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 nmol/L [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±343 962 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 adrenocorticotropic hormone-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 photoplethysmography, we have found impaired cholinergic vasodilatation after cortisol administration. Cortisol did not affect the response to sodium nitroprusside, and although N⁶-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 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, and radioactivity associated with uptake was measured by scintillation counting. Uptake studies were performed in duplicate for each individual. Parallel protocols were performed for each individual in the added presence of 10 mmol/L L-lysine, a specific L-arginine uptake inhibitor. Arginine uptake was calculated as the difference between uptake in the absence and presence of the inhibitor.

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% trichloroacetic 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 trichloroacetic acid and combined in equal volume with 20 mmol/L HEPES, pH 6. Samples were then applied to a column (Dowex 50W-X8) that had been pre-equilibrated 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, FPF (mL/min) = FBF×FPS×Hct/FV, where FBF = forearm blood flow, FPS = forearm systolic pressure, Hct = hematocrit, and FV = forearm volume.

[3H]-L-arginine uptake was calculated according to the formula

\[ \text{Uptake} = \frac{\text{Radioactivity}}{\text{Plasma flow}} \]

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

\[ \text{Uptake} = \frac{\text{Radioactivity}}{\text{Plasma flow}} \]
concentrations used (Figure 2a). Cortisol had no effect on the rate of accumulation of arginine by peripheral blood mononuclear cells when compared with placebo. Cortisol treatment had no effect on resting forearm blood flow (data not shown). When [ 3 H]- L-arginine uptake was calculated in the forearm vasculature, cortisol had no effect on the uptake of arginine in that vasculature (Figure 2b). Direct addition of cortisol (10 μmol/L) into the incubating medium similarly had no effect on L-arginine uptake in peripheral blood mononuclear cells (Figure 3).

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.

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 either 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.

### Pleiotropic Effects of Hydrocortisone Acetate (Cortisol)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Preplacebo</th>
<th>Postplacebo</th>
<th>Preactive</th>
<th>Postactive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium, mmol/L</td>
<td>142±0.7</td>
<td>141±0.6</td>
<td>142±0.6</td>
<td>141±0.8</td>
</tr>
<tr>
<td>Potassium, mmol/L</td>
<td>3.7±0.1</td>
<td>3.8±0.1</td>
<td>3.9±0.1</td>
<td>3.6±0.1</td>
</tr>
<tr>
<td>Chloride, mmol/L</td>
<td>103.6±0.6</td>
<td>104.3±0.6</td>
<td>103.4±0.6</td>
<td>104.5±0.8</td>
</tr>
<tr>
<td>Bicarbonate, mmol/L</td>
<td>28.8±0.4</td>
<td>28.1±0.9</td>
<td>28.8±0.9</td>
<td>27.8±1.1</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>4.1±0.3</td>
<td>3.9±0.3</td>
<td>4.4±0.4</td>
<td>5.3±0.3</td>
</tr>
<tr>
<td>Cortisol, nmol/L</td>
<td>342±32</td>
<td>290±40</td>
<td>323±43</td>
<td>1082±246*</td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>145.6±4.5</td>
<td>141.6±4.0*</td>
<td>146.4±3.8</td>
<td>140.0±3.4*</td>
</tr>
<tr>
<td>White blood cells, 10^9/L</td>
<td>5.12±0.40</td>
<td>5.33±0.39</td>
<td>5.84±0.52</td>
<td>9.60±1.02*</td>
</tr>
<tr>
<td>Platelets, 10^9/L</td>
<td>197±32</td>
<td>217±14</td>
<td>218±13</td>
<td>205±34*</td>
</tr>
<tr>
<td>Red blood cells, 10^12/L</td>
<td>4.73±0.15</td>
<td>4.61±0.12*</td>
<td>4.75±0.14</td>
<td>4.54±013*</td>
</tr>
<tr>
<td>Hematocrit, L/L</td>
<td>0.42±0.01</td>
<td>0.41±0.01*</td>
<td>0.43±0.01</td>
<td>0.40±0.01*</td>
</tr>
<tr>
<td>Neutrophils, 10^9/L</td>
<td>2.94±0.30</td>
<td>3.37±0.48</td>
<td>3.39±0.43</td>
<td>7.69±0.86*</td>
</tr>
<tr>
<td>Lymphocytes, 10^9/L</td>
<td>1.64±0.15</td>
<td>1.64±0.17</td>
<td>1.77±0.21</td>
<td>1.44±0.27*</td>
</tr>
<tr>
<td>Monocytes, 10^9/L</td>
<td>0.39±0.04</td>
<td>0.38±0.03</td>
<td>0.46±0.04</td>
<td>0.43±0.05</td>
</tr>
<tr>
<td>Eosinophils, 10^9/L</td>
<td>0.14±0.04</td>
<td>0.13±0.03</td>
<td>0.19±0.05</td>
<td>0.02±0.01*</td>
</tr>
<tr>
<td>Basophils, 10^9/L</td>
<td>0.02±0.00</td>
<td>0.01±0.00</td>
<td>0.03±0.01</td>
<td>0.02±0.01</td>
</tr>
</tbody>
</table>

*Cortisol dosage: 50 mg every 6 hours for 24 hours.

*P<0.05 when compared to pretreatment.
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. 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, exogenous L-arginine supplementation is nevertheless able to overcome the observed endothelium dysfunction. This has certainly been reported in many models of experimental hypertension, including glucocorticoid-induced hypertension, although it was not apparent in cortisol-induced hypertension in humans. 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–synthesizing and –secreting cells. Although we have previously demonstrated that some of the extracted L-arginine is associated with L-citrulline release, 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 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, diminished nitric oxide synthase activity and/or expression, 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.

**Acknowledgments**

The authors would like to acknowledge the technical expertise of Ann-Maree Jefferis, Jennifer Starr, and Margaret Vincent.

**References**

I-Arginine Transport in Humans With Cortisol-Induced Hypertension
Jaye P.F. Chin-Dusting, Belinda A. Ahlers, David M. Kaye, John J. Kelly and Judith A. Whitworth

Hypertension. 2003;41:1336-1340; originally published online April 21, 2003;
doi: 10.1161/01.HYP.0000070024.59313.AE
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2003 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://hyper.ahajournals.org/content/41/6/1336

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org/subscriptions/