The Vascular Effects of L-Arginine in Humans

The Role of Endogenous Insulin

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Abstract

This study aimed at evaluating whether increased availability of the natural precursor of nitric oxide, L-arginine, could influence systemic hemodynamic and rheologic parameters in humans and whether the effects of L-arginine are mediated by endogenous insulin. 10 healthy young subjects participated in the following studies: study I, infusion of L-arginine (1 g/min for 30 min); study II, infusion of L-arginine plus octreotide (25 μg as i.v. bolus + 0.5 μg/min) to block endogenous insulin and glucagon secretion, plus replacement of basal insulin and glucagon; study III, infusion of L-arginine plus octreotide plus basal glucagon plus an insulin infusion designed to mimic the insulin response of study I. L-Arginine infusion significantly reduced systolic (11±3, mean±SE) and diastolic (8±2 mmHg, $P < 0.001$) blood pressure, platelet aggregation (20±4%), and blood viscosity (1.6±0.2 centipois, $P < 0.01$), and increased leg blood flow (97±16 ml/min), heart rate, and plasma catecholamine levels ($P < 0.01$). In study II, plasma insulin levels remained suppressed at baseline; in this condition, the vascular responses to L-arginine were significantly reduced, except for plasma catecholamines which did not change significantly. In study III, the plasma insulin response to L-arginine was reestablished; this was associated with hemodynamic and rheologic changes following L-arginine not significantly different from those recorded in study I. These findings show that systemic infusion of L-arginine in healthy subjects induces vasodilation and inhibits platelet aggregation and blood viscosity. These effects are mediated, in part, by endogenous released insulin. (*J. Clin. Invest.* 1997. 99:433–438.) Key words: blood pressure • platelet aggregation • blood viscosity • octreotide • leg blood flow

Introduction

The endothelium participates in the local regulation of vascular smooth muscle tone by releasing nitric oxide (NO)[1] and other factors (1). NO is synthetised from the physiologic precursor L-arginine by the stereospecific enzyme NO synthase in the so called L-arginine/NO pathway (1). NO causes relaxation of the smooth muscle by activating soluble guanylate cyclase to rise cyclic 3′,5′-guanosine monophosphate (GMP) levels. Inhibition of NO production with the arginine analogue N^G-monomethyl-L-arginine (L-NMMA) acutely raises vascular resistances in humans (2), demonstrating the role of NO in the regulation of vascular tone. Increased availability of the natural precursor of NO formation (L-arginine) has been shown to decrease peripheral arterial resistance and inhibit platelet aggregation in healthy subjects (3–6). All of this seems compatible with the known effects of NO on vasculature and is likely to be the consequence of enhanced NO synthesis from L-arginine. However, other factors may be involved too. In particular, L-arginine is known to stimulate in a direct way endogenous insulin secretion from the pancreatic beta-cells (7): this hormone has a vasodilating capacity which may be mediated by endogenous NO release (8, 9). This study was undertaken to examine whether endogenous insulin is implicated in the hemodynamic and rheologic effects brought about by L-arginine in healthy humans.

Methods

Subjects

Informed consent was obtained from 10 young subjects (5 males and 5 females) who volunteered for the study after a clear explanation of its nature and potential hazards involved. Their age was 24±1 (SE) yr (range 21–29), body weight was 67±1.8 kg (range 57–80) and body mass index was 23±0.4 (wt/ht^2, range 21–25). All had a negative family history of diabetes and hypertension. All of the subjects were screened by clinical history, physical examination, electrocardiogram, and routine chemical analyses and had no evidence of present or past hypertension, hyperlipidemia, or any systemic conditions. They were consuming a diet containing at least 200 g of carbohydrate and 90 g of protein/d during the week preceding the study. The subjects were asked not to modify their dietary regimen during the time involved in this specific study. Dietary history and constancy of body weight over the time period during which the study was conducted documented their compliance to the dietary advice. None was taking any medication. Special care was taken to ensure against the recent use of drugs that contained aspirin or related compounds. The study was reviewed and approved by the ethical committee of our institution.

Experimental protocol

All studies were performed in the morning, after an overnight fast, in a quiet room with a temperature ~ 21–24°C. All subjects were instructed to refrain from smoking and drinking alcohol or caffeine-containing beverages from the night before. After their arrival to the metabolic ward, they were put in bed in a supine comfortable position. Intravenous lines were inserted in a large antecubital vein of one arm for infusions and in a dorsal vein of the contralateral arm for blood sampling. Patency was preserved by a slow saline infusion (0.9% NaCl). The subjects were then instrumented for automatic

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1. *Abbreviations used in this paper:* AUC, area under the curve; LBF, leg blood flow; L-NMMA, N^G-monomethyl-L-arginine; LVR, leg vascular resistance; MAP, mean arterial pressure; NO, nitric oxide.

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measurements of blood pressure and heart rate. The study started after the subjects had rested for at least 30 min and after three consecutive measurements of blood pressure and heart rate different by < 5% were recorded. Each subject participated in three study protocols. Study I was always performed first. Study II and III were performed in random order at 7–10-d intervals.

Study I. L-Arginine (30% L-arginine monochloride solution; Damor Pharmaceuticals, Naples, Italy) was infused at the constant rate of 3.3 ml/min (1 g/min) over 30 min with an electrically driven motor pump. L-Arginine elicited a monophasic pattern of insulin secretion with circulating insulin levels approximately four- to fivefold higher than baseline that persisted for the duration of the infusion.

Study II. The L-arginine infusion was performed as in study I and the secretion of endogenous insulin and glucagon was inhibited with the somatostatin analogue octreotide (Sandoz, Milan, Italy), 25 μg as i.v. bolus followed by a 0.5 μg/min infusion. At the same time that octreotide was initiated (−5 min), an infusion of glucagon (Novo-Nordisk, Rome, Italy, 0.2 ng/kg/min) and insulin (Humulin R; Eli Lilly, Florence, Italy, 6 mU/min) was administered to replace the basal plasma insulin and glucagon concentrations. All three hormones were dissolved in saline containing 0.3 g/dl human serum albumin (Human Albumin ISI, Milan, Italy).

Study III. The L-arginine infusion was performed in combination with octreotide and glucagon as described in study II. At the start of L-arginine, a variable insulin infusion was started and adjusted on an empiric bases to mimic the plasma insulin response that was observed in study I. Specifically, an infusion of insulin was started at an initial rate of 25±2 mU/min (concentration, 20 mU/ml) for the first 10 min and then increased to 40±4 mU/min for the remaining 20 min of the test. To keep plasma glucose close to the values observed during study I, a variable amount of a 20% glucose was also delivered, based on the results of bedside blood glucose level monitoring.

Hemodynamic measurements

Heart rate and finger arterial pressure were measured with a noninvasive technique (Finapres; Ohmeda, Englewood, CA) that has been shown to be as accurate as intraarterial blood pressure measurements (10). Data were elaborated by a software that allowed systolic, diastolic, mean arterial pressure (MAP), and heart rate to be expressed in graphs. Blood flow in the femoral artery was determined by image-directed duplex ultrasonography combining B-mode imaging and pulsed Doppler beams (Apogeé CX 200; Interspec ATL, Ambler, PA). Blood flow volumes were automatically calculated as the vessel cross-sectional area multiplied by the time average volume from five repeated measurements for each volume flow estimation. Blood flow did not differ between the two legs and pooled data are presented accordingly. Leg vascular resistance (LVR) was calculated by dividing the MAP (mmHg) by the leg blood flow (LBF) and expressed in arbitrary units.

Blood rheology

Platelet aggregation response induced by ADP (1.25 μM) was determined according to Born (11). Aliquots of blood anticoagulated with 0.77 M EDTA (ratio blood/EDTA was 1:20) were used to assess blood viscosity at different rates of shear (225 and 45 s^{-1}) using a digital viscosimeter (Brookfield Engineering Laboratories, Stoughton, MA). Hematocrit values were determined by centrifuging blood samples in glass capillary tubes for 5 min at 12,000 rpm. All determinations were made in duplicate by a person blinded to subjects and treatments. Coefficients of variations were 4% for blood viscosity and 5% for platelet aggregation.

Analytical methods

Samples for plasma glucose were collected in tubes containing a trace of sodium fluoride and those for insulin and glucagon in tubes containing a mixture (0.1 ml/ml of blood) of EDTA-Trasylol solution (5,000 U/ml, Trasylol; Bayer, Leverkusen, Germany; 1.2 g/liter di-sodium EDTA). Samples for plasma catecholamines were collected in iced heparanized tubes and the plasma was separated within 120 min. Plasma was stored at −70°C as perchloric acid extracts until assayed. Plasma glucose was determined with the glucose oxidase method on an autoanalyzer (Beckman, Fullerton, CA). Plasma insulin and glucagon levels were determined by RIA, as described previously (12). Plasma catecholamines were measured with high pressure liquid chromatography. In our laboratory the assay has a detection limit of 20 ng/liter; the intraassay and interassay coefficients of variation are 8.1 and 8.8%, respectively. Plasma arginine levels were determined according to Bacchus and London (13).

Data were analyzed by ANOVA for repeated measures. Post-hoc comparisons were made with Scheffé's test. The area under the curve (AUC) for MAP and LVR was calculated as the decremental (or incremental) values from baseline obtained during the 30 min of L-arginine infusion and expressed in arbitrary units. A value of $P < 0.05$ was considered statistically significant. All statistical analyses were on IBM computers with the SOLO software package (BMDP, statistical software). All data are presented as means±SE.

Results

L-Arginine infusion (study I). Basal plasma glucose levels averaged 4.5±0.2 nmol/liter (range 4.2–5). After L-arginine administration, glucose levels increased to 6±0.35 nmol/liter at 15 min and remained elevated until the end of the infusion. Afterward, they returned to prestimulatory values at 50 min (Fig.

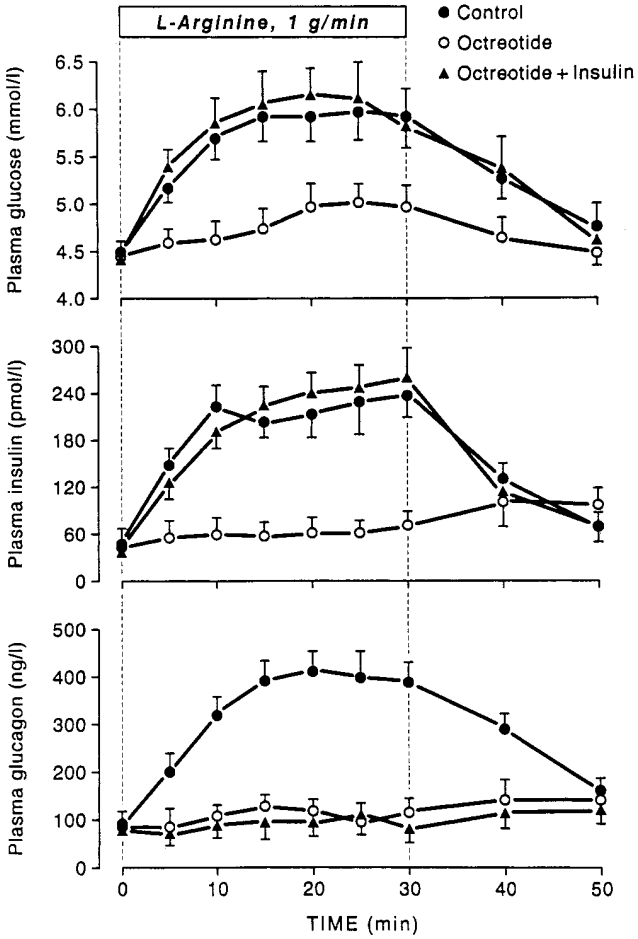
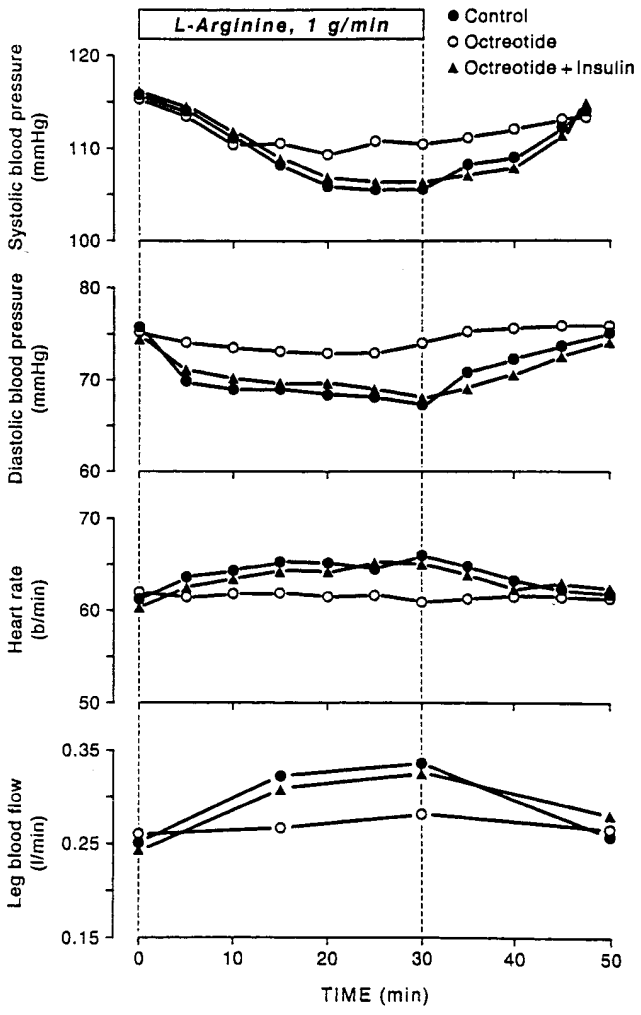


Figure 1. Changes in plasma glucose, insulin, and glucagon concentrations after L-arginine infusion in 10 healthy subjects. *Control*, study I; *Octreotide*, study II; *Octreotide + Insulin*, study III.

Table I. SBP, DBP, HR, and LBF in Response to L-Arginine in 10 Normal Subjects

	Time (min)								
	0	5	10	15	20	25	30	40	50
SBP (mmHg)									
Study I	116±3	114±3	112±3*	108±2*	106±2*	105±2*	106±3*	109±4*	114±4
Study II	115±3	113±3	111±3*	111±2*	110±2*‡	111±3*‡	111±3*‡	112±4*	113±4
Study III	116±3	114±3	113±3	109±3*	107±2*	106±2*	106±2*	107±3*	115±4
DBP (mmHg)									
Study I	76±1	70±1*	69±1*	69±1*	68±1*	68±1*	67±1*	72±2*	75±2
Study II	75±1	74±1‡	74±1‡	73±1‡	72±1‡	73±1‡	74±1‡	75±2	76±1
Study III	75±1	71±1*	70±1*	70±1*	69±1*	69±1*	68±1*	71±1*	74±1
HR (beats/min)									
Study I	61±1	63±1	64±1*	65±1*	65±1*	65±1*	66±1*	63±1	62±1
Study II	62±1	62±1	62±1	62±1‡	62±1‡	61±1‡	61±1‡	62±1	62±1
Study III	61±1	62±1	63±1	64±1*	64±1*	65±1*	65±1*	62±1	62±1
LBF (liters/min)									
Study I	0.25±0.03			0.33±0.03*			0.34±0.04*		0.26±0.03
Study II	0.26±0.03			0.27±0.03‡			0.28±0.03‡		0.27±0.03
Study III	0.25±0.03			0.31±0.03*			0.33±0.03*		0.28±0.04

Data are mean±SEM. *SBP*, systolic blood pressure; *DBP*, diastolic blood pressure; *HR*, heart rate. *Significantly different from basal values ($P <$ 0.05–0.01); ‡Significantly different from the corresponding values of study I ($P < 0.05$–0.01).



1). Basal plasma insulin levels were 55±9 pmol/liter (range 35–87). L-Arginine elicited a monophasic pattern of insulin secretion with a peak at 10 min followed by a plateau that lasted until the end of the infusion. Prestimulatory insulin levels were reestablished 20 min after stopping the infusion (Fig. 1). The fasting plasma glucagon levels averaged 97±11 ng/liter (range 53–138); there was a progressive rise of plasma glucagon concentrations which plateaued (~ 400 ng/liter) at 15 min and remained constant until the end of the L-arginine infusion. Afterward, plasma glucagon levels returned to baseline values at 50 min (Fig. 1).

L-Arginine infusion caused systolic and diastolic blood pressure to decrease within 5 min, with the lowest value occurring between 25 and 30 min (−11±3 mmHg for systolic, and −9±2 mmHg for diastolic, $P < 0.001$). Heart rate and LBF increased by 10% ($P < 0.01$) and 40% ($P < 0.01$), respectively (Table I, Fig. 2). Basal LVR was 356±34 and fell by 34±4% (235±24) at 30 min ($P < 0.01$). Hemodynamic parameters returned to baseline values within 20 min after the end of the L-arginine infusion.

Plasma norepinephrine rose from a basal value of 137±13 to 182±20 ng/liter ($P < 0.01$) at 30 min; plasma epinephrine rose from a basal value of 46±5 to 70±8 ng/liter ($P < 0.02$). Plasma arginine levels increased from a basal level of 111±18 μmol/liter to 1,885±245 μmol/liter at 30 min.

Platelet aggregation induced by ADP decreased by ~ 40% ($P < 0.01$) at the end of the L-arginine infusion. Similarly, blood viscosity was also significantly reduced (Table II).

Figure 2. Changes in hemodynamic parameters after L-arginine infusion in 10 healthy subjects. For clarity of presentation, the standard error of the means has been omitted, but can be found in Table I.

Table II. Platelet Aggregation to ADP and Blood Viscosity at Two Different Rates of Shear after L-Arginine Infusion in 10 Healthy Subjects

	Basal	*P* value	30 min	Change
Platelet aggregation (%)				
Study I	51±8	< 0.01	30±6	−20±4.5
Study II	48±7	< 0.05	39±6	−9±2.1*
Study III	50±8	< 0.01	31±6	−19±5.2
Blood viscosity (cp)				
225 s^{-1}				
Study I	4.3+0.3	< 0.02	3.6+0.4	−0.7+0.1
Study II	4.3+0.3	NS	4.0+0.4	−0.3+0.1*
Study III	4.3+0.3	< 0.02	3.5±0.3	−0.8±0.2
45 s^{-1}				
Study I	7.0±0.6	< 0.01	5.4±0.5	−1.6±0.2
Study II	6.9±0.6	< 0.05	6.2±0.5	−0.7±0.15*
Study III	7.0±0.6	< 0.01	5.5±0.5	−1.5±0.2

Data are mean±SEM. The values of *P* indicate significant differences versus baseline. *Significant differences versus the corresponding values of study I.

L-Arginine plus octreotide (study II). The plasma glucose, insulin, and glucagon concentrations in the basal state were not significantly different from those observed in study I. After L-arginine infusion, plasma glucose levels showed only modest changes from baseline, with a maximal rise at 25 min (0.5±0.3 mmol/liter, $P < 0.05$). Both plasma insulin and glucagon responses were equally suppressed by octreotide (Fig. 1).

Blood pressure fall was significantly lower during the combined infusion of L-arginine and octreotide (maximal systolic decrease: 5±1.4 mmHg; diastolic decrease: 3±0.4 mmHg) than during L-arginine alone ($P < 0.01$). The increase of heart rate and LBF after L-arginine administration was significantly reduced by octreotide (Table I, Fig. 2). Basal LVR was 338±41 and did not change significantly at 30 min (307±32, $P = 0.09$). Octreotide infusion reduced the effect of L-arginine on MAP (AUC) by 64% (95% CI, 41–78, $P < 0.01$) and on LVR (AUC) by 77% (95% CI, 51–92, $P < 0.001$) (Table III).

Plasma catecholamine concentrations did not show any significant change in response to the combined infusion of L-arginine and octreotide (norepinephrine: basal value 139±14 ng/liter, 30 min value 152±17 ng/liter; epinephrine: 67±8 and 60±6 ng/liter, respectively).

Platelet aggregation and blood viscosity in the basal state were not significantly different from the corresponding values obtained in study I. The infusion of octreotide was associated with a significant reduction of rheological responses to L-arginine (Table II). Plasma arginine levels were 103±12 μmol/liter at baseline and 1,997±215 μmol/liter at 30 min.

Table III. MAP and LVR in Response to L-Arginine in 10 Normal Subjects

	MAP (AUC)	LVR (AUC)
	mmHg	
Study I	−192±18	−2527±239
Study II	−98±10*	−577±52*
Study III	−170±18	−2167±284

Data are mean±SEM. The AUC is the decremental area under the curve (0–30 min). *$P < 0.01$ versus the corresponding value of study I.

L-Arginine plus octreotide plus insulin (study III). Basal plasma glucose, insulin, and glucagon levels were similar to those recorded in studies I and II. The plasma insulin response to L-arginine was closely mimicked in each individual subject (Fig. 1). The plasma glucose rise was similar to that observed in study I. It was necessary to infuse small intravenous amounts of glucose in order to compensate for the lacking hyperglycemic effect of glucagon which remained suppressed throughout the study by the ongoing infusion of octreotide. The reintroduction of the plasma insulin response to L-arginine was associated with hemodynamic and rheologic changes indistinguishable from those recorded during L-arginine alone (Fig. 2, Tables I and III). Plasma norepinephrine rose from 149±15 ng/liter (baseline) to 179±19 ng/liter (30-min value, $P < 0.02$); plasma epinephrine rose from 52±6 to 75±8 ng/liter ($P < 0.05$). Plasma arginine levels increased from 122±14 to 2,002±214 μmol/liter (30-min value).

Discussion

In this study, L-arginine infusion in healthy volunteers reduces blood pressure and raises blood flow in the femoral artery. This is associated with significant increases of heart rate and plasma catecholamine levels, a likely consequence of systemic vasodilation induced by L-arginine. In addition, L-arginine inhibits platelet aggregation and decreases blood viscosity. These vascular effects of L-arginine are partially dependent upon the simultaneous secretion of endogenous insulin elicited by the amino acid.

Previous studies in humans have shown that systemic infusion of L-arginine reduces blood pressure in normal subjects (3–6, 14) and patients with hypertension (15) or uncomplicated insulin-dependent diabetes mellitus (4). In addition, local intraarterial infusion of L-arginine has been shown to potentiate vasodilation induced by acetylcholine in the forearm of healthy subjects (15) and patients with hypercholesterolemia (16). In general, L-arginine appears to exert beneficial effects on vasculature similar to those currently being attributed to NO, which also include vasodilation and antiplatelet activity (1). Moreover, inhibition of blood viscosity has been observed recently in normal subjects during administration of both short- and long-term nitrates, whose vascular effects are thought to derive from metabolic conversion to NO (17). Caution has been raised in attributing the vascular effects of L-arginine to the mediation of raised NO synthesis. In particular, two studies (18, 19) were unable to find any significant decrement of blood pressure during L-arginine infusion, although this might be related to the different doses of L-arginine used. Systemic L-arginine administration has been found to be associated with increases of both plasma (4) and urinary (3) excretion of cyclic GMP, urinary NO_3^-, the stable end product of NO metabolism (4), as well as exhaled NO (5, 20), which suggests an improvement in the release of NO.

The mechanism by which L-arginine can increase the production of NO is still debated. A popular view claims that the enhanced vasodilation is due to increased availability of intracellular substrate for the endothelial NO synthase. However, the K_m of the constitutive endothelial NO synthase is far below

the ambient intracellular L-arginine levels. In cultured cells, for example, intracellular L-arginine is 30–800-fold higher than the K_m for NO synthase (21), which questions the validity of the assumption that L-arginine serves merely as a substrate for NO synthase. There also is some recent evidence suggesting that L-arginine may enhance NO release via reversal of the inhibitory effect of L-glutamine, but again apparently independent of its role as a substrate for NO synthase (22). A third possibility is that the vasodilating effect of L-arginine may be nonspecific. The enantiomer D-arginine, which is not used for the synthesis of NO (1), has been shown to have no effects on endothelium-dependent vascular relaxation induced by intraarterial infusion of acetylcholine (15, 19, 23), although high doses (160 μmol/min) can augment the vasodilating response to the agonist (23). To our knowledge, no study has specifically addressed the systemic hemodynamic response to peripheral administration of other basic amino acids; we found that, unlike L-arginine, the infusion of equimolecular amounts of D-arginine (1 g/min) did not contrast the vascular effects (increase of blood pressure and reduction of peripheral blood flow) brought about by acute hyperglycemia in normal subjects (6). Further studies are urgently needed to clarify this important topic.

We considered the possibility that endogenous substances released during L-arginine infusion might have participated in or mediated the vascular changes observed. As insulin demonstrates vasodilating properties (8, 9), L-arginine–induced hyperinsulinemia might be involved in the vascular effects observed during infusion of L-arginine. This was found to be the case. We blocked insulin secretion with octreotide, a somatostatin analogue shown to be an easier substitute than somatostatin in some metabolic studies (24). When L-arginine was infused along with octreotide and basal insulin and glucagon secretion was maintained by exogenous hormone replacement, the hemodynamic and rheologic effects of L-arginine were reduced. In particular, the vasodilating effect of L-arginine was reduced by 77% and the antiplatelet effect by 55%. This difference in the vascular effects of L-arginine is unlikely to be explained by a difference in the plasma glucose rise observed on the two experimental conditions, because the smaller hyperglycemia obtained in study II would be expected to have lesser negative influence on hemodynamic parameters (25).

Octreotide is known to suppress a number of other hormones, including glucagon, which in theory could modify the hemodynamic effects of L-arginine. To examine this possibility, the subjects participated in a third study during which exogenous insulin was infused with octreotide to replace the plasma insulin levels observed in study I. In this condition, the vascular responses to L-arginine were nearly identical to those observed in study I. These results strongly suggest that the blunted vasodepressor effects of L-arginine observed during octreotide infusion are a likely consequence of insulin suppression.

The finding that at least one half of the vascular responses to L-arginine is mediated by endogenous insulin secretion seems also consistent with the reported effects of L-arginine to increase NO synthesis in humans (3–5, 20). Recent human data indicate that insulin-induced vasodilation is mediated by NO, being blunted by the NO synthase inhibitor L-NMMA (8). Furthermore, insulin can also increase the content of cyclic GMP in both human vascular smooth muscle cells (26) and human platelets (27), an effect blunted by L-NMMA. On a speculative note, a synergistic mechanism may be operative in which the signal (L-arginine) increases the availability of mediators (NO and insulin), which in turn facilitate the uptake of L-arginine by cells, further raising its intracellular availability. In vascular endothelial cells, for example, the increased production of NO after exposure to bradykinin and ATP is associated with the enhanced uptake of L-arginine (28), while in rat cardiac myocytes insulin augments L-arginine uptake by a selective upregulation of the isoform 1 of the cationic amino acid transporter (29).

It is noteworthy that sympathetic activation did not occur during suppression of insulin secretion with octreotide, as suggested by the unchanged heart rate and plasma catecholamine levels. This may be related to the lesser drop of blood pressure, not enough for triggering sympathetic discharge. However, acute physiological increments in plasma insulin concentrations can stimulate the sympathetic noradrenergic activity (9) and this might not have occurred when insulin was blocked with octreotide. At present, we cannot distinguish between these two possibilities.

In conclusion, systemic L-arginine infusion in normal subjects decreases blood pressure, inhibits platelet aggregation, and also reduces blood viscosity, which is now regarded as a major cardiovascular risk factor (30). A substantial part of the vascular effects of L-arginine is mediated by endogenous insulin. If L-arginine acts by stimulating NO synthesis, through the mediation of insulin or via some other still unidentified mechanisms, it may be an easy and simple tool to assess endothelium-dependent vascular function in pathophysiological conditions, including states of insulin resistance where the ability of insulin to modulate vascular reactivity may be impaired (31, 32).

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