A Polymorphism of the 5′-Flanking Region of the Glucocorticoid Receptor Gene Locus Is Associated With Basal Cortisol Secretion in Men

Roland Rosmond, Yvon C. Chagnon, Monique Chagnon, Louis Pérusse, Claude Bouchard, and Per Björntorp

There is growing evidence that cortisol secretion and/or metabolism is associated with cardiovascular risk factors. Previous studies have shown that cardiovascular risk factors are associated with stimulated cortisol secretion and not with basal cortisol secretion. With the restriction enzyme *Tth111*I, a variant of the 5′-flanking region of the glucocorticoid receptor gene locus (GRL) was identified. The genotypes were compared for measurements of cortisol secretion and cardiovascular risk factors in a cohort (N = 284) of randomly selected middle-aged men. The frequency of the 3.4/3.4-, 3.4/3.8-, and 3.8/3.8-kilobase (kb) genotypes was 49.6%, 41.4%, and 9.0% respectively. The 3.8-kb homozygotes showed higher total and evening cortisol levels with a trend for elevated levels over the day. Neither stimulated or suppressed cortisol secretion nor anthropometric, endocrine, metabolic, and hemodynamic cardiovascular risk factors were significantly different among the genotypes. Since the polymorphism studied herein is localized at the 5′-flanking region of the GRL, the results suggest that elevated basal cortisol secretion is associated with a polymorphism of the promoter region.

AN INCREASING body of evidence indicates that cortisol may play a pathogenic role in patients with visceral obesity, insulin resistance, dyslipidemia, and hypertension. Stressful events during everyday life, food intake, or laboratory-induced stress enhances cortisol secretion in such patients,[1-6] suggesting an increased sensitivity of the hypothalamic-pituitary-adrenal (HPA) axis. There is also evidence that the peripheral metabolism of cortisol might be involved.[7]

The feedback control of cortisol is partly exerted via glucocorticoid receptors (GRs).[8] In general, the actions through GRs are suppressive.[8] Upon frequent or chronic challenge of the HPA axis, the sensitivity to cortisol feedback diminishes due to a downregulation of GR density, followed by presumably irreversible neurodegenerative damage.[9]

The recent discovery that a *Bcl*I GR gene locus (GRL) polymorphism is associated with elevated cortisol concentrations in response to metabolic stress[10] has raised the possibility that mutations may decrease the sensitivity to cortisol feedback. While the *Bcl*I restriction enzyme cleaves the GRL in the first intron, the functional role of the polymorphism, if any, is uncertain.[11] However, a polymorphism in an intron may interfere with splicing of primary mRNA or serve as an index for functionally important polymorphisms in neighboring gene domains, including the promoter region.

Observations on genetic alterations of the GRL may shed light on important aspects of a dysregulated HPA axis, and perhaps the neuroendocrine background to several common diseases. To elucidate this issue further, we examined a polymorphism in the 5′-flanking region of the GRL using the restriction enzyme *Tth111*I.[12]

SUBJECTS AND METHODS

For the present study, we recruited subjects from an ongoing cohort study of men born in 1944,[13] which was initiated in 1992. Based on a self-measured waist to hip ratio (WHR), the following 3 subgroups, each with 150 men, were selected for further studies: the lowest WHR (≤0.885), the highest WHR (≥1.01), and a WHR around the arithmetic mean (0.94 to 0.96). We examined these men in 1995 at the age of 51 years, and 284 (63%) volunteered to participate. All men provided written informed consent before participating in the study, which was approved by the Göteborg University ethics committee. There was no difference in measures of health status between nonresponders and responders in terms of hypertension, diabetes mellitus, myocardial infarction, stroke, and angina pectoris.

The salivary cortisol level was measured repeatedly over a random working day, including morning and evening and before, during, and after a standardized lunch. Stressful events were reported each hour and related to cortisol secretion.[5] The arithmetic mean of all cortisol measurements was calculated, providing an estimate of the total diurnal cortisol secretion. The cortisol variability, essentially determined by the difference in morning and evening cortisol, was also calculated. In addition, an overnight low-dose (0.5 mg) dexamethasone suppression test was performed at home with cortisol analyzed in saliva. The details of these procedures have been published previously.[5]

Anthropometric measurements included the body mass index, WHR, and abdominal sagittal diameter (centimeters). Endocrine measurements besides cortisol included testosterone, insulin-like growth factor-I (IGF-I), and leptin as described in detail previously.[5] Insulin, glucose, triglycerides, total, high-density lipoprotein, and low-density lipoprotein cholesterol, systolic and diastolic blood pressure, and heart rate were measured in the overnight fasting state as detailed previously.[5]

DNA was extracted using Qiagen kits (Qiagen, Hilden, Germany). One microgram of DNA from each subject was digested with 20 U *Tht111*I restriction enzyme (New England Biolabs, Beverly, MA) at 65°C for 16 to 20 hours in 20 μL total volume. The digested DNA was separated in a 1.2% agarose gel for 16 to 20 hours at 50 mA and transferred to a nylon membrane by alkaline blotting for hybridization. Prehybridization was performed in a 3-mL solution of 0.25 mol/L Na_2HPO_4 (pH 7.4), 1 mmol/L EDTA, 7% sodium dodecyl sulfate (SDS), and 100 μg/mL salmon sperm DNA (Stratagene, La Jolla, CA) for 4 to 18 hours at 65°C in a hybridization incubator (Robins Scientific, Sunnyvale, CA). The membranes were then hybridized with a GRL 2.86-kb human genomic fragment DNA probe labeled with [α-^{32}P]dCTP by random priming using a T7 DNA polymerase kit (T7 Quick Prime; Pharmacia Biotech, Baie dÚrfe, Quebec, Canada) to at least 1×10^9

From the Department of Heart and Lung Diseases, Göteborg University, Göteborg, Sweden; Physical Activity Sciences Laboratory, Laval University, Quebec, Canada; and Pennington Biomedical Research Center, Louisiana State University, Baton Rouge, LA.

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Address reprint requests to Roland Rosmond, MD, PhD, Pennington Biomedical Research Center, 6400 Perkins Rd, Baton Rouge, LA 70808.

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Table 1. Comparisons Between the 3.4/3.4-kb, 3.4/3.8-kb, and 3.8/3.8-kb Genotypes of the GR Gene Identified by *Tth111*I in Measurements of Salivary Cortisol

Variable (nmol/L)	3.4/3.4 (n = 127, 49.6%)	3.4/3.8 (n = 106, 41.4%)	3.8/3.8 (n = 23, 9.0%)	*P*
Diurnal cortisol secretion	6.9 ± 2.1[a]	7.6 ± 3.7[a]	9.8 ± 8.2[b]	.014
Cortisol level				
Morning	14.2 ± 7.3	14.8 ± 6.9	17.6 ± 9.8	.194
15 min before lunch	6.9 ± 5.1	7.5 ± 5.8	7.9 ± 4.9	>.20
30 min after lunch	8.1 ± 8.9	8.1 ± 6.7	10.8 ± 10.0	>.20
45 min after lunch	7.2 ± 4.3	7.2 ± 5.2	10.0 ± 10.2	.112
60 min after lunch	6.5 ± 4.8	6.9 ± 5.5	8.8 ± 7.2	>.20
Average of 30, 45, and 60 min	7.2 ± 5.8	7.4 ± 5.8	9.9 ± 9.0	>.20
5:00 PM	4.6 ± 2.3	4.9 ± 2.2	5.4 ± 4.1	>.20
Before bedtime	2.8 ± 2.5[a]	3.4 ± 3.4[a]	5.8 ± 11.2[b]	.026
Stress-related cortisol secretion	0.22 ± 0.5	0.16 ± 0.4	0.06 ± 0.4	>.20
Cortisol variability	28.8 ± 54.3	31.0 ± 76.1	30.0 ± 37.3	>.20
Dexamethasone suppression test	11.6 ± 5.5	12.1 ± 5.1	13.5 ± 6.3	>.20

NOTE. Results are the mean ± SD. Values with different superscripts are significant at the .05 level.

cpm/μg DNA specific activity. After hybridization at 65°C for 18 to 20 hours, nonspecific binding was washed off 2 to 4 times in a solution of 0.1 mol/L SSPE (20 mmol/L NaCl, 1 mmol/L Na_2HPO_4, and 0.1 mmol/L EDTA) plus 0.1% SDS. Occasionally, the membranes were washed in a more stringent solution containing 10 times less salt. Subsequently, the membranes were exposed to Kodak (New Haven, CT) X-OMAT AR film for 70 to 120 hours at −70°C.

Spjotvoll-Stoline post hoc tests were performed to test the statistical significance of differences among the mean scores of 2 or more groups. *P* values are 2-sided throughout, and a *P* value .05 was considered significant. The statistical analyses were performed with SPSS for Windows, release 9.0 (SPSS, Chicago, IL).

RESULTS

The *Tth111*I allelic frequency was 0.30 and 0.70 for the 3.8- and 3.4-kb allele, respectively. The frequency of the 3.4/3.4-, 3.4/3.8-, and 3.8/3.8-kb genotypes was 49.6%, 41.4%, and 9.0% (Table 1). The total diurnal cortisol secretion and the cortisol level before bedtime were significantly higher in 3.8-kb homozygotes versus 3.4-kb homozygotes and the heterozygotes. Cortisol levels in the morning and at 5 PM, as well as before and after lunch, were also higher in the 3.8-kb homozygotes, but the difference with the other genotypes did not reach statistical significance.

There were no statistically significant differences in anthropometry, testosterone, IGF-I, or metabolic and hemodynamic measures among the genotypes (not shown). However, there was a stepwise increase from the 3.4-kb to 3.8-kb GRL allele in testosterone, IGF-I, leptin, insulin, glucose, cholesterol, and systolic blood pressure, but these trends were not significantly different.

Finally, the carriers of the 3.8-kb allele (n = 129) and the 3.4/3.4-kb homozygotes (n = 127) were compared. The results for selected endocrine, metabolic, and hemodynamic variables are displayed in Table 2. In comparison to the 3.4-kb homozygous men, men with the 3.8-kb allele had borderline nonsignificantly higher diurnal cortisol secretion and cortisol levels before bedtime.

DISCUSSION

The main finding of this study is that there is an association between a *Tth111*I GRL polymorphism and basal cortisol secretion. Individuals who were homozygous for the larger allele (3.8/3.8 kb) had elevated diurnal cortisol levels, which were most pronounced and significant at bedtime. Cortisol levels in the morning and at 5 PM were higher in the 3.8-kb homozygotes, although the difference versus the other genotypes did not reach statistical significance. Stimulated or suppressed cortisol secretion did not reveal any differences, which suggests that basal cortisol secretion was primarily affected. No significant differences in anthropometric, endocrine, metabolic, and hemodynamic measurements emerged.

Several studies suggest that anthropometric, endocrine, metabolic, and hemodynamic risk factors are associated with stimulated cortisol secretion after stressful events[5] or food intake,[14] rather than basal, unstimulated cortisol secretion.[14] The results of the present study indicate that the *Tth111*I GRL polymorphism is associated with basal cortisol concentrations, but not or only weakly with stimulated cortisol levels and other variables.

Previous genetic studies have identified a *Bcl*I restriction fragment length polymorphism at the GRL, localized at a known *Bcl*I cleave site in intron 1 and a putative site in intron 2, discoverable as a 4.5-kb allele.[11,15] This 4.5-kb allele is associated with elevated postprandial cortisol secretion,[10] abdominal obesity, insulin resistance, and elevated blood pressure.[10,11,15]

Table 2. Comparisons Between Carriers of the 3.8-kb Allele Versus the 3.4/3.4-kb Homozygotes

Variable	3.8-kb Allele (n = 129)	3.4/3.4 (n = 127)	*P*
Diurnal cortisol secretion (nmol/L)	8.0 ± 4.7	6.9 ± 2.1	.053
Cortisol level before bedtime (nmol/L)	3.8 ± 5.6	2.8 ± 2.5	.079
Insulin (mU/L)	12.6 ± 10.7	12.9 ± 11.6	>.20
Glucose (mmol/L)	4.6 ± 1.1	4.5 ± 0.9	>.20
Cholesterol (mmol/L)	6.2 ± 1.1	6.1 ± 1.1	>.20
Systolic blood pressure (mm Hg)	129.2 ± 17.4	129.2 ± 17.8	>.20
Diastolic blood pressure (mm Hg)	82.9 ± 9.5	83.7 ± 11.4	>.20

NOTE. Values are the mean ± SD.

Hence, this *Bcl*I polymorphism is associated with a decreased sensitivity of the HPA axis to elevated cortisol levels, as well as several cardiovascular risk factors.

In summary, stimulated cortisol secretion after stressful events during everyday life or in the laboratory, as well as after food intake, seems to be associated with abnormal anthropometric, endocrine, metabolic, and hemodynamic variables.[1,2,5,14] Stimulated cortisol secretion and cardiovascular risk factors are associated with the *Bcl*I polymorphism in the first intron of the GRL.[10,11,15] In contrast, there is no evidence linking basal, unstimulated cortisol secretion to anthropometric, endocrine, metabolic, and hemodynamic risk factors.[5,14] However, as demonstrated herein, the basal cortisol concentration is associated with a *Tth111*I GRL polymorphism. This polymorphism is localized in the 5′-flanking region of the GRL,[12] likely in the promoter region of the gene, and may therefore be involved in the regulation of GR density.

Recently, a hyposensitive dexamethasone dose-response curve was found to be associated with the central GR,[4] presumably as a result of a perturbed regulation of GR density. In contrast, the peripheral GR appears to be associated with an increased responsiveness to cortisol. This suggests different regulatory mechanisms of the central and peripheral GRs, perhaps due to varying regulatory gene elements. However, one cannot conclude that the *Bcl*I or *Tth111*I GRL polymorphism is associated with differential regulation of central and peripheral GR functions, a topic for which further studies are needed.

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