Cortisol Inhibits Cholinergic Vasodilatation in the Human Forearm

George J. Mangos, Brian R. Walker, John J. Kelly, Jane A. Lawson, David J. Webb, and Judith A. Whitworth

Exogenous cortisol raises blood pressure (BP) in humans and there is accumulating evidence of abnormalities of glucocorticoid activity in essential hypertension. In this study we tested the hypothesis that exogenous cortisol attenuates the cholinergic dilator response in the forearm circulation. Fourteen healthy normotensive men were studied. Using bilateral forearm venous plethysmography, we examined forearm blood flow responses to intra-arterial acetylcholine (ACh) and sodium nitroprusside (SNP) pre- and post-NG-monomethyl-L-arginine (LNMMA) after 2 or 5 days of oral cortisol or placebo in a randomized, double-blind crossover study.

Exogenous cortisol increased supine systolic (P < .05) and standing systolic (P < .05) BP and produced expected metabolic changes and suppressed serum cortisol concentration (P < .001). Baseline forearm blood flow did not differ between placebo and cortisol treatments at 2 or 5 days. Cholinergic vasodilatation was impaired after cortisol administration, reaching statistical significance at 5 days (P < .05). Cortisol did not affect responses to SNP. NG-monomethyl-L-arginine inhibited cholinergic vasodilatation in placebo-treated groups but had no additional effect in the presence of cortisol.

These results support our hypothesis and suggest that the mechanism of impaired cholinergic dilatation in glucocorticoid-treated subjects involves abnormalities of the endothelial nitric oxide system. Am J Hypertens 2000; 13:1155–1160 © 2000 American Journal of Hypertension, Ltd.

KEY WORDS: Glucocorticoid, hypertension, nitric oxide, forearm blood flow, acetylcholine.

Hypertension is a recognized complication of endogenous or exogenous cortisol excess. In healthy human subjects, oral cortisol administration reproducibly increases blood pressure (BP) within 24 h. Changes in cortisol secretion or metabolism contribute to the pathogenesis of a number of rare forms of hypertension including Cushing’s syndrome, the syndrome of apparent mineralocorticoid excess, and liquorice abuse. Cortisol has also been implicated in the hypertension of chronic renal failure and in some subjects with essential hypertension.

The mechanism of cortisol-induced hypertension is not known. The hypertension is amplified by—but not

Received December 6, 1999. Accepted April 4, 2000.
From the Departments of Medicine (GJM, JJK, JAL) and Renal Medicine (JJK), St. George Hospital, University of New South Wales, Kogarah, Australia; Endocrinology Unit, Department of Medical Sciences (BRW), and Department of Medicine (DJW), University of Edinburgh, Western General Hospital, Edinburgh, UK; and John Curtin School of Medical Research (JAW), Canberra, Australia.

This work was supported by the National Health and Medical Research Council of Australia and Dr. J. Kelly was supported by a Wellcome-Ramaciotti Research Travel Grant.

Address correspondence and reprint requests to Dr. George J Mangos, Department of Medicine, St. George Hospital, Gray St Kogarah Sydney NSW 2217, Australia; e-mail: g.mangos@unsw.edu.au

© 2000 by the American Journal of Hypertension, Ltd.
Published by Elsevier Science, Inc.
dependent on—sodium intake\(^9\) and is not prevented by blockade of mineralocorticoid receptors by spironolactone.\(^1\) Sympathetic nervous system activity is unchanged or reduced\(^10,11\) and the renin-angiotensin system is suppressed by cortisol.\(^12\) Vascular responsiveness to phenylephrine\(^13\) and angiotensin II\(^14\) is enhanced but this may simply reflect a compensatory response to suppression of the sympathetic nervous system.

In the model of adrenocorticotrophin (ACTH)-induced hypertension in the rat, we have recently demonstrated that dietary supplementation with l-arginine but not D-arginine prevents the onset of hypertension.\(^15\) This suggests a role for the nitric oxide (NO) system in ACTH-induced hypertension and, by extension, in cortisol-induced hypertension. To examine the relevance of the NO system to cortisol-induced hypertension in humans, we measured the vasodilator responses to intra-arterial ACh in the forearm vascular bed in subjects treated with oral cortisol for 2 or 5 days.

**METHODS**

**Subjects** Fourteen healthy normotensive male volunteers without contraindications to corticosteroid therapy were studied. Healthy volunteers were enrolled to minimize confounding variables such as vascular and metabolic disease, as may occur in conditions of chronic endogenous glucocorticoid excess, such as Cushing’s disease. Smokers were included in the 2-day but not in the 5-day study. Each subject gave written informed consent and the studies were approved by the Lothian Research Ethics Committee (2-day study) or the Committee on Experimental Procedures Involving Human Subjects of the University of New South Wales and the South Eastern Sydney Area Health Service Ethics Committee (5-day study) and conformed to the guidelines for conduct of human experimentation of the National Health and Medical Research Council of Australia.

**Study Design** The experiments were two-phase, randomized, placebo-controlled, double-blind crossover studies comparing placebo with cortisol (hydrocortisone acetate, Alphapharm, Sydney, Australia or Merck, Sharp and Dohme, Hoddesdon, United Kingdom) 20 mg 6 hourly by mouth for 2 days (n = 6) or 5 days (n = 8). At the end of each treatment phase (ie, placebo or hydrocortisone), forearm blood flow measurements were performed as described later. Treatment phases were separated by washout periods of at least 2 weeks (2-day study) or 5 weeks (5-day study).

The 2-day study was performed initially, the results of which were suggestive (but not conclusive) of a suppressive effect of cortisol on cholinergic vasodilation. Subsequently, a more detailed 5-day study was undertaken in a different population of nonsmoking individuals.

**Two-Day Study** This study was performed in Edinburgh. Subjects received the blinded treatment in four divided doses daily for 2 days, then reported for measurements of forearm blood flow. Subjects abstained from nonsteroidal anti-inflammatory agents and other proprietary drugs for the duration of the study and avoided caffeine and tobacco for 12 h before the study.

**Five-Day Study** This study was performed in Sydney. Subjects were asked to maintain a fixed sodium diet (150 mmol/day) from 2 days before and for the duration of the study, to abstain from alcohol, and to consume no more than two caffeine-containing beverages daily during each phase of the study. Subjects reported to the test area at 7:00–8:00 AM daily on each of 3 control days, 5 treatment days, and 3 post-treatment observation days. After 30 min of supine recumbency in a quiet room (temperature 23–24°C), BP and pulse rate were measured, then repeated after 5 min standing. All BP measurements were performed on the same arm, using a Hawksley random-zero sphygmomanometer (Hawksley and Sons Ltd., Lancing, England) by the same observer. The mean of three measures of pulse and BP was recorded in each position. A right antecubital vein was cannulated every second day with subjects recumbent and blood was collected for measurement of electrolytes, urea and creatinine, albumin, glucose, calcium, phosphate, hematocrit, hemoglobin, leukocyte count, platelet count (routine automated methods), and cortisol concentration (Amerlex Cortisol RIA, Amersham, United Kingdom). Twenty-four-hour urine collections were performed on control days 2 and 3 and treatment days 4 and 5 for measurement of electrolyte excretion and creatinine clearance.

**Forearm Blood Flow Measurements After 2 and 5 Days of Treatment** This technique has been described in detail elsewhere.\(^16,17\) Briefly, subjects lay supine with their arms inclined at 30° to improve venous drainage. Wrist cuffs were applied and, during the recording period, were inflated to 200 to 220 mm Hg to exclude hand circulation from the measurements. Upper arm congesting cuffs, at the midhumerus level, were alternately inflated to 40 mm Hg and deflated during measurement. Multiple measures were taken over 3-minute periods and the slopes of the final five recordings averaged to determine the forearm blood flow. Forearm blood flow was derived as the ratio of flow in the infused arm to that of the control arm, expressed as the percentage change from baseline. This method allows for correction for nonspecific changes that arise during and between studies, and has been reviewed elsewhere.\(^16\)

Blood pres-
Hemodynamic and Metabolic Effects of 5 Days of Cortisol Treatment

There was no change in supine or erect systolic or diastolic BP with placebo treatment (Fig. 1). Cortisol treatment increased supine systolic (P < .05) and erect systolic (P < .01) BP with no effect on diastolic BP in either position. There was no change in heart rate with either treatment.

Results

Statistical Analysis

Data are reported as mean ± SEM. Distribution of data was assessed by the Lilliefors’s test. Change in BP from the pooled control value (PC, mean of control days 1–3) over the treatment period was tested by repeated-measures analysis of variance, with significance accepted as P < .05, followed by Student t test when appropriate. Changes in metabolic parameters were tested by repeated-measures analysis of variance, where more than two measures were taken, or Student t test where there were two measurements. To detect differences in baseline forearm blood flow between treatment groups, we compared the baseline forearm blood flow in each limb between placebo and cortisol treatments by Student t test. To detect differences in forearm blood flow responses to vasodilators we compared the area under the curve (calculated by the trapezoidal rule) of the dose-response curve to each drug by Student t test.18

FIG. 1. Supine and erect systolic blood pressure in subjects (n = 8) treated with placebo (solid circles) and cortisol 80 mg/day (solid triangles). Hatched area indicates treatment period. PC = pooled control. *P < .05, **P < .01 for comparison of treatment day 5 v PC. SBP = systolic blood pressure.

Effect of 5 Days of Cortisol on Hemodynamic and Metabolic Parameters

The plasma cortisol concentration increased by cortisol treatment (387 ± 30 to 849 ± 54 nmol/L, P < .001, control to treatment day 5) and was unaffected by placebo (431 ± 17 to 390 ± 38 nmol/L). Cortisol treatment increased body weight (70.4 ± 2.8 kg, P < .001), white cell count (5.1 ± 0.3 to 7.7 ± 0.7 × 10⁹/L, P < .01), absolute neutrophil count (2.7 ± 0.3 to 5.4 ± 0.6 × 10⁹/L, P < .01), platelet count (220 ± 11 to 244 ± 10 × 10⁹/L, P < .01), and serum glucose (5.9 ± 0.5 to 6.8 ± 0.6 mmol/L, P = .05) and reduced eosinophil count (0.16 ± 0.04 to 0.08 ± 0.03 × 10⁹/L, P < .01), as previously reported.19 Placebo had no effect on these parameters. There were no changes in urinary sodium and potassium excretion, or serum sodium, potassium, albumin, cholesterol or triglyceride concentrations with either treatment.

Forearm Blood Flow After 2 and 5 Days of Cortisol

Baseline forearm blood flow was not affected by cortisol or by LNMMA at 2 or 5 days (Tables 1, and 2). Cholinergic vasodilatation (Fig. 2a and b) was impaired after cortisol administration, reaching statistical significance at 5 days. Cortisol did not affect responses to SNP. N⁶-monomethyl-L-arginine inhibited cholinergic vasodilatation in placebo-treated groups,
DISCUSSION

These studies show that cortisol inhibited cholinergic vasodilatation in the human forearm circulation. This was only a trend after 2 days of cortisol administration but was more apparent after 5 days of cortisol administration. The effect of cortisol on cholinergic dilatation involves inhibition of NO synthesis (NOS) rather than sensitivity to NO, as cortisol abolished the incremental effect of LNMMA both at 2 and 5 days and responses to SNP were not different.

Cholinergic dilatation is mediated, at least in part, by endothelial NOS. In vitro, glucocorticoids do not influence endothelial NOS expression, although they do inhibit inducible NOS. We infer that the effect of cortisol on cholinergic dilatation may be dependent on inhibited endothelial NOS; however, indirect mechanisms (eg, altered prostanoid synthesis) cannot be excluded. Prostanoids do not contribute directly to forearm cholinergic dilatation in health, however, there is evidence for interactions between prostacyclin and NOS activity in vitro and in other vascular beds.

Glucocorticoids are known to inhibit prostacyclin production, however, we have previously demonstrated that coadministration of indomethacin does not alter pressor responsiveness in cortisol subjects. Alternatively, the effect on cholinergic dilatation may be an indirect effect of altered sensitivity to norepinephrine or angiotensin II, both of which are affected by glucocorticoids and have actions on endothelium as well as vascular smooth muscle. Finally, glucocorticoids may reduce endothelial NO production by mechanisms other than NOS inhibition. Glucocorticoids have been demonstrated to inhibit synthesis of tetrahydrobiopterin, which is a necessary cofactor for maximal activity of all NOS isoforms.

NG-

\[ \text{monomethyl-L-arginine} \]

has previously been shown to reduce basal forearm blood flow, in doses similar to that used in our study. The failure of LNMMA to reduce basal forearm blood flow in the present study was probably a consequence of the brief duration of infusion of the drug before blood flow measurements were made (we measured basal forearm blood flow during the final 3 min of a 6-min infusion of LNMMA). Although there was a trend for vasodilatation to SNP to be reduced by LNMMA at 2 days, this nonsignificant effect was not altered by cortisol and may be explained by a coincidental fall in blood flow in the later part of the Edinburgh study (Table 1).

These changes in vascular responsiveness occurred in the context of a small but significant increase in BP during 5 days of cortisol treatment, accompanied by glucocorticoid (hyperglycemia, leukocytosis, and eosinopenia) and mineralocorticoid (increased body weight) effects consistent with those we have previously documented. Placebo treatment did not change BP, plasma cortisol concentrations, or other metabolic parameters. The effect of cortisol on cholinergic vasodilatation was more obvious at day 5 than at day 2, suggesting that there is progressive impairment in endothelial function after BP has started to rise.

### TABLE 1. TWO-DAY STUDY: FOREARM BLOOD FLOW PRE- AND POST-LNMMA (n = 6)

<table>
<thead>
<tr>
<th></th>
<th>Basal BP (mm Hg)</th>
<th>Basal FBF (mL/100 mL/min)</th>
<th>LNMMA FBF (mL/100 mL/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo, 2-day</td>
<td>134/66</td>
<td>Infused 3.38 ± 0.51</td>
<td>3.16 ± 0.23</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>2.98 ± 0.53</td>
<td>4.05 ± 0.45</td>
</tr>
<tr>
<td>Cortisol, 2-day</td>
<td>125/70</td>
<td>Infused 4.14 ± 1.02</td>
<td>2.79 ± 0.92</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>3.80 ± 0.83</td>
<td>2.87 ± 1.2</td>
</tr>
</tbody>
</table>

**BP** = blood pressure; **LNMMA** = NG-

\[ \text{monomethyl-L-arginine} \]; **FBF** = forearm blood flow.

### TABLE 2. FIVE-DAY STUDY: FOREARM BLOOD FLOW PRE- AND POST-LNMMA (n = 8) *

<table>
<thead>
<tr>
<th></th>
<th>Basal BP (mm Hg)</th>
<th>Basal FBF (mL/100 mL/min)</th>
<th>LNMMA FBF (mL/100 mL/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo, 5-day</td>
<td>127/55</td>
<td>Infused 4.25 ± 0.53</td>
<td>4.85 ± 0.63</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>4.11 ± 0.5</td>
<td>4.23 ± 0.6</td>
</tr>
<tr>
<td>Cortisol, 5-day</td>
<td>131/55</td>
<td>Infused 4.05 ± 0.39</td>
<td>4.99 ± 0.82</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>4.06 ± 0.41</td>
<td>4.23 ± 0.29</td>
</tr>
</tbody>
</table>

* Blood pressure was recorded by oscillometric method as described. There was no difference in basal FBF between cortisol and placebo treatments. In this study, LNMMA did not produce significant vasoconstriction from baseline FBF after either 2 or 5 days of either treatment.
studies on day 2 and day 5 were not strictly comparable, however, because there were differences in statistical power (n = 6 vs n = 8) and subject characteristics (ie, the 2-day study included smokers, who, on the evidence of impaired cholinergic dilatation in Fig. 2, had relative endothelial dysfunction). Endothelial dysfunction may complicate hypertension, though this effect has not been universally reported. It is possible that the observed endothelial dysfunction was secondary to hypertension. However, the rise in BP was small (≈6 mm Hg systolic) and occurred over days, in contrast to patients with essential hypertension in whom BP elevation is usually greater and in whom structural vascular wall changes occur over a prolonged period. The increase in serum glucose during cortisol treatment may have contributed to endothelial dysfunction, as acute hyperglycemia has been shown to impair endothelial-dependent vasodilation in healthy humans.

These data support the hypothesis that impaired endothelium-dependent dilatation is important in glucocorticoid-induced hypertension, as suggested by our recent human and animal observations. Plasma nitrate/nitrite concentrations are reduced in cortisol-treated human males. In the Sprague Dawley rat, l-arginine supplementation prevents ACTH-induced hypertension. No matter whether the effect of cortisol is direct or indirect, these data also raise the possibility that enhanced vascular action of glucocorticoids may contribute to endothelial dysfunction and to the pathophysiology of essential hypertension.

ACKNOWLEDGMENTS

Dr. B.R. Walker is a British Heart Foundation Senior Research Fellow. Pathology services were provided by SEALS courtesy of the Division of Medicine, St. George Hospital.

REFERENCES

