Inhibition of Cortisol Production With Metyrapone Prevents Mental Stress-Induced Endothelial Dysfunction and Baroreflex Impairment

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OBJECTIVES

This study was designed to investigate the role of cortisol in stress-induced endothelial dysfunction and impaired baroreflex sensitivity (BRS) by blocking cortisol production with metyrapone before subjecting healthy volunteers to mental stress.

BACKGROUND

Mental stress raises cortisol levels and is associated with increased coronary heart disease (CHD) morbidity and mortality, especially from sudden cardiac death. It also causes endothelial dysfunction and impaired BRS.

METHODS

We measured brachial artery flow-mediated dilation (FMD), a measure of endothelial function, and BRS in 36 subjects without CHD risk factors who were then randomized in a double-blind fashion to oral metyrapone 750 mg × 2 or placebo. Five hours later we subjected subjects to mental stress and then remeasured endothelial function and BRS.

RESULTS

Prestress cortisol levels were significantly higher in the placebo group at 270.5 (30.9) nmol/l versus 89.1 (11.8) nmol/l (p = 0.01), and the increase with stress was higher at 57.9 (17.9) nmol/l versus 11.2 (2.2) nmol/l (p < 0.001). In the placebo group, compared to baseline, FMD and BRS fell significantly from 4.5% (0.7%) to 1.4% (1.1%) (p = 0.02) and 21.4 (2.3) ms/mm Hg to 16.3 (1.5) ms/mm Hg (p = 0.04), respectively. In the metyrapone group, FMD and BRS were unchanged from baseline: 4.3% (0.9%) versus 5.1% (0.8%) (p = 0.48) and 26.4 (2.9) ms/mm Hg versus 24.9 (2.6) ms/mm Hg (p = 0.62), respectively. Analysis of covariation showed a significant effect of metyrapone on change in both FMD (p = 0.009) and BRS (p = 0.024).

CONCLUSIONS

Stress-related endothelial dysfunction and BRS impairment can be prevented by blocking cortisol production with metyrapone, demonstrating a direct or facilitative role for cortisol in these phenomena and suggesting mechanisms by which stress contributes to CHD and sudden cardiac death. (J Am Coll Cardiol 2005;46:344–50) © 2005 by the American College of Cardiology Foundation

Conversion of 11-deoxycortisol to cortisol by adrenal 11-beta-hydroxylase.

METHODS

Subjects. We conducted a randomized, double-blind placebo-controlled trial. Ethical permission was obtained from our local research ethics committee. Thirty-six healthy nonsmoking subjects gave informed consent and participated. Exclusion criteria included a history of heart disease or any traditional risk factor for coronary heart disease (CHD) (smoking within the past 10 years, hypertension, diabetes, or hypercholesterolemia). Other exclusion criteria were a body mass index >30; current pregnancy or menopause; or any other condition, drug treatment, or dietary supplements known or likely to affect the measured variables. Subjects were ages 18 to 55 years.

Baroreflex sensitivity. Subjects were initially studied in the morning beginning at 8:30 AM, fasting and having avoided alcohol for at least 24 h and caffeine for at least 12 h. On arrival their height and weight were measured, their cardiovascular systems were examined, and a 12-lead electrocar-
diagram (ECG) was performed. After attachment to an ECG monitor and fitting of a Portapres noninvasive continuous blood pressure monitor (TNO Biomedical Instrumentation, Affilgem, Belgium), subjects rested supine in a quiet, temperature-controlled environment for 20 min. For the next 10 min heart rate and blood pressure were recorded. Analog output from the ECG monitor and the Portapres were fed into an analog-to-digital converter (Biopac, Biopac Systems Inc., Goleta, California), and output from the converter was fed into a personal computer and recorded at 500 Hz using AcqKnowledge software (Biopac Systems Inc.) for later calculation of BRS by the “spontaneous sequence” method (7–9). Briefly, each systolic blood pressure measurement was matched with the following RR interval. A proprietary program was then used to seek “sequences” in which both systolic pressure and RR interval increased or decreased together for three or more beats. Minimum increments were 1 mm Hg and 2 ms. A second program selected those sequences in which systolic blood pressure and RR interval had a correlation coefficient of 0.85 or higher. The mean gradient of these sequences, expressed in ms/mm Hg, was taken as BRS.

**Endothelial function.** Flow-mediated dilation (FMD) of the brachial artery was then measured using a technique previously validated in our department (10). Briefly, high-resolution ultrasound (7.5 MHz) was used to visualize the brachial artery above its bifurcation at the elbow. An M-mode of the ultrasound image was fed to a wall tracking system (Vadirec 101, Medical Systems Arnhem, Oosterbrek, the Netherlands), which is able to determine the diastolic diameter of the artery to within 10 μm. A wrist cuff was inflated to and maintained at between 250 and 300 mm Hg for 5 min and then released, causing reactive hand hyperaemia, which in turn led to increased flow through the observed segment of brachial artery. Increased flow through an artery increases the shear stress at the blood-endothelium interface and stimulates healthy endothelial cells to produce the vasodilator nitric oxide (NO). Some of the NO produced diffuses into the vascular smooth-muscle layer adjacent to the endothelium, inducing relaxation and vessel dilation. The dilation of the brachial artery in response to increased flow (the FMD) was measured at 1-min intervals for the next 8 min. The greatest change from baseline within the first 3 min was taken to be FMD and was expressed as percent of baseline diameter. Once the artery had returned to baseline diameter, 1 ml of glyceryl trinitrate (GTN) solution containing 50 μg of GTN was administered sublingually, and brachial artery dilation was measured 3 min later as a measure of glyceryl trinitrate-mediated endothelium-independent dilation (GTNMD). In our laboratory the coefficient of variation for repeated measures of FMD is 7%.

**Blood sampling.** With the subjects still supine, blood was taken for urea and electrolytes, full blood count, glucose, triglycerides, cholesterol (total, high-density, and low-density) and cortisol, all of which were analyzed in our hospital’s main laboratory. Further samples were immediately placed in a centrifuge that had been prechilled to 4°C and spun at 3,000 rpm for 10 min. Plasma was then pipetted off and stored at −70°C for later measurement of catecholamines. After venesection, the subjects were allowed to sit up and were given a standard low-fat snack and something to drink.

**Placebo/metyrapone.** Before their visit, subjects were randomized to one of two groups: the metyrapone group was given metyrapone 750 mg with their snack and a further 750 mg dose to be taken with their midday meal 3.5 h later. The control group was given placebos. We chose this dosing schedule because earlier work has shown that this regime produces a significant reduction in plasma cortisol levels at 6 h and is well tolerated (11). Subjects were then allowed to leave the laboratory and were asked to return 5 h later. They were asked to avoid heavy physical work or exercise in the interval between their visits. They were also asked to continue abstaining from alcohol and caffeinated drinks and to avoid fatty foods.

**Return visit.** Subjects returned 5 h after taking the first dose of metyrapone or placebo. They lay down and were connected to the ECG and Portapres as before. A 23-G cannula was sited in the right antecubital fossa for later blood sampling and flushed with saline. After 20 min of supine rest, as before, blood samples were taken from the cannula for catecholamine and cortisol levels. Subjects were then asked if they would be willing to undertake a simulated public speaking task designed to place them under a degree of mental stress before repeating the morning’s measurements. All subjects agreed and were then played taped instructions asking them to imagine that they had been falsely accused of a crime and were defending themselves to the police. These instructions took 3 min and were followed by 2 min of “thinking time” and 3 min of talking. We chose this test because it has previously been shown to produce endothelial dysfunction in the brachial artery (12).

Blood pressure and heart rate were recorded continuously by ECG and Portapres throughout the stress test and for 10 min afterwards, and poststress BRS was calculated from the final 10 min of this recording. Once this recording was complete, blood samples were again taken from the cannula for cortisol and catecholamines. Finally, FMD of the
brachial artery was measured again. On completion of the second FMD study, subjects graded the intensity of mental stress induced by the test on a 10-point scale ranging from “no stress at all” to “very severe mental stress.”

In vitro studies. Metyrapone has been reported to have generally, but not exclusively, inhibitory effects on several P-450 enzyme systems, and endothelial nitric oxide synthase (eNOS) is a P-450 enzyme. In order to exclude a direct effect of metyrapone of NO production independent of any effect of cortisol lowering, we undertook an in vitro study with cultured human umbilical vein endothelial cells (HUVECs). Human umbilical vein endothelial cells were isolated from umbilical cords as previously described (13) and cultured in medium 199 (Gibco Invitrogen Compounds, Paisley, United Kingdom), supplemented with 20% fetal calf serum, 10 ng/ml epithelial growth factor, 35 μg/ml gentamycin, 1 μg/ml hydrocortisone (all from Sigma-Aldrich, Poole, United Kingdom) and 2.5 μg/ml amphotericin B (Gibco Invitrogen Compounds). Confluent primary HUVECs were dissociated using trypsin/EDTA (Sigma), seeded into six-well culture plates (Falcon; Becton Dickinson Labware, New Jersey) and grown until confluent (∼3 days). The release of NO in response to 100 μM acetylcholine (ACh) was measured using an NO-sensitive electrode with a 2-mm diameter tip (ISO-NOP, WPI, Florida) connected to a NO meter (ISO-NO Mark II, WPI, Berlin, Germany). The device was calibrated on each experimental day by chemical generation of NO (14) and the response to ACh was expressed as the change in NO output from the baseline level 1 min before to the level reached in the 10th minute after ACh administration. Human umbilical vein endothelial cells were tested before and 180 min after the administration of metyrapone (5 μg/ml) and at the same times in parallel untreated control patients and in cultures treated with the vehicle for metyrapone (0.5% methanol).

Statistics. Group results are expressed as mean ± SEM. Repeated measures within individuals were examined by Student paired t test; comparison of means between groups was by Student nonpaired t test. Repeated measures analysis of variation adjusted for baseline values was used to examine the effect of metyrapone upon changes in measured parameters. A p value of <0.05 was considered significant.

RESULTS

Baseline. Baseline demographic, hemodynamic, and hematologic characteristics did not differ significantly between placebo (n = 17) and metyrapone groups, although systolic blood pressure was nonsignificantly higher in the placebo group at baseline (Table 1). There was no difference between placebo and metyrapone groups for FMD, GTNMD, or BRS.

Effect of metyrapone. CORTISOL. Afternoon prestress cortisol was significantly higher in the placebo group than in the metyrapone group (270.5 [30.9] nmol/l vs. 89.1 [11.8] nmol/l, p = 0.01). As expected, the increment in cortisol response with stress was significantly greater in the placebo group (57.9 [17.9] nmol/l vs. 11.2 [2.2] nmol/l, p < 0.001).

BLOOD PRESSURE AND HEART RATE RESPONSE TO STRESS. Afternoon prestress systolic and diastolic blood pressure and heart rate did not differ significantly between groups. The blood pressure response to stress was slightly (but not significantly) blunted in the metyrapone group, but at no point was the difference in systolic or diastolic blood pressure between the groups significant (Fig. 1A). Within minutes of the end of the stress test, blood pressure had stabilized in both groups and remained stable until the end of the 10-min BRS recording, albeit at a slightly higher level than before the test (Fig. 1A). In the placebo group, poststress systolic and diastolic blood pressures were slightly but significantly higher than prestress values (systolic: 121 [4.6] mm Hg vs. 127 [3.7] mm Hg, p = 0.001; diastolic: 58 [2.2] mm Hg vs. 61 [2.2] mm Hg, p = 0.001). In the metyrapone group there was no significant difference between pre- and poststress blood pressure values. Heart rate trended to be lower prestress in the metyrapone group, and the stress heart rate (at 6 min) was significantly lower in the metyrapone group (76 [3.2] beats/min vs. 89 [4.3] beats/min, p = 0.03) (Fig. 1B). Poststress heart rates also trended to be lower in the metyrapone group, but this difference was not significant.

FMD AND GTNMD. Brachial artery diameter did not differ following mental stress in the metyrapone versus placebo groups (3.03 [0.13] mm vs. 2.99 [0.13] mm, respectively, p = NS). Poststress FMD was lower than baseline FMD in the placebo group (p = 0.019) but was similar to baseline in the metyrapone group (p = 0.49). Analysis of variation using baseline FMD as covariate showed a significant effect of metyrapone on poststress FMD (p = 0.009). In contrast, GTNMD was not significantly different following stress compared with baseline in either group.

BRS. As shown in Table 2, poststress BRS was lower than baseline BRS in the placebo group (p = 0.042) but not in
the metyrapone group (p = 0.620). Analysis of variance using baseline BRS as covariate showed a significant effect of metyrapone on poststress BRS (p = 0.024).

**Subjective stress.** There was no difference in subjective assessment of stress between groups (placebo 4.9 of 10 vs. 4.6 of 10, p = 0.56).

**Other effects.** There was no difference in the catecholamine response to stress between groups (data not shown). Before beginning the study, subjects were asked to report any side effects after taking each tablet. Four subjects reported minor lightheadedness of short duration about 20 min after taking the first tablets. Of these, two had taken

### Table 2. Effect of Metyrapone on FMD, GTNMD, and BRS

<table>
<thead>
<tr>
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<th>Baseline vs. Post-Stress</th>
<th>Student's Paired t Test</th>
<th>Effect of Metyrapone ANCOVA (Covariate: Baseline)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMD (%)</td>
<td>4.5 [0.71] vs. 1.4 [1.11]</td>
<td>4.3 [0.93] vs. 5.1 [0.79]</td>
<td>p = 0.009</td>
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<tr>
<td></td>
<td>p = 0.019</td>
<td>p = 0.49</td>
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<tr>
<td>GTNMD (%)</td>
<td>10.7 [1.33] vs. 10.1 [1.73]</td>
<td>12.9 [2.14] vs. 11.6 [1.77]</td>
<td>p = 0.990</td>
</tr>
<tr>
<td></td>
<td>p = 0.89</td>
<td>p = 0.35</td>
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<tr>
<td>BRS (ms/mm Hg)</td>
<td>21.4 [2.25] vs.16.3 [1.48]</td>
<td>26.4 [2.91] vs. 24.9 [2.60]</td>
<td>p = 0.024</td>
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<tr>
<td></td>
<td>p = 0.042</td>
<td>p = 0.620</td>
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ANCOVA = analysis of covariance; other abbreviations as in Table 1.
placebo. One subject, who had taken metyrapone, reported similar lightheadedness after both doses of tablets. There were no other reported effects.

**In vitro studies.** Metyrapone did not alter nitric oxide release from cultured endothelial cells. Acetylcholine-evoked release of NO from HUVECs (45.7 [7.1] nM) was not significantly changed 180 min after metyrapone administration (42.4 [7.3] nM; mean ± SEM from 6 paired experiments). In addition, in matched untreated time controls, the NO response to ACh (38.9 [2.2] nM) was unchanged after 180 min (41.2 [1.8] nM; n = 6). Finally, the vehicle for metyrapone, 0.5% methanol, had no effect on the NO release evoked by ACh after 180 min (n = 2; data not shown).

**DISCUSSION**

The principal findings of this study are that pretreatment with metyrapone, an inhibitor of cortisol synthesis, reduced prestress plasma cortisol, blunted the cortisol response to mental stress, and prevented mental stress-induced endothelial dysfunction and impairment of BRS.

**Cortisol and endothelial function.** Endothelial dysfunction in response to mental stress was first shown by Ghiadoni et al. (15) using a similar FMD technique to our group and the same mental stress test. In their healthy volunteers, FMD fell significantly from a mean of 5.0% [2.1%] to 2.8% [2.3%] at the 30-min poststress time point, which is approximately the same time point at which we remeasured FMD. In their study FMD was still impaired 90 min after the stress test but had returned to normal after 240 min. This relatively prolonged time course, which contrasts with the rapid return of heart rate and blood pressure to prestress values, may imply mediation by humoral factors.

More recently, Spieker et al. (16) showed that mental stress impaired radial artery endothelial function for at least 45 min. In their study, infusion of an endothelin (ET) A receptor antagonist prevented endothelial dysfunction. Their control group showed a reduction in radial artery FMD from 8.0% [1.1%] to 4.1% [1.0%] at the 10-min time point. In the group in whom receptor blocker was infused before, during, and after the stress test, baseline FMD was 8.6% [1.3%] beforehand and nonsignificantly increased at 9.4% [1.4%] afterwards.

The findings that suppression of cortisol production with metyrapone prevented mental stress-induced endothelial dysfunction are actually consistent with the observation that ET receptor blockade also blocks it (16). Recent evidence suggests that the hypothalamic-pituitary-adrenal axis and ET system interact closely in their effects on the vasculature in health and disease. Endothelin-1 is produced by vascular endothelial and smooth muscle cells and is a potent vasoconstrictor that antagonizes the vasodilator effects of NO (17). Glucocorticoids stimulate the production and release of ET by vascular smooth muscle cells in cell culture and in animals and seem to exercise this effect primarily by promoting transcription of prepro-ET mRNA (18,19), which is probably the major regulatory step in the production of ET (20). In addition, in cultured human endothelial cells, inhibition of 11-beta-hydroxysteroid dehydrogenase, which in endothelial cells probably acts to convert cortisol into inactive cortisone, led to increased levels of ET, but only in the presence of corticosterone (a cortisol analog) (21). This has been interpreted as evidence that the activation of the vascular ET system is dependent on the presence of glucocorticoids. If that is so, then, given the marked reduction in cortisol levels in our metyrapone group to approximately 30% that of the control group over the 5 h preceding the mental stress test, our metyrapone group may have been unable to produce ET-1 in response to mental stress in the normal manner owing to lack of the facilitating presence of normal levels of glucocorticoids in the preceding hours.

Another emerging strand of evidence links cortisol and the NO system, the key factor involved in the phenomenon of FMD. Glucocorticoids are known to inhibit the NO synthase (NOS) isoforms nNOS and eNOS (22,23), both directly by decreasing their production (24) and indirectly by reducing production of the essential eNOS cofactor tetrahydrobiopterin (25), thereby impairing “endothelial function” in cell and tissue models (26,27).

As the NO and ET systems are functionally antagonistic in their effects on ET function, and as glucocorticoids have been shown to inhibit the NO and induce ET, it is hardly surprising that cortisol impairs endothelial function in humans. Our data are strong evidence that cortisol mediates mental stress-induced impairment of endothelial function in humans.

**Cortisol and BRS.** Reduced BRS is an important independent predictor of mortality in patients post-myocardial infarction, and is particularly associated with sudden cardiac death (28). In these circumstances it is likely that reduced BRS both promotes and reflect sympatoexcitation and a predisposition to ventricular arrhythmias (29). Acute mental stress also impairs BRS. Thus, it is possible that a reduction in BRS and an associated reduction in arrhythmia threshold may partly explain the link between acute mental stress and cardiac events, especially SCD. Intuitively, this would apply to those with previously clinically silent CHD as well as to those with known disease, and may be the mechanism underlying some cases of SCD in those with no previously known heart disease. A direct role for cortisol in mental stress-induced impairment of BRS has not previously been described in humans. Animal studies support the theory that glucocorticoids may act centrally to modify BRS, independent of any effect on blood pressure, and show that such actions are of rapid onset, being detectable within hours (30–32). The administration of cortisol to normal subjects, though it raises serum cortisol and produces hypertension after several days, has not consistently been shown to reduce BRS (33), and this suggests that in humans
any negative influence of cortisol on BRS during the stress response may be acute and somewhat situation specific.

Our finding that suppression of cortisol production with metyrapone protects against the mental stress-induced impairment of BRS, in the absence any significant hemodynamic effect, supports an acute role for cortisol in this process.

Study limitations. It is a necessary limitation of our study that FMD was not measured immediately before the mental stress test in order to assess the effect on FMD of metyrapone alone. We chose not to do this in order to keep the second visit protocol as close as possible to that of the baseline visit, and in particular to standardize the length of time for which volunteers lay supine, an important issue when examining the impact of stress on FMD. We considered the possibility that metyrapone may have a direct beneficial effect on endothelial function that is independent of its cortisol-lowering action. In vitro, metyrapone appears to have generally inhibitory, but in some cases stimulatory, effects on some P-450 enzymes. For example, it was shown to have direct inhibitory effect on endothelium derived hyperpolarizing factor-mediated vasorelaxation via its inhibitory action on the cytochrome P-450 enzyme system (34). Because eNOS is a P-450 enzyme, we were concerned to exclude a direct stimulatory effect of metyrapone on NO production via direct effects on eNOS gene expression or enzyme activity. Our in vitro studies in HUVECs make this very unlikely because metyrapone had no evident effect on the basal release of NO (data not shown) or, more importantly, on acetylcholine-stimulated NO release.

The long interval between tests introduces an element of diurnal variation but was dictated by the pharmacodynamics of metyrapone and could not be avoided. Diurnal variation in FMD/endothelial function has been described previously (35,36), with lower values in the morning than the evening, in FMD/endothelial function has been described previously of metyrapone and could not be avoided. Diurnal variation but was dictated by the pharmacodynamics of metyrapone and was not measured immediately before the mental stress test in order to assess the effect on FMD of metyrapone alone. We chose not to do this in order to keep the second visit protocol as close as possible to that of the baseline visit, and in particular to standardize the length of time for which volunteers lay supine, an important issue when examining the impact of stress on FMD. We considered the possibility that metyrapone may have a direct beneficial effect on endothelial function that is independent of its cortisol-lowering action. In vitro, metyrapone appears to have generally inhibitory, but in some cases stimulatory, effects on some P-450 enzymes. For example, it was shown to have direct inhibitory effect on endothelium derived hyperpolarizing factor-mediated vasorelaxation via its inhibitory action on the cytochrome P-450 enzyme system (34). Because eNOS is a P-450 enzyme, we were concerned to exclude a direct stimulatory effect of metyrapone on NO production via direct effects on eNOS gene expression or enzyme activity. Our in vitro studies in HUVECs make this very unlikely because metyrapone had no evident effect on the basal release of NO (data not shown) or, more importantly, on acetylcholine-stimulated NO release.

The long interval between tests introduces an element of diurnal variation but was dictated by the pharmacodynamics of metyrapone and could not be avoided. Diurnal variation in FMD/endothelial function has been described previously (35,36), with lower values in the morning than the evening, in both a group with variant angina and in a control group. This pattern mirrors the normal diurnal variation in cortisol levels, which, in view of our results, may suggest an effect of “ordinary” stress/cortisol levels on FMD even without added laboratory mental stress. However, even if underlying FMD had improved over the course of the day, it was still markedly reduced following stress in the placebo group compared with the morning baseline, but not in the metyrapone group.

In addition to its inhibitory effect on 11-beta-hydroxylase, metyrapone also inhibits the enzyme 11-beta-HSD type I, which catalyzes the peripheral conversion of inactive cortisone to active cortisol. Accordingly, it is possible that the impact of metyrapone on tissue cortisol levels may have been greater than that on plasma cortisol levels. However, this does not weaken our conclusion that cortisol plays a role in the endothelial dysfunction induced by mental stress.

Sympathetic activation has previously been shown to impair brachial artery FMD (37). Although our data suggest that cortisol plays an important role in the FMD response to stress, we cannot exclude an additional effect of sympathetic activation. Indeed, our observations of a blunting of the mental stress-induced impairment of BRS by metyrapone suggest that there is also an interaction between cortisol and baroreflex function. Finally, we did not measure brachial artery flow. We cannot exclude an increase in the reactive hyperemia flow stimulus in the metyrapone group versus placebo group, but this is highly unlikely. Even if this were so, the most likely explanation would be greater microvascular NO activity.

Summary. We present data that suggest that cortisol may mediate mental stress-induced endothelial dysfunction and impairment of BRS. A deleterious direct effect of cortisol on endothelial function and BRS may contribute to the link between mental stress and acute cardiac events.

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