistance, such as obese subjects or patients with diabetes mellitus. It should be borne in mind that antihypertensive drugs that cause insulin resistance may attenuate the anti-vasoconstrictive effects of insulin.

In summary, the present study demonstrated that the anti-vasoconstrictive effects of insulin on the peripheral vessels are lost in patients with hypertension. Hyperinsulinemia may contribute to the development and maintenance of hypertension not only in patients with hypertension, but also in any subject with insulin-resistance. In these latter subjects, the balance between the pressor and de-pressor effects of insulin may be altered in favor of its pressor effects.

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