L-Arginine Transport in Humans With Cortisol-Induced Hypertension

Jaye P.F. Chin-Dusting, Belinda A. Ahlers, David M. Kaye, John J. Kelly, Judith A. Whitworth

Abstract—A deficient L-arginine–nitric oxide system is implicated in cortisol-induced hypertension. We investigate whether abnormalities in L-arginine uptake contribute to this deficiency. Eight healthy men were recruited. Hydrocortisone acetate (50 mg) was given orally every 6 hours for 24 hours after a 5-day fixed-salt diet (150 mmol/d). Crossover studies were performed 2 weeks apart. Thirty milliliters of blood was obtained for isolation of peripheral blood mononuclear cells after each treatment period. L-Arginine uptake was assessed in mononuclear cells incubated with L-arginine (1 to 300 μmol/L), incorporating 100 nCi/mL [3H]-L-arginine for a period of 5 minutes at 37°C. Forearm [3H]-L-arginine extraction was calculated after infusion of [3H]-L-arginine into the brachial artery at a rate of 100 nCi/min for 80 minutes. Deep forearm venous samples were collected for determination of L-arginine extraction. Plasma cortisol concentrations were significantly raised during the active phase (323±43 to 1082±245 mmol/L, P<0.05). Systolic blood pressure was elevated by an average of 7 mm Hg. Neither L-arginine transport into mononuclear cells (placebo vs active, 26.3±3.6 vs 29.0±2.1 pmol/10 000 cells per 5 minutes, respectively, at an L-arginine concentration of 300 μmol/L) nor L-arginine extraction in the forearm (at 80 minutes, placebo vs active, 1 868 904±434 962 vs 2 013 910±770 619 disintegrations per minute) was affected by cortisol treatment; ie, that L-arginine uptake is not affected by short-term cortisol treatment. We conclude that cortisol-induced increases in blood pressure are not associated with abnormalities in the L-arginine transport system. (Hypertension. 2003;41:1336-1340.)

Key Words: cortisol ■ hypertension, mineralocorticoid ■ nitric oxide ■ arginine ■ human

Cortisol, the major naturally occurring human glucocorticoid, is implicated in the pathogenesis of some forms of essential hypertension, but the mechanisms by which cortisol raises blood pressure are not fully defined. Cardiac output is increased but is not essential for the increase, and sympathetic activity is, if anything, decreased.

A role for the nitric oxide system in cortisol-induced hypertension has been implicated in animal models of cortisol-induced hypertension; L-arginine, the substrate precursor to nitric oxide, prevents and reverses the development of adrenocorticotropin-induced hypertension in rats. In humans, cortisol-increases in blood pressure occur in association with reductions in plasma nitrate/nitrite concentrations, but no change in plasma arginine or symmetric or asymmetric dimethyl arginine has been observed, indicating that the reductions in nitrate could not be explained by changes in substrate availability or endogenous nitric oxide synthase inhibitors. More recently, using bilateral forearm plethysmography, we have found impaired cholinergic vasodilatation after cortisol administration. Cortisol did not affect the response to sodium nitroprusside, and although N°-monomethyl-L-arginine inhibited cholinergic vasodilation in placebo-treated subjects, it had no additional effect in the presence of cortisol. Taken together, these results are consistent with a role for abnormalities of the nitric oxide system in cortisol-induced hypertension in humans.

In the current study, we explored whether cortisol reduces the cellular uptake of L-arginine and whether this mechanism might provide an explanation for the decreased synthesis or secretion of nitric oxide. The study was performed in healthy adult men against a background of a restricted-nitrate diet. L-Arginine transport was assessed in peripheral blood mononuclear cells isolated from these subjects as well as in vivo in the forearm vascular bed.

Methods

Subjects
Healthy (or disease-free) men were recruited by advertisement. All underwent a physical medical examination and had their medical history recorded. Persons with a history of major illness, including diabetes, cardiovascular disease, respiratory illness, and any contraindication to corticosteroid therapy, were excluded, as were smokers, heavy alcohol drinkers (ie, >3 standard alcohol drinks per day), and persons who had a body mass index >25. All participants gave informed consent and were aware of their right to withdraw from the study.

Crossover studies were performed 2 weeks apart. Thirty milliliters of blood was obtained for isolation of peripheral blood mononuclear cells after each treatment period. L-Arginine uptake was assessed in mononuclear cells incubated with L-arginine (1 to 300 μmol/L), incorporating 100 nCi/mL [3H]-L-arginine for a period of 5 minutes at 37°C. Forearm [3H]-L-arginine extraction was calculated after infusion of [3H]-L-arginine into the brachial artery at a rate of 100 nCi/min for 80 minutes. Deep forearm venous samples were collected for determination of L-arginine extraction. Plasma cortisol concentrations were significantly raised during the active phase (323±43 to 1082±245 mmol/L, P<0.05). Systolic blood pressure was elevated by an average of 7 mm Hg. Neither L-arginine transport into mononuclear cells (placebo vs active, 26.3±3.6 vs 29.0±2.1 pmol/10 000 cells per 5 minutes, respectively, at an L-arginine concentration of 300 μmol/L) nor L-arginine extraction in the forearm (at 80 minutes, placebo vs active, 1 868 904±434 962 vs 2 013 910±770 619 disintegrations per minute) was affected by cortisol treatment; ie, that L-arginine uptake is not affected by short-term cortisol treatment. We conclude that cortisol-induced increases in blood pressure are not associated with abnormalities in the L-arginine transport system. (Hypertension. 2003;41:1336-1340.)

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study at any time of their choosing. The study protocol was approved by the Alfred Hospital Institutional Ethics Committee, and all procedures followed were in accordance with institutional guidelines.

Experimental Design

Visit 1
Participants were required to make 5 separate visits to the Alfred Hospital. At visit 1, a medical examination was conducted to assess the suitability of the participant for the study. This was followed by a detailed discussion of the study protocol, and the participant was given the opportunity to discuss any queries. After formal consent was obtained, participants were given a dietary program to which they were required to adhere for 5 days before visits 3 and 5. The diet was calculated to contain 150 mmol/d sodium, and subjects were asked to refrain from alcohol and to maintain a constant caffeine intake, with no caffeine 12 hours before visits 2 through 5.

Visit 2
At 8 AM, participants rested in a supine position, when blood pressure recordings were taken 3 times (Dinamap) after a 15-minute rest. A blood sample was obtained for full blood examination, including glucose, cortisol, and electrolyte levels. In a double-blind, randomized protocol, participants were then given hydrocortisone acetate (50 mg; cortisol) or placebo tablets, which they commenced taking at 9 AM and then every 6 hours thereafter for 24 hours (a total of 5 tablets in all).

Visit 3
Visit 3 was conducted 24 hours after visit 2 at 9 AM (after the final morning dose). As in visit 2, 3 supine blood pressure recordings (Dinamap) were taken after a 15-minute rest. A blood sample was obtained for full blood examination, glucose, cortisol, and electrolyte levels. L-Arginine transport was then assessed in vitro (in peripheral blood mononuclear cells) and in vivo (forearm).

Visit 4
Visit 4 was conducted at least 3 weeks after visit 3 to allow for recovery from arterial puncture and was identical in protocol to visit 2.

Visit 5
Visit 5 was scheduled 24 hours after visit 4. The protocol was the same as in visit 3, but subjects were given the treatment other than that received in visit 2.

Study Protocol for Investigation of L-Arginine Uptake in Peripheral Blood Mononuclear Cells
Thirty milliliters of blood was collected into tubes containing EGTA and diluted in equal volume with a balanced salt solution. Peripheral blood mononuclear cells were isolated by standard density gradient centrifugation (Ficoll-Paque, Pharmacia) as per the manufacturer’s instruction. Trypan blue staining was used to assess cell viability (90%) according to standard methods. Isolated peripheral blood mononuclear cells were isolated by standard density gradient centrifugation (Ficoll-Paque, Pharmacia) as per the manufacturer’s instruction. Trypan blue staining was used to assess cell viability (90%) according to standard methods.

In a separate series of experiments, 60 mL blood was collected from 5 healthy individuals (3 men, 2 women). The direct effect of cortisol on L-arginine uptake in peripheral blood mononuclear cells was examined by repeating the assay described previously in the absence and presence of cortisol (10 μmol/L) added to the incubating medium. The concentration of cortisol was based on the average plasma concentration of cortisol in the subjects after the active phase of cortisol ingestion.

Study Protocol for Investigation of L-Arginine Transport in the Forearm
This procedure has been described previously. In brief, the brachial artery and a retrograde deep venous cannula were placed in the nondominant arm. [3H]-L-Arginine was infused into the brachial artery at a rate of 100 nCi/min for 80 minutes. Deep forearm venous samples were collected at 40 and 80 minutes for the determination of forearm arginine kinetics. Forearm blood flow was measured by venous occlusion plethysmography. The plasma concentration of [3H]-L-arginine was determined by ion-exchange chromatography. Plasma proteins were removed from 750 μL plasma by the addition of 250 μL of 20% trichloracetic acid, followed by cooling on ice and subsequent removal of the precipitated proteins by centrifugation. Samples were then extracted 5 times in ether to remove trichloracetic acid and combined in equal volume with 20 mmol/L HEPES, pH 6. Samples were then applied to a column (Dinamap 50W-X8) that had been preequilibrated with HEPES. After repeated washes, [3H]-L-arginine was eluted from the column with 1N NaOH.

Radioactivity was determined by liquid scintillation spectroscopy.

Forearm plasma flow (FPF) was calculated according to the formula

\[
\text{FPF} = \frac{\text{FBF} \times FV}{\text{Hct}}
\]

where FBF = forearm blood flow, Hct = hematocrit, and FV = forearm volume.

The effect of active and placebo phases on blood parameters was examined by repeating the assay described previously in the absence and presence of cortisol (10 μmol/L) added to the incubating medium. The concentration of cortisol was based on the average plasma concentration of cortisol in the subjects after the active phase of cortisol ingestion.

Statistical Analysis
Data are presented as mean±SEM. Between-treatment comparisons were performed by repeated-measures ANOVA followed, where indicated, by paired Student t test. A probability value <0.05 was taken as a measure of statistical significance. No order effect was observed.

Results

Blood Pressure and Blood Parameters
Eight men (mean±SEM age, 24.1±1.6 years; range, 20 to 31 years) were recruited and completed the study protocol. Confirmation of protocol compliance was obtained by measurement of a significant increase in plasma cortisol levels during the active phase (323±43 to 1082±245 nmol/L, P<0.01) but not the placebo phase. Cortisol, but not placebo, significantly increased systolic blood pressure (from 112±4 to 119±4 mm Hg, P=0.01; Figure 1). When the comparison was made between postcortisol and postplacebo phases, both systolic and mean arterial blood pressures were significantly higher during the cortisol phase. Cortisol also significantly increased glucose, white blood cell, and neutrophil concentration levels and decreased potassium and eosinophil levels. The effect of active and placebo phases on blood parameters is shown in the Table.

L-Arginine Transport in Isolated Peripheral Blood Mononuclear Cells and in the Intact Forearm
In peripheral blood mononuclear cells, accumulation of [3H]-L-arginine was detected over the physiological range of...
Glucocorticoid excess raises blood pressure in association with abnormalities in the L-arginine–nitric oxide pathway in both animals and humans. Exogenous cortisol inhibits forearm vascular responses to acetylcholine but not to sodium nitroprusside and decreases nitric oxide metabolites in humans, and both adrenocorticotropic hormone and corticosterone downregulate expression of endothelial nitric oxide synthases in the rat. Furthermore, L-arginine, but not D-arginine, prevents corticotropin-induced increases in blood pressure in the rat, suggesting a relative insufficiency of substrate availability. This beneficial effect of L-arginine was, however, not observed in humans. It was thus unclear from studies of L-arginine supplementation whether the insufficiency was extracellular or intracellular and/or whether cellular L-arginine transport was impaired.

The current protocol was designed to examine whether cortisol-induced hypertension is associated with dysfunctional cellular L-arginine transport. In the current study, orally administered cortisol, sufficient to provoke an increase in systolic blood pressure, had no effect on cellular L-arginine transport in peripheral blood mononuclear cells or in the forearm vasculature. Similarly, despite a small trend toward a marginal effect, direct addition of cortisol to the incubating medium had no significant effect on L-arginine uptake in peripheral blood mononuclear cells.

The hypertensive effects of adrenocorticotropic hormone injection or infusion can be reproduced by oral cortisol administration. Systolic but not diastolic or mean blood pressure increased in all 8 subjects, who were confirmed to be capsule-compliant (by plasma cortisol levels); the increase in blood pressure averaged 7 mm Hg. The increase in blood pressure was accompanied by other previously documented pleiotropic and plasma biochemistry alterations. These changes were not observed after placebo supplementation.

### Discussion

Glucocorticoid excess raises blood pressure in association with abnormalities in the L-arginine–nitric oxide pathway in both animals and humans. Exogenous cortisol inhibits forearm vascular responses to acetylcholine but not to sodium nitroprusside and decreases nitric oxide metabolites in humans, and both adrenocorticotropic hormone and corticosterone downregulate expression of endothelial nitric oxide synthases in the rat. Furthermore, L-arginine, but not D-arginine, prevents corticotropin-induced increases in blood pressure in the rat, suggesting a relative insufficiency of substrate availability. This beneficial effect of L-arginine was, however, not observed in humans. It was thus unclear from studies of L-arginine supplementation whether the insufficiency was extracellular or intracellular and/or whether cellular L-arginine transport was impaired.

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We are therefore confident that the cortisol treatment was adhered to and effective.

The majority of l-arginine is transported into mammalian cells by cationic amino acid transporters with the properties of system \( y^+ \) transport.\(^{15} \) Impairment of the l-arginine transport system is a plausible explanation for the "l-arginine paradox," wherein despite the fact that intracellular concentrations of l-arginine are in excess of the \( K_m \) for nitric oxide synthase,\(^{16} \) exogenous l-arginine supplementation is nevertheless able to overcome the observed endothelium dysfunction. This has certainly been reported in many models of experimental hypertension,\(^{17,18} \) including glucocorticoid-induced hypertension,\(^{4,19} \) although it was not apparent in cortisol-induced hypertension in humans.\(^{12} \) This might be due to species differences in the entry of exogenous arginine into active versus passive intracellular pools with regard to access to synthesis pathways for nitric oxide. The current study reports the finding that cortisol had no effect on l-arginine uptake.

A possible limitation of the current study is in directly relating the observed results to actions within nitric oxide–

Figure 2. a, \(^{[3}H\)-l-arginine uptake in peripheral blood mononuclear cells over the concentration range from 0 to 300 \( \mu \)mol/L was not altered by oral after acetate (50 mg every 6 hours for 24 hours). b, \(^{[3}H\)-l-arginine uptake in the forearm following 40 and 80 minutes of infusion (steady state) was not altered by oral hydrocortisone acetate (50 mg every 6 hours for 24 hours). Results are expressed as mean±SEM.

Figure 3. \(^{[3}H\)-l-arginine uptake in peripheral blood mononuclear cells over the concentration range from 0 to 300 \( \mu \)mol/L was not altered by addition of cortisol (10 \( \mu \)mol/L) to the incubating medium.

synthesizing and –secreting cells. Although we have previously demonstrated that some of the extracted l-arginine is associated with l-citrulline release,\(^{20} \) we cannot discount the effects of renal l-arginine uptake, transport, or metabolizing systems, although this would appear unlikely, particularly in the context of the local infusions of l-arginine across the forearm.

Given the findings that l-arginine supplementation did not lower the cortisol-induced increase in blood pressure\(^{12} \) and furthermore, that l-arginine transport was intact, we conclude that inadequate substrate (extracellular or intracellular) is not a contributory factor in cortisol-induced hypertension. It is therefore likely that the impairment is downstream of the l-arginine–nitric oxide cascade and includes such potential targets as diminished cofactor availability,\(^{11} \) diminished nitric oxide synthase activity and/or expression,\(^{9} \) or increased nitric oxide metabolism.

Perspectives
The effect of exogenous cortisol on l-arginine was assessed in young, healthy men. Using a random crossover design, we measured the effect of cortisol on blood pressure, peripheral monocyte uptake of labeled l-arginine, and forearm extraction of infused, labeled arginine. Compared with placebo, cortisol raised systolic pressure by 7 mm Hg but had no effect on l-arginine uptake or extraction. We conclude that cortisol-related elevations in blood pressure are not associated with alterations in l-arginine transport.

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References


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