Glucocorticoids, Sympathetic Activity, and Presynaptic $\alpha_2$-Adrenoceptor Function in Humans

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ABSTRACT

The sympathetic nervous system and the pituitary-adrenocortical system are two of the body's main stress effector systems. Animal studies have indicated that exogenously administered glucocorticoids inhibit sympathetic outflows and interfere with the function of presynaptic $\alpha_2$-adrenoceptors modulating neuronal norepinephrine (NE) release. The present study tested whether glucocorticoids produce similar effects in humans. In a randomized, double-blind, placebo-controlled cross-over experiment, 15 healthy subjects took 20 mg prednisone or placebo orally daily each morning for 1 week, followed by the other drug after a 1-week washout. On the last day of each treatment week, blood samples were drawn for assays of plasma levels of catechol and ACTH before and after iv infusion of the $\alpha_2$-adrenoceptor antagonist yohimbine (YOH) (0.125 mg/kg bolus, 0.001 mg/kg min$^{-1}$ infusion). In 7 subjects, directly recorded peroneal skeletal muscle sympathetic nerve activity (MSNA) was also measured at baseline and after YOH infusion at the end of both treatment weeks. Prednisone decreased plasma NE levels and MSNA compared with levels after placebo (1.09 ± 0.11 nmol/L vs. 1.40 ± 0.13 nmol/L, P < 0.01; 30 ± 4 bursts/min vs. 36 ± 3 bursts/min, P < 0.05) without affecting blood pressure or pulse rate. YOH increased mean arterial blood pressure by 12% (P < 0.001) and heart rate by 7% (P < 0.05); prednisone did not alter these effects of YOH. YOH-induced proportionate increments in plasma NE levels averaged about 10 times those in MSNA. Prednisone did not affect the YOH-induced proportionate increments in plasma NE levels (placebo, 243%; prednisone, 237%) or MSNA (placebo, 22%; prednisone, 23%). The decrements in MSNA and plasma NE levels after prednisone treatment indicate that glucocorticoids inhibit sympathoneural outflows in humans. The 10-fold larger NE than MSNA response to YOH confirms substantial inhibitory modulation of NE release by $\alpha_2$-adrenoceptors on noradrenergic terminals, and the similarity of responses to YOH after prednisone or placebo suggests that glucocorticoid-induced sympathoinhibition occurs independently of altered modulatory function of $\alpha_2$-adrenoceptors on noradrenergic terminals. (J Clin Endocrinol Metab 80: 1804–1808, 1995)
blind, placebo-controlled, randomized cross-over study of healthy adult volunteers. The study was designed to address whether glucocorticoid administration affects plasma NE and MSNA levels at baseline and in response to α2-adrenoceptor blockade and if so whether the extent of attenuation of the MSNA and plasma NE responses are proportionately similar. If the extent of attenuation of the plasma NE response to YOH were the same as the extent of attenuation of the MSNA response to YOH, this would suggest that glucocorticoids interfere with YOH-induced increases in sympathetic nerve traffic, whereas if the extent of attenuation of the plasma NE response to YOH were larger than the extent of attenuation of the MSNA response to YOH, this would suggest that glucocorticoids interfere with YOH-induced NE release from sympathetic terminals for a given amount of nerve traffic. Thus, the study design enabled separate examination of effects of glucocorticoids on α2-adrenoceptor restraint of central sympathetic outflow and on NE release from sympathetic terminals in the periphery.

Subjects and Methods

Subjects

Fifteen healthy volunteers (12 men and 3 women; mean age 33 yr, range 21–54 yr) participated in the study after giving written informed consent. All subjects had a normal medical history, physical examination, screening laboratory tests of blood and urine, and electrocardiogram. The participants refrained from smoking and from drinking alcohol or caffeinated beverages for 24 h before each YOH challenge test. The study protocol was approved by the Intramural Research Board of the National Institute of Neurological Disorders and Stroke.

Study design

All subjects were studied twice, after 20 mg prednisone per day for 7 days and after placebo for 7 days. The sequence of the double-blind treatments was randomized. Between the two treatments there was a washout period of 7 days. On the last day of each treatment, a YOH challenge test was performed. The subjects were advised to take the last dose 1 to 2 h before the YOH infusion. The chosen dose of prednisone is known to suppress hypothalamo-pituitary-adrenocortical activity (17).

Setup

The experiments were conducted in a quiet room with constant temperature (~24°C) and with the subjects in the supine position. Blood pressure was measured by an automated cuff device (Critikon, Tampa, FL). Heart rate was recorded continuously by electrocardiogram. An iv catheter was inserted in each arm, one iv for infusing YOH and one for sampling blood.

Microneurography

In seven subjects, multifiber recordings of MSNA were obtained successfully from a muscle fascicle of the peroneal nerve at the head of the fibula after each drug treatment. The course of the nerve was mapped by transcutaneous electrical stimulation (40–60 volts, 0.2 millisecond, 1 hertz) with a pencil-shaped electrode. A steel, tungsten wire micro-electrode was then inserted in the region of the nerve. A similar reference electrode was inserted at 1–3 cm from the recording electrode. Weak electrical stimuli (2–4 volts, 0.2 millisecond, 1 hertz) were delivered to the recording electrode by a stimulator connected to an isolation unit. The elicitation of involuntary twitches in the foot indicated that the electrode tip was located within or was close to the muscle nerve fascicle. The electrode was then moved until there was acceptable recording from sympathetic nerve fibers. Recordings of MSNA were accepted if tapping or stretching the muscles or tendons supplied by the innervated nerve produced afferent mechanoreceptor discharges and rubbing the skin in the sensory field of the nerve evoked no afferent response and if spontaneous, pulse synchronous bursts of muscle sympathetic nerve activity were observed, with the frequency and amplitude of the bursts increasing during held expiration but not during arousal stimuli. Electrodes remained in place throughout the study. The electrodes were connected to a preamplifier (gain 1000) and an amplifier (gain 70). The nerve signals were played through a loudspeaker, displayed on a monitor, and recorded using a MacLab/8 data recording system (MacLab, ADInstruments Pty, Ltd, Castle Hill, Australia) controlled by a Macintosh computer. The number and the amplitude of sympathetic bursts were measured during 5-min periods and expressed as bursts per min and µvolts per min. The measurements were performed by Peaks software from MacLab.

YOH infusion

After the subject had been at supine rest for 20 min, blood pressure and heart rate were recorded every 2 min for 10 min, and baseline blood samples (~10 cm³) were drawn. YOH hydrochloride was obtained from Sigma F and D Division (St. Louis, MO) and administered under Investigative New Drug number 21,220. YOH was injected as a bolus (0.125 mg·kg⁻¹·h⁻¹ for 3 min) followed by a continuous infusion at 0.001 mg·kg⁻¹·min⁻¹ for 12 min. Blood pressure and heart rate were recorded every 2 min during the infusion; the last three values were used for data analysis. In the last min of the infusion, another blood sample was drawn.

Assays

Blood samples were collected into chilled heparinized glass tubes and centrifuged at 4°C and 3,500 × g for 15 min before storage of the plasma at −75°C until assayed. Plasma levels of catechols were measured by liquid chromatography with electrochemical detection after adsorption on alumina (18). The limits of detection were about 0.04 nmol/L for NE and about 60 pmol/L for epinephrine.

ACTH assays were conducted using a dual antibody immunoradiometric assay (Allegro HS-1CTH; Nichols Institute, Los Angeles, CA), as previously described (19). The intrassay coefficients of variation were 20% and 6.5% at concentrations of 1.5 and 9.0 pmol/L, and the minimum detectable concentration was 0.44 pmol/L (range 0.22–1.1 pmol/L).

Data analysis

Results are presented as means ± SE. Responses were expressed both as absolute and as percentage changes from the resting value. The Wilcoxon signed rank test was used to compare the effects of prednisone and placebo on basal values and to assess the effects of YOH during placebo treatment. Two-way analyses of variance were used to test for interactions between prednisone and YOH. P value less than 0.05 defined statistical significance.

Results

Effects of prednisone on basal values

Prednisone administration for 1 week did not affect systolic, diastolic, or mean blood pressure, heart rate, or body weight (Table 1). Plasma ACTH levels decreased significantly from 21.7 ± 5.6 pmol/L to 10.6 ± 3.7 pmol/L (P < 0.05). Plasma levels of NE decreased by 20% (P < 0.01), and DHPG levels decreased by 15% (P < 0.01). MSNA (bursts per min) decreased by 21% (P < 0.05, Table 1); the proportionate decrease in MSNA was larger (51%) when MSNA was expressed as µvolts per min (P < 0.05). Prednisone did not affect plasma levels of epinephrine, 3,4-dihydroxyphenylalanine, or dihydroxyphenylacetic acid. Urinary sodium excretion was not significantly affected by
TABLE 1. Effects of 1 week of treatment with placebo or prednisone (20 mg orally each morning) on responses of blood pressure, heart rate, plasma levels of catechols, and skeletal muscle sympathetic nerve activity (MSNA) responses to yohimbine (YOH) in healthy volunteers

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
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<th>Prednisone</th>
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<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>YOH</td>
<td>Baseline</td>
<td>YOH</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>121 ± 4</td>
<td>136 ± 5</td>
<td>119 ± 3</td>
<td>133 ± 4</td>
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<tr>
<td>Diastolic BP (mm Hg)</td>
<td>70 ± 2</td>
<td>77 ± 2</td>
<td>70 ± 1</td>
<td>76 ± 2</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>87 ± 2</td>
<td>97 ± 3</td>
<td>86 ± 2</td>
<td>95 ± 2</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>63 ± 2</td>
<td>67 ± 3</td>
<td>64 ± 3</td>
<td>67 ± 3</td>
</tr>
<tr>
<td>Plasma NE (nmol/L)</td>
<td>1.40 ± 0.13</td>
<td>4.83 ± 0.56</td>
<td>1.09 ± 0.11</td>
<td>3.47 ± 0.38</td>
</tr>
<tr>
<td>Plasma E (pmol/L)</td>
<td>150 ± 30</td>
<td>220 ± 40</td>
<td>110 ± 20</td>
<td>150 ± 30</td>
</tr>
<tr>
<td>Plasma DHPG (nmol/L)</td>
<td>6.10 ± 0.37</td>
<td>7.59 ± 0.49</td>
<td>5.02 ± 0.24</td>
<td>6.37 ± 0.29</td>
</tr>
<tr>
<td>Plasma DOPAC (nmol/L)</td>
<td>9.08 ± 0.71</td>
<td>9.16 ± 0.82</td>
<td>7.68 ± 0.39</td>
<td>8.24 ± 0.38</td>
</tr>
<tr>
<td>Plasma Dopac (nmol/L)</td>
<td>8.05 ± 1.09</td>
<td>8.70 ± 1.24</td>
<td>8.90 ± 1.48</td>
<td>9.06 ± 1.56</td>
</tr>
<tr>
<td>MSNA (bursts/min)</td>
<td>36 ± 3</td>
<td>44 ± 4</td>
<td>30 ± 4</td>
<td>35 ± 4</td>
</tr>
<tr>
<td>MSNA (μvolts/min)</td>
<td>6.56 ± 0.93</td>
<td>9.45 ± 1.72</td>
<td>3.26 ± 0.31</td>
<td>4.69 ± 0.65</td>
</tr>
</tbody>
</table>

BP, blood pressure; MAP, mean arterial blood pressure; DHPG, dihydroxyphenylglycol; DOPAC, dihydroxyphenylacetic acid.

a MSNA recording was carried out in 7 out of the 15 subjects.

b $P < 0.001$ (YOH vs. baseline).
c $P < 0.05$ (YOH vs. baseline).
d $P < 0.01$ (baseline value, prednisone vs. placebo).
e $P < 0.001$ (YOH vs. baseline).
f $P < 0.05$ (baseline value, prednisone vs. placebo).

**Effects of YOH infusion during placebo phase**

During placebo treatment, YOH increased systolic, diastolic, and mean arterial blood pressure significantly by 13%, 11%, and 12% (all $P < 0.001$), respectively, and heart rate by 7% ($P < 0.05$; Table 1). YOH also increased plasma NE levels and MSNA (bursts per min) in all subjects. The mean increment in plasma NE levels was 243% (Fig. 1), about 10-fold larger than the 22% increase in MSNA (Fig. 2). Plasma DHPG levels increased by 25% (Table 1). MSNA (μvolts per min) increased by about 40%. Plasma epinephrine levels did not increase significantly, and plasma 3,4-dihydroxyphenylalanine and dihydroxyphenylacetic acid levels were unchanged.

**Effects of YOH during prednisone treatment phase**

Prednisone did not affect absolute or percentage responses of blood pressure or heart rate to YOH (Table 1). The absolute increment in plasma NE levels during YOH infusion was not significantly smaller after prednisone than after placebo (2.38 ± 0.34 nmol/L and 3.44 ± 0.46 nmol/L, $0.05 < P < 0.10$); the proportionate increments did not differ (237 ± 41% and 243 ± 24%, Fig. 1). Prednisone also did not influence the YOH-induced proportionate increments in plasma DHPG levels (Table 1).

Analogously, neither absolute nor proportionate responses of MSNA to YOH differed between placebo (7 ± 3 bursts/min, 22 ± 7%) and prednisone (6 ± 1 bursts/min, 23 ± 7%) treatments (Fig. 2). The proportionate plasma NE response to YOH remained about 10 times the MSNA response. Analyses of variance revealed no interaction between the effects of prednisone and of YOH for any of the dependent neuronal or neurochemical measures. The relative responses of NE levels and MSNA ($\Delta$NE/$\Delta$MSNA) also did not differ between the prednisone and placebo treatments.

**Discussion**

The results show that oral prednisone treatment for 1 week at a dose of 20 mg each morning produces sympathoinhibition, because directly recorded MSNA and plasma levels...
of the sympathetic neurotransmitter NE and of the intraneuronal NE metabolite DHPG were significantly lower after prednisone treatment than after placebo treatment in the same subjects. The double-blind, placebo-controlled, randomized cross-over design excluded important sequence effects or observer biases. The sympathoinhibition probably resulted at least partly from effects of prednisone in the central nervous system or sympathetic ganglia, because prednisone decreased the rate of directly recorded sympathetic nerve traffic in the peroneal nerve, and because peroneal sympathetic fibers are postganglionic (16).

Studies of laboratory animals have indicated that \( \alpha_2 \)-adrenoceptors in the brain tonically inhibit sympathoneural outflows (20). The same may hold true for humans, because patients with spinal cord transections with disruption of descending pathways from the brain to the intermediolateral columns of the thoracolumbar spinal cord do not demonstrate a decrease in blood pressure in response to clonidine (21), and iv infusion of the \( \alpha_2 \)-adrenoceptor blocker YOH in healthy volunteers increases MSNA (16). An increase in the number or affinity of central \( \alpha_2 \)-adrenoceptors during prednisone treatment could therefore inhibit sympathoneural outflows. Consistent with this view, elevated plasma cortisol levels in anorexic patients are associated with an increased number of platelet \( \alpha_2 \)-adrenoceptors (22); however, such an effect of prednisone would be expected to augment sympathoneural responses to \( \alpha_2 \)-adrenoceptor blockade, and the present results failed to confirm this hypothesis.

Other animal studies have suggested that glucocorticoids interfere with the modulatory function of \( \alpha_2 \)-adrenoceptors in the brain and periphery (5, 10). The failure in the present study to detect attenuation of responses of either MSNA or of plasma NE levels after prednisone treatment casts doubt on the generalization of these findings in rats to healthy adult humans.

In the present study, the proportionate increases in antecubital venous plasma NE levels during YOH infusion averaged about 10 times those in MSNA, confirming previous clinical reports that the human forearm possesses abundant, functional \( \alpha_2 \)-adrenoceptors on sympathetic nerves (23).

Prednisone treatment failed to attenuate the marked effects of YOH on plasma NE or MSNA levels. The fact that there was no significant difference in the relative plasma NE response for a given neural response (\( \Delta\%\text{NE}/\Delta\%\text{MSNA} \)) to YOH between the prednisone and placebo treatments suggests the absence of an effect of prednisone on the modulatory function of presynaptic \( \alpha_2 \)-adrenoceptors in the periphery. Therefore the findings are consistent with the view that the sympathoinhibition produced by prednisone occurs independently of effects of prednisone on the function of peripheral and central neural \( \alpha_2 \)-adrenoceptors.

Plasma DHPG levels reflect NE turnover in sympathetic nerves (24, 25). The fall in plasma DHPG levels after prednisone treatment therefore confirms glucocorticoid-induced sympathoinhibition. YOH increases plasma DHPG levels in humans because of reuptake and intraneuronal oxidative demamination of endogenously released NE (24). The finding that increments in plasma DHPG levels during YOH infusion did not differ after placebo or prednisone treatment suggests that prednisone does not interfere with neuronal uptake of NE (Uptake-1) or with monoamine oxidase. Because of the relatively small proportion of endogenously released NE that is removed by nonneuronal uptake (Uptake-2, reference 26), prednisone treatment would not be expected to alter plasma NE responses to YOH, even though exogenously administered steroids inhibit Uptake-2 (4).

Several reports noted glucocorticoid-induced augmentation of pressor or vasoconstrictor responses to exogenously administered NE and augmentation of tachycardic responses to \( \beta \)-adrenoceptor agonists (5, 27). In the present study, YOH-induced pressor and tachycardic responses were similar after placebo and after prednisone. These results suggest that glucocorticoids may not accentuate responses for a given amount of endogenously released NE in humans, but a simultaneous effect of prednisone on other vasopressor or depressor hormone systems could have obscured an augmented pressor response to YOH.

In conclusion, administration of prednisone to humans causes sympathoinhibition. In contrast to previous findings in laboratory animals, this inhibition seems to occur independently of inhibitory modulation of sympathoneural outflows by \( \alpha_2 \)-adrenoceptors in the central nervous system and of inhibitory modulation of NE release by \( \alpha_2 \)-adrenoceptors on noradrenergic terminals in the periphery.
Acknowledgments
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References