Vasodilator effects of L-arginine are stereospecific and augmented by insulin in humans

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Dallinger, Susanne, Anna Sieder, Jeanette Strametz, Michaela Bayerle-Eder, Michael Wolzt, and Leopold Schmetterer. Vasodilator effects of L-arginine are stereospecific and augmented by insulin in humans. Am J Physiol Endocrinol Metab 284: E1106–E1111, 2003; 10.1152/ajpendo.00292.2002.—The amino acid L-arginine, the precursor of nitric oxide (NO) synthesis, induces vasodilation in vivo, but the mechanism behind this effect is unclear. There is, however, some evidence to assume that the L-arginine membrane transport capacity is dependent on insulin plasma levels. We hypothesized that vasodilator effects of L-arginine may be dependent on insulin plasma levels. Accordingly, we performed two randomized, double-blind crossover studies in healthy male subjects. In protocol 1 (n = 15), subjects received an infusion of insulin (6 mU·kg−1·min−1 for 120 min) or placebo and, during the last 30 min, L-arginine or D-arginine (1 g/min for 30 min). In protocol 2 (n = 8), subjects received L-arginine in stepwise increasing doses in the presence (1.5 mU·kg−1·min−1) or absence of insulin. Renal plasma flow and glomerular filtration rate were assessed by the para-aminohippurate and inulin plasma clearance methods, respectively. Pulsatile choroidal blood flow was assessed with laser interferometric measurement of fundus pulsation, and mean flow velocity in the ophthalmic artery was measured with Doppler sonography. L-arginine, but not D-arginine, significantly increased renal and ocular hemodynamic parameters. Coinfusion of L-arginine with insulin caused a dose-dependent leftward shift of the vasodilator effect of L-arginine. This stereospecific renal and ocular vasodilator potency of L-arginine is enhanced by insulin, which may result from facilitated L-arginine membrane transport, enhanced intracellular NO formation, or increased NO bioavailability.

renal plasma flow; ocular blood flow; nitric oxide

The mechanism underlying this vasodilator effect has not yet been completely elucidated, since biochemical considerations indicate that L-arginine concentrations present in the plasma and within endothelial cells are far in excess of the membrane L-arginine transport capacity, and NO synthesis under normal situations is therefore not limited by substrate availability (3). On the other hand, a nonenzymatic pathway for NO synthesis may be responsible for vasodilation induced by L-arginine in vivo (4). Thus it is unclear whether the hemodynamic effects of L-arginine are the result of increased NO synthesis or attributable to endothelium-independent vasodilatory effects. Previous in vitro and in vivo studies indicate, however, that the L-arginine transport capacity may depend on insulin (5, 20, 23).

The aim of the present study was to gain further insight into the mechanism underlying the vasodilator effect of L-arginine. For this purpose, systemic hemodynamics and renal and ocular blood flow were measured in response to intravenous L-arginine in the presence or absence of euglycemic hyperinsulinemia. The vascular beds of the eye and the kidney were chosen for two reasons. On the one hand, a vasodilator effect of insulin is documented for both vascular beds (17). On the other hand, the eye and the kidney are end organs of diabetic damage.

SUBJECTS AND METHODS

Subjects

Protocol approval was obtained by the Ethics Committee of Vienna University School of Medicine. After written informed consent was signed, 15 healthy male subjects were studied in protocol 1 (age range: 20–32 yr; 26 ± 3 yr), and 8 healthy volunteers were studied in protocol 2 (age range: 23–35 yr; 30 ± 3 yr). All volunteers passed a prestudy screening during the 4 wk before the first study day, which included a physical examination and medical history, 12-lead electrocardiogram, complete blood cell count, clinical chemistry, 24-h creatinine clearance and urine analysis, and an ophthalmic examination. Subjects with normal findings in the screening examinations and ametropia of fewer than three diopters were included in the trial.

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Table 1. Baseline parameters on the 3 study days (protocol 1)

<table>
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<tr>
<th></th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
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<tbody>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>80 ± 2</td>
<td>80 ± 2</td>
<td>83 ± 3</td>
</tr>
<tr>
<td>Pulse rate, beats/min</td>
<td>68 ± 2</td>
<td>68 ± 2</td>
<td>62 ± 2</td>
</tr>
<tr>
<td>Renal plasma flow, ml/min</td>
<td>813 ± 21</td>
<td>796 ± 23</td>
<td>833 ± 31</td>
</tr>
<tr>
<td>Glomerular filtration rate, ml/min</td>
<td>131 ± 4</td>
<td>129 ± 3</td>
<td>132 ± 3</td>
</tr>
<tr>
<td>Fundus pulsation amplitude, μm</td>
<td>4.2 ± 0.4</td>
<td>4.2 ± 0.4</td>
<td>4.2 ± 0.4</td>
</tr>
<tr>
<td>Mean flow velocity, cm/s</td>
<td>17.7 ± 0.8</td>
<td>16.7 ± 1.1</td>
<td>16.2 ± 0.5</td>
</tr>
<tr>
<td>Insulin, μU/ml</td>
<td>4.4 ± 0.4</td>
<td>3.8 ± 0.3</td>
<td>4.0 ± 0.3</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>93 ± 2</td>
<td>87 ± 2</td>
<td>89 ± 3</td>
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</table>

Values are means ± SE; n = 15.

Study Design

All subjects were asked to refrain from alcohol and caffeine for ≥12 h before trial days. To standardize the sodium balance, all subjects received 2 g of sodium chloride for 2 days before trial days in addition to the usual salt intake.

Protocol 1. The study was performed in a randomized, double-blind, three-way crossover design. Subjects were assigned to coinfusion protocols of placebo plus D-arginine, placebo plus L-arginine, and insulin (euglycemic clamp) plus L-arginine in a balanced order on different trial days with a washout period of 5 days between the study days.

After an initial resting period, a continuous intravenous infusion of para-aminohippurate (PAH; Clinalfa, Laufelfingen, Switzerland) and inulin (Inutest; Laevosan, Linz, Austria) was started. A subsequent 45-min equilibration period was scheduled, and baseline hemodynamic readings were obtained from the comfortably seated subjects. Subjects received primed, constant infusions of glucose (1.5 mU·kg⁻¹·min⁻¹) under euglycemic conditions or placebo over 120 min. After 60 min, L-arginine was coadministered in stepwise increasing doses of 10, 30, 100, and 300 mg/min. Each dose of L-arginine was administered for 15 min.

Methods

Euglycemic insulin clamps. Each clamp was started with a primed infusion of insulin for 8 min followed by a constant infusion rate of insulin of 6 mU·kg⁻¹·min⁻¹ (protocol 1) or 1.5 mU·kg⁻¹·min⁻¹ (protocol 2). KCl was infused at a rate of 150 ml/h to prevent hypokalemia. Glucose was infused at a rate necessary to maintain the blood glucose level constant. Arterialized venous blood samples were drawn every 5 min from the contralateral arm placed in a heating blanket for measurement of glucose concentration.

Systemic hemodynamics. Systolic and diastolic blood pressures were measured on the upper arm by an automated oscillometric device. Pulse rate (PR) was automatically recorded from a finger pulse oxymetric device (HP-CMS patient monitor; Hewlett-Packard, Palo Alto, CA).

Renal plasma flow and glomerular filtration rate. Renal plasma flow (RPF) and glomerular filtration rate (GFR) were estimated using the PAH and the inulin plasma clearance method (18). All subjects received primed, constant infusions of glucose during hyperinsulinemia.

Dynamic measurements were performed at frequent intervals in a predetermined sequence during drug administration.

Protocol 2. The study was performed in a randomized, double-blind, two-way crossover design. Subjects were assigned to coinfusion of placebo plus L-arginine and insulin (euglycemic clamp) plus L-arginine in a balanced order on different trial days with a washout period of 5 days between the study days.

After an initial resting period, a continuous intravenous infusion of PAH and inulin was started. After the subsequent 45-min equilibration period, baseline hemodynamic values were obtained. Subjects received intravenous infusions of insulin (1.5 mU·kg⁻¹·min⁻¹) under euglycemic conditions or placebo over 120 min. After 60 min, L-arginine was coadministered in stepwise increasing doses of 10, 30, 100, and 300 mg/min. Each dose of L-arginine was administered for 15 min.

Fig. 1. Effects of D-arginine and L-arginine in the presence or absence of exogenous insulin. RPF, renal plasma flow; FPA, fundus pulsation amplitude; GFR, glomerular filtration rate; MFV, mean flow velocity. Data are presented as % change of preinfusion value (means ± SE; protocol 1, n = 15). *, Significantly different from the % change during hyperinsulinemia. Data indicate a synergistic effect of L-arginine and insulin, because the effect of coinfusion of L-arginine and insulin is significantly higher than the sum of the individual agent effects.
of PAH and inulin on trial days. After an intravenous loading dose, a continuous infusion of PAH and inulin to attain a plasma concentration of 0.02 and 0.25 mg/ml, respectively, was started. The infusion rate was calculated as estimated clearance of PAH and inulin (750 and 140 ml/min, respectively) times the target plasma concentration. PAH and inulin plasma concentrations were measured at baseline and during drug administration by photometric analysis and a commercially available test (Inutest), respectively. Subjects were asked to drink 300 ml water/h during renal hemodynamic studies.

Ocular hemodynamics. In all subjects, the left eye was studied. Ocular fundus pulsation was assessed by laser interferometry (16). Briefly, the eye is illuminated by the beam of a single-mode laser diode (λ = 783 nm) along the optical axis. The light is reflected at both the front side of the cornea and the retina. The two reemitted waves produce interference fringes from which the distance changes between cornea and retina during a cardiac cycle can be calculated. These distance changes are caused by the pulsatile inflow of blood through the arteries and by the nonpulsatile outflow through the veins. The maximum change in corneoretinal distance is called the fundus pulsation amplitude (FPA). This method has been shown to estimate the pulsatile blood flow in the choroidal vasculature (14).

Mean flow velocity (MFV) and resistive index in the ophthalmic artery were measured by color Doppler imaging. This noninvasive method is based on the backscattering of ultrasound by the formed elements in the blood vessels. Measurement of the frequency shift due to the Doppler effect yields information about the blood velocity. Peak-systolic flow velocity and end-diastolic flow velocity in the ophthalmic artery were assessed with a 3.25-MHz probe with pulsed Doppler device and simultaneous electrocardiogram recording (10) (CPM 750; Vingmed Sound, Horten, Norway). From these parameters, MVF (= integral of the Doppler curve/duration of the cardiac cycle) was calculated.

Laboratory analysis. Insulin plasma levels were measured by routine procedures. Glucose concentrations during euglycemic hyperinsulinemia were measured using a glucose analyzer (Beckman Glucose Analyzer 2; Beckman Instruments, Fullerton, CA).

Data Analysis
All statistical analyses were done using the Statistica software package (release 4.5; StatSoft, Tulsa, OK). Data are presented as means ± SE. Drug effects on main outcome variables were assessed by repeated-measure ANOVA. Post hoc analysis was done with a paired t-test. Effects of L-arginine and D-arginine were expressed as percent change from pretreatment values. A P value of <0.05 was considered significant.

RESULTS
Protocol 1
Baseline values of the measured parameters are given in Table 1. There were no significant differences between the baseline readings on the three study days. Insulin did not affect mean arterial pressure (MAP) and PR but increased RPF (14 ± 1%, P < 0.001), GFR (8 ± 1%, P < 0.001), and FPA (9 ± 1%, P < 0.001). Insulin also tended to increase MFV in the ophthalmic artery.

Table 2. Effects of insulin, L-arginine and D-arginine on insulin and glucose plasma levels (protocol 1)

<table>
<thead>
<tr>
<th></th>
<th>Placebo + L-Arginine</th>
<th>Placebo + D-Arginine</th>
<th>Insulin + L-Arginine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin, μU/ml</td>
<td>33.9 ± 3.1</td>
<td>5.4 ± 0.4</td>
<td>683.3 ± 28.5</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>91 ± 2</td>
<td>97 ± 3</td>
<td>99 ± 3</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 15.

Fig. 2. Effects of stepwise increasing doses of L-arginine in the presence (●) or absence (○) of exogenous insulin on fundus pulsation amplitude, mean flow velocity, renal plasma flow, and glomerular filtration rate. Data are presented as absolute values (means ± SE; protocol 2, n = 8). BL, baseline. Insulin infusion period is indicated by an arrow. *Significant differences between L-arginine and placebo study days as evidenced from post hoc testing.

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artery, but this effect was not significant (8 ± 3%, P = 0.29).

D-Arginine did not cause any significant hemodynamic effect but tended to increase MFV in the ophthalmic artery (Fig. 1). L-Arginine did not affect MAP or PR but increased regional hemodynamic parameters. The effect of L-arginine on RPF (11 ± 2%, P = 0.002 vs. D-arginine), GFR (7 ± 2%, P = 0.016 vs. D-arginine), and FPA (12 ± 1%, P < 0.001 vs. D-arginine) was more pronounced than the effect of D-arginine. L-Arginine also increased MFV in the ophthalmic artery (9 ± 2%), but this effect was comparable to the effect of D-arginine (P = 0.44).

L-Arginine also had no effect on systemic hemodynamics during coinfusion of insulin, but the regional hemodynamic responses were augmented compared with L-arginine alone (Fig. 1). L-Arginine increased RPF (21 ± 3%, P = 0.010 vs. L-arginine alone), GFR (12 ± 1%, P = 0.031), FPA (19 ± 2%, P < 0.001), and MFV (23 ± 3%, P = 0.002) during hyperinsulinemia.

L-Arginine significantly increased insulin plasma levels, whereas D-arginine had no effect (Table 2; P < 0.001). As expected, administration of exogenous insulin (1.5 mU·kg⁻¹·min⁻¹) increased insulin plasma levels to values observed in the high postprandial range (Table 3). Glucose plasma levels were within the euglycemic range throughout the experiments.

**DISCUSSION**

In the present study, intravenous L-arginine caused a significant increase in renal and ocular hemodynamics.

![Fig. 3](http://ajpendo.physiology.org/)

![Fig. 4](http://ajpendo.physiology.org/)

**Table 3. Effects of insulin and L-arginine on insulin and glucose plasma levels (protocol 2)**

<table>
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<tr>
<th></th>
<th>Placebo + L-Arginine</th>
<th>Insulin + L-Arginine</th>
</tr>
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<tbody>
<tr>
<td>Insulin, µU/ml</td>
<td>39.6 ± 5.1</td>
<td>132.3 ± 12.9</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>86 ± 2.8</td>
<td>101 ± 3.2</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8.
parameters, confirming that L-arginine exerts vasodilator effects in vivo. This is in keeping with a variety of previous studies in humans (2, 7, 15, 24). The key finding of the present study is that these vasodilator effects of L-arginine are augmented in the presence of exogenous insulin. Insulin caused a significant leftward shift of the regional hemodynamic dose-response curve to L-arginine (protocol 2), and the vasodilator effect of L-arginine was stereospecific (protocol 1).

It has previously been speculated that the administration of L-arginine stimulates NO synthesis in vivo (9, 15, 19), because intravenous or oral administration of the amino acid causes an increase in the concentration of NO in exhaled air and increases nitrate plasma levels and urinary nitrate excretion in humans. The present study provides several lines of evidence that exogenous L-arginine increases NO synthesis in vivo. D-Arginine, the enantiomer of L-arginine, did not exert significant systemic renal or ocular hemodynamic effects. Previous studies compared the vasoactive actions of D-arginine and L-arginine in the human forearm. Whereas L-arginine enhanced forearm blood flow, D-arginine administered at the same dose showed no vasodilator effect (8). Ueda et al. (23) reported that, in the presence of exogenous insulin, L-arginine induced a more pronounced vasodilator effect than D-arginine. On the other hand, other investigators found that high doses of L-arginine as well as D-arginine, locally infused into a vein on the back of the hand or in the brachial artery, increase venous blood flow (11). This latter result could, however, also point toward a different vasodilator mechanism in the venous vasculature.

In addition, L-arginine, but not D-arginine, increased insulin plasma levels in the present study. This is in keeping with a previous study showing that insulin-mediated glucose uptake in healthy subjects during L-arginine infusion is stereospecific (12). Moreover, there is evidence that part of the vasodilator effect of L-arginine is mediated via the increase in insulin plasma levels (6). It appears, however, that insulin secretion induced by L-arginine is caused by membrane depolarization and is independent of NO (22).

Insulin has previously been shown to induce forearm, renal, and ocular vasodilation in humans (1, 17), which can be blunted by NO synthase inhibition (13, 17, 21). Hence, it appears that at least part of insulin-induced vasodilation is NO dependent. In addition, there is evidence from in vitro studies in endothelial cells and isolated gastric glands that insulin stimulates L-arginine transport and NO synthesis (5, 20). Our data also indicate that high plasma insulin augments the vasodilator effects of L-arginine in vivo. This is seen with high doses of L-arginine and pharmacological insulin plasma levels (protocol 1) as well as with lower doses of L-arginine and insulin plasma levels in the high postprandial range (protocol 2). This finding is compatible with previous data in the human forearm (23) and suggests that L-arginine may become rate limiting for NO production at high insulin plasma levels. However, the present study does not provide direct evidence that the synergistic vasodilator effects of L-arginine and insulin are coupled to increased NO production, because we have not shown that an NO synthase inhibitor modifies this effect in the eye or kidney. A study using an insulin clamp, intravenous infusion of L-arginine, and intravenous infusion of an NO synthase inhibitor does not seem, however, to be feasible in healthy humans.

A limitation of the present study is that effects of L-arginine in the presence or absence of exogenous insulin are not directly comparable, because hyperinsulinemia per se increased renal and ocular hemodynamic parameters. Hence, effects of L-arginine expressed as percent change from preadministration values refer to a slightly different vascular tone. However, the effects of insulin on peripheral hemodynamics were small in the present study, and predilatation should rather lead to a blunted L-arginine response. Hence, our data rather underestimate the elevated L-arginine response during hyperinsulinemia.

In conclusion, the renal and ocular vasodilator potency of L-arginine is enhanced by insulin. Whether this results from facilitated L-arginine membrane transport, enhanced intracellular NO formation, or increased NO bioavailability remains to be shown.

REFERENCES