Effects of Gender and Diagnosis on Growth Hormone Response to Clonidine for Major Depression: A Large-Scale Multicenter Study

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Objective: *The authors' goal was to establish, in a large multicenter sample of patients classified according to gender and menopausal status, if the growth hormone (GH) response to clonidine discriminated patients with episodes of major depression from patients with episodes of minor depression.* *Method:* *The GH response to intravenous clonidine administration (150 μg) was compared in 71 male and 140 female patients with major depressive episodes and 47 male and 53 female patients with minor depressive episodes. These patients were diagnosed according to Research Diagnostic Criteria.* *Results:* *Differences in the GH response to clonidine between diagnostic groups occurred only between male patients. These results were found in the group as a whole and in each center. The GH responses to clonidine of premenopausal women differed significantly from those of postmenopausal women in each diagnostic group.* *Conclusions:* *These results confirm that gender and menopausal status are of the utmost importance in the interpretation of the clonidine GH test.*

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The blunted GH response to intravenous clonidine administration in depressed patients is well-established. Five groups have independently reported that patients with depression (mainly of the endogenous subtype) manifest smaller GH responses to clonidine than control subjects (1). This blunted GH response to clonidine in depressed patients has been proposed as an indirect index of dysrhythmia central adrenoceptor function (2) and as a possible biological marker of primary major depression (3).

One methodological problem raised by the studies of GH response to clonidine in depressed patients is the relatively small number of patients and control subjects used. Results obtained on small numbers of patients could be partly related to the influence of variables that themselves may alter GH response. Elsewhere, we reported that long-lasting effects of tricyclics (4, 5) and gender (6) interfere with the interpretation of the clonidine GH test in depressed patients. Interestingly, Horton et al. (7) and Katona et al. (8), who studied the largest groups of control subjects (N=31 and N=42, respectively) and depressed patients (N=34 and N=30, respectively) found no between-group difference in GH response to clonidine. Unfortunately, however, both studies used a fixed dose of 1.3 μg/kg of clonidine, which has been shown to be inadequate to differentiate clear GH response from nonresponse in normal volunteers (9).

Another methodological problem is that a possible biological marker may be given a falsely high value for its "rule in" and "rule out" performance if the spectrum of subjects with the disease and the control subjects is not broad enough (10).

Nierenberg and Feinstein (11) suggested five phases in evaluating a potential biological marker. In the fifth phase, a relatively unselected group of patients and control subjects, similar to the group of patients who would be encountered when the test is applied in a typical clinical setting, should be used.

The control subjects have to fulfill two requirements: 1) they should have symptoms that resemble the presenting features of the principal disease and that must be diagnostically distinguished from it, and 2) the comorbid component of the control group and group of

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subjects with the disease should include potential interfering conditions, along with the therapeutic agents used to treat those conditions.

The purpose of our present study was to evaluate the diagnostic performance of the clonidine GH test in a large group of patients with major and minor depressive episodes and to answer the following questions: 1) Does this test discriminate between patients with major and minor depressive episodes and/or between patients with endogenous and nonendogenous major depression? 2) Is there a gender difference in the GH response to clonidine in depressed patients? 3) What is the influence of menopausal status on this test?

METHOD

Subjects

In a retrospective study, we collected the results of clonidine GH tests of depressed patients from three hospitals in Belgium. Hospital A was the Department of Psychiatry of the Vincent Van Gogh Hospital in Charleroi, hospital B was the Psychiatric Unit of the University Hospital in Liège, and hospital C was the Department of Psychiatry of the Brugmann University Hospital in Brussels. All of the patients had been assessed by clinicians (G.C., M.A., and G.H.) using the Schedule for Affective Disorders and Schizophrenia (SADS) (12, 13). The clinicians had been trained in the use of the SADS. The patients were separated into subgroups by diagnosis, gender, and menopausal status. Patients' scores on the Hamilton Depression Rating Scale (14) were recorded as severity ratings.

Patients with at least one baseline GH value of 5 ng/ml or more were excluded from study (15, 16). This left 211 patients who met Research Diagnostic Criteria (RDC) (17, 18) for primary major depressive episodes (72 endogenous) and 100 patients who met RDC for minor depressive episodes.

Analysis of variance (ANOVA) revealed a small but significant age difference between the patients with major (mean=45.2 years, SD=10) and minor (mean=39.2 years, SD=11) depressive episodes ($F=9.3$, df=1, 308, $p<0.01$). Patients with major depressive episodes had significantly higher Hamilton depression scores than patients with minor depressive episodes (mean=25.8, SD=8.9, versus mean=16.5, SD=5.6) ($F=76.1$, df=1, 269, $p<0.001$).

All patients were free of antidepressant and neuroleptic drugs for at least 15 days and free of benzodiazepines for at least 3 days before the clonidine GH test was administered. Patients with body weights exceeding their ideal weights by more than 20% were excluded (15). The protocol was approved by the ethical committees of all three hospitals, and all patients gave their informed consent.

Procedure

At 8:00 a.m., after an overnight fast, an intravenous catheter was inserted into a forearm of the reclining patient. Five ml of blood were sampled for hormone determination 30 minutes before the infusion of clonidine at hospital A, 40 and 20 minutes before the infusion of clonidine at hospital B, and immediately before the infusion of clonidine (time 0) at hospitals A, B, and C, starting at 9:00 a.m. Clonidine was administered intravenously at a fixed dose of 150 µg dissolved in 9 ml of saline solution over a 10-minute period according to the methodology of the study of Matussek et al. (15).

Subsequent blood samples were collected 30, 45, and 60 minutes after the infusion at hospital A; 20, 30, 40, 60, and 80 minutes after the infusion at hospital B; and 20, 30, 40, and 60 minutes after the infusion at hospital C. Blood samples were centrifuged within 2 hours, and the plasma was stored at –40 °C. GH determinations were performed by using a radioimmunoassay according to a methodology described elsewhere (19, 20). We calculated a mean baseline GH value (the mean of the time 0 value and the preceding GH values) and a maximum change in GH value (the difference between the highest value following the injection and the mean baseline value). GH response was also measured as the area under the response curve following the injection of the drug.

The areas under the curve and the mean GH and maximum change in GH values were compared by using a Kruskal-Wallis one-way ANOVA (21). A blunted response to clonidine was defined as a maximum change in GH value of less than 5 ng/ml (3), and Fisher's exact tests were used for the two-by-two comparisons. Analyses were performed on the group as a whole and on the subjects in each center. All statistical procedures, except the Spearman's rank correlation coefficient, were performed by using SPSS/PC+ V2.0 (22).

Finally, assays for GH concentrations were performed on the same 30 blood samples at the three hospitals. The assay methods were highly correlated ($r=0.987$, N=30, $p<0.001$, and $r=0.99$, N=30, $p<0.001$). We calculated the regression lines between the GH values in the three hospitals by using GH values at hospital A as the dependent variable (GH at hospital A=–0.26+1.39×GH at hospital B and GH at hospital A=0.06+0.71×GH at hospital C). GH values from hospitals B and C were then transformed by these equations to hospital A GH values.

RESULTS

In the whole sample of 311 patients, there was no significant difference between the baseline GH values of patients with major depressive episodes and patients with minor depressive episodes ($\chi^2=2.5$, N=311, n.s.) (table 1). The 211 patients with major depressive episodes had a smaller mean GH response to clonidine than the 100 patients with minor depressive episodes as measured by maximum change in GH ($\chi^2=25.7$, N=311, $p<0.001$) and area under the curve ($\chi^2=27$, N=311, $p<0.001$) (table 1).

Male patients with major depressive episodes (N=71) had smaller GH responses to clonidine than male patients with minor depressive episodes (N=47) as measured by maximum change in GH ($\chi^2=23.6$, N=118, $p<0.001$) and area under the curve ($\chi^2=22.8$, N=118, $p<0.001$) (table 1). This difference was also noted in each center independently (all p values <0.01).

Premenopausal and postmenopausal female patients with major depressive episodes did not significantly differ from those with minor depressive episodes in their GH responses to clonidine, however (table 1). These findings also applied in each center separately (all p values were nonsignificant).

Postmenopausal female patients with major depressive episodes had smaller GH responses to clonidine than premenopausal female patients with major depressive episodes as measured by maximum change in GH ($\chi^2=13.2$, N=140, $p<0.005$) and area under the curve ($\chi^2=15.5$, N=140, $p<0.05$). Postmenopausal female patients with minor depressive episodes had smaller GH responses to clonidine than premenopausal female patients with minor depressive episodes as measured by maximum change in GH ($\chi^2=5.7$, N=53, $p<0.02$) and area under the curve ($\chi^2=9.8$, N=53, $p<0.002$) (table 1).

The maximum change in GH values and area under the curve values showed unimodal distributions in each of the subgroups.

Using a cutoff maximum change in GH value of 5

TABLE 1. Effects of Clonidine on Plasma GH Concentrations in Depressed Subjects Classified According to RDC

Subjects	Mean Baseline GH Value (ng/ml)		Maximum Change in GH (ng/ml)		Area Under the Curve		Number of Subjects With Blunted Response[a]
	Mean	SD	Mean	SD	Mean	SD	
All patients (N=311)							
Major depressive episodes (N=211)	1.0	1.0	3.8[b]	8.3	10.7[b]	18.7	175[c]
Minor depressive episodes (N=100)	0.8	1.0	7.1	8.0	16.3	16.0	50
Male patients (N=118)							
Major depressive episodes (N=71)	0.8	1.0	3.2[b]	6.9	8.3[b]	15.0	62[c]
Minor depressive episodes (N=47)	0.5	0.5	9.7	9.3	20.4	19.3	16
Female patients (N=193)							
Major depressive episodes (N=140)							
Premenopausal (N=74)	1.3	1.3	6.2[d]	10.6	17.5[b]	25.0	49
Postmenopausal (N=66)	0.9	0.8	1.7	5.8	5.6	9.8	64
Minor depressive episodes (N=53)							
Premenopausal (N=44)	1.1	1.0	5.4[e]	5.7	14.4[d]	11.9	26
Postmenopausal (N=9)	0.5	0.4	1.3	2.2	3.5	3.4	8

[a]Maximum change in GH less than 5 ng/ml.
[b]Significant difference between groups ($p<0.001$, Kruskal-Wallis one-way ANOVA).
[c]Significant difference between groups ($p<0.001$, Fisher exact test).
[d]Significant difference between groups ($p<0.01$, Kruskal-Wallis one-way ANOVA).
[e]Significant difference between groups ($p<0.05$, Kruskal-Wallis one-way ANOVA).

ng/ml, the difference in GH response between patients with major and minor depressive episodes was seen only in men: 62 (87%) of 71 male patients with major depressive episodes versus 16 (34%) of 47 male patients with minor depressive episodes had a blunted response ($p=0.0001$, Fisher's exact test) (table 1).

In the whole sample of 311 patients, the sensitivity and specificity of the test were 83% and 50%, respectively. The cutoff maximum change in GH value of 5 ng/ml had as good or better diagnostic specificity as cutoff values of 4 or 6 ng/ml.

Severity of the depression, measured by scores on the Hamilton Depression Rating Scale, correlated positively with the maximum change in GH values in male patients with major ($r_s=0.24$, N=71, $p<0.05$) or minor depressive episodes ($r_s=0.32$, N=47, $p<0.05$) and in premenopausal female patients with major depressive episodes ($r_s=0.25$, N=74, $p<0.05$) but not in the premenopausal female patients with minor depressive episodes or in the postmenopausal female patients with either major or minor depressive episodes. No correlation was observed between age and maximum change in GH values in any subgroups (all p values were nonsignificant).

Among all of the patients with major depressive episodes (men and pre- and postmenopausal women), there was no statistical difference in GH response to clonidine between patients with endogenous versus nonendogenous depression (all p values were nonsignificant by Kruskal-Wallis one-way ANOVA).

DISCUSSION

This large-scale multicenter study showed that the clonidine GH test 1) discriminates between male patients with major and male patients with minor depressive episodes, 2) does not discriminate between female patients with major and female patients with minor depressive episodes, 3) does not discriminate between patients with endogenous and patients with nonendogenous major depression, and 4) is dependent on menopausal status.

We did not compare our patients with a control group because our purpose was not to establish that patients were different from control subjects. Our goal was to examine the differences in subgroups (classified according to diagnosis, gender, and menopausal status) that fulfilled the criteria of Nierenberg and Feinstein (11) for evaluating a potential biological marker—relatively unselected subgroups of patients, similar to patients who would be encountered when the test is applied in a typical clinical setting.

The clonidine GH tests were conducted from 1982 according to the methodology of the study of Matussek et al. (15). In that study and in our present study, clonidine was administered at a fixed dose of 150 µg, and information about the time of the menstrual cycle and the duration of medication-free period were not recorded. Our finding that female patients with major and minor depressive episodes do not differ in their GH responses to clonidine should be considered with caution because it is at odds with the findings of some other studies (15, 23). Confounding variables (24, 25), such as hormonal status, duration of medication-free period prior to testing, severity of depressive symptoms, dose of clonidine, and means of administering clonidine could partly explain some discrepancies.

It is interesting to note that the two studies that found no difference between depressed and control subjects in GH response to intravenous administration of 1.3 µg/kg of clonidine (7, 8) included more female than male patients. In the study of Horton et al. (7), 25 of the 34 patients and 22 of the 31 control subjects were women; in the study of Katona et al. (8), 24 of

the 30 patients and 29 of the 42 control subjects were women.

Our results are in accordance with our previous prospective study (6), which found a gender difference in the GH response to clonidine in a small group of carefully selected untreated depressed patients. A gender difference in the GH response to 5 μg/kg per oz of clonidine has also been observed by Charney et al. (26), who found a difference between their male patients and control subjects but not their female patients and control subjects.

Elsewhere (6), we suggested that steroidal hormones acting at multiple levels of the α_2 receptor/growth hormone-releasing hormone (GH-RH)/GH axis could be responsible for the differences found in the GH response to clonidine in men and women, as it was found in male and female animals (27). Sex differences in the patterns of GH secretion have been recognized in rats for many years and are associated with differential responsiveness to exogenous GH-RH (28), suggesting a differential somatostatin secretion. It has been shown that in man, the clonidine effect appears to be dependent on a decreased somatostatinergic input to the pituitary, resulting as a consequence of α_2 adrenergic agonism (29).

Our finding of a smaller GH response to clonidine in postmenopausal depressed women than in premenopausal depressed women replicates the results of two other studies (30, 31).

Finally, in our sample of depressed outpatients the clonidine GH test did not discriminate between patients with endogenous and nonendogenous depression. One study (32) found a difference between endogenous and nonendogenous depressed patients, but another study (33) found no significant differences between these two groups.

We think that three main conclusions result from this study: 1) At least a subgroup of male patients with major depressive episodes have smaller GH responses to clonidine than patients with minor depressive episodes, supporting in these patients the hypothesis of reduced α_2 adrenergic sensitivity. 2) The specificity of the clonidine GH test to discriminate patients with primary major affective illness from a control group is very poor, even after excluding patients with elevated baseline GH values. The relatively high sensitivity observed in our sample suggests that a negative test could be useful to rule out major depressive episodes in male patients. 3) Other experimental strategies than the GH response to clonidine are needed as indexes of central adrenoceptor function in depressed patients and as biological markers of primary major depression (34).

REFERENCES

1. Schittecatte M, Charles G, Machowski R, Wilmotte J: Tricyclic washout and growth hormone response to clonidine. Br J Psychiatry 1989; 154:858–863
2. Siever LJ, Davis KL: Overview: toward a dysregulation hypothesis of depression. Am J Psychiatry 1985; 142:1017–1031
3. Ansseau M, Scheyvaerts M, Doumont A, Legros JJ, Franck G: Concurrent use of REM latency, dexamethasone suppression, clonidine and apomorphine tests as biological markers of endogenous depression: a pilot study. Psychiatr Res 1984; 12:261–272
4. Schittecatte M, Charles G, Machowski R, Garcia-Valentin J, Wilmotte J: Controversies about the clonidine test, in Biological Markers of Depression: State of the Art: Excerpta Medica International Congress Series 932. Edited by Ansseau M, von Frenkell R, Franck G. New York, Excerpta Medica, 1991
5. Schittecatte M, Charles G, Nefve C, Garcia-Valentin J, Machowski R, Wilmotte J: Long-term downregulation of central adrenoceptor function by desipramine treatment: a clonidine study in normal subjects. Biol Psychiatry 1992; 31:856–858
6. Schittecatte M, Charles G, Machowski R, Wilmotte J: Growth hormone response to clonidine in untreated depressed patients. Psychiatry Res 1989; 29:199–206
7. Horton RW, Katona CL, Theodorou AE, Hale AS, Davies SL, Tunnicliffe C, Yamaguchi C, Paykel ES, Kelly JS: Platelet radioligand binding and neuroendocrine challenge tests in depression. Ciba Found Symp 1986; 123:84–105
8. Katona CL, Healy D, Paykel ES, Theodorou AE, Lawrence KM, Whitehouse A, White B, Horton RW: Growth hormone and physiological responses to clonidine in depression. Psychol Med 1993; 23:57–63
9. Hoehe M, Valido G, Matussek N: Growth hormone, noradrenaline, blood pressure and cortisol responses to clonidine in healthy male volunteers: dose-response relations and reproducibility. Psychoneuroendocrinology 1988; 13:409–418
10. Ransohoff DF, Feinstein AR: Problems of spectrum and bias in evaluating the efficacy of diagnostic tests. N Engl J Med 1978; 229:926–930
11. Nierenberg AA, Feinstein AR: How to evaluate a diagnostic marker test. JAMA 1988; 259:1699–1702
12. Spitzer RL, Endicott J: Schedule for Affective Disorders and Schizophrenia—Lifetime Version, 3rd ed. New York, New York State Psychiatric Institute, Biometrics Research, 1978
13. Charles G, Ansseau M: Guide pour le diagnostic des troubles affectifs et de la schizophrénie. Acta Psychiatr Belg 1987; 87: 361–516
14. Hamilton M: A rating scale for depression. J Neurol Neurosurg Psychiatry 1960; 23:56–62
15. Matussek N, Ackenheil M, Hippius H, Muller F, Schroder HT, Schultes H, Wasilewski B: Effect of clonidine on growth hormone release in psychiatric patients and controls. Psychiatr Res 1980; 2:25–36
16. Laackman G: Psychopharmacology and Depression Research. Berlin, Springer-Verlag, 1990
17. Spitzer RL, Endicott J, Robins E: Research Diagnostic Criteria (RDC) for a Selected Group of Functional Disorders, 3rd ed. New York, New York State Psychiatric Institute, Biometrics Research, 1980
18. Ansseau M: Critères de diagnostic pour la recherche en psychiatrie. Acta Psychiatr Belg 1985; 85:253–324
19. Ansseau M, von Frenkell R, Cerfontaine JL, Papart P, Timsit-Berthier M, Geenen V, Legros JJ: Blunted response of growth hormone to clonidine and apomorphine in endogenous depression. Br J Psychiatry 1988; 153:65–71
20. Schittecatte M, Charles G, Depauw Y, Mesters P, Wilmotte J: Growth hormone response to clonidine in panic disorder patients. Psychiatr Res 1988; 23:147–151
21. Theodorsson-Norheim E: Kruskal-Wallis test: BASIC computer program to perform nonparametric one-way analysis of variance and multiple comparisons on ranks of several independent samples. Comput Methods Programs Biomed 1986; 23:57–62
22. Hull CH, Nie NH: SPSS Update 7-9: New Procedures and Facilities for Releases 7-9. New York, McGraw-Hill, 1981
23. Boyer P, Davila M, Schaub C, Nassiet J: Growth hormone response to clonidine stimulation in depressive states, I: a two-part study. Psychiatrie et Psychobiologie 1986; 1:189–195
24. Trestman RL, Siever LJ: Growth hormone response to clonidine in depression, in Biological Markers of Depression: State of the Art: Excerpta Medica International Congress Series 932. Edited

by Ansseau M, von Frenkell R, Franck G. New York, Excerpta Medica, 1991
25. Matussek N, Ackenheil M, Herz M: The dependence of the clonidine growth hormone test on alcohol drinking habits and the menstrual cycle. Psychoneuroendocrinology 1984; 9:173–177
26. Charney DS, Heninger GG, Sternberg DE, Hafstad KM, Giddings S, Landis H: Adrenergic receptor sensitivity in depression. Arch Gen Psychiatry 1982; 39:290–294
27. Eriksson E, Modigh K, Jansson J-O: Effects of sex steroids on growth hormone responses to clonidine and GHRH in reserpine pretreated rats. J Neurotransmission 1988; 71:99–113
28. Bercu BB, Weidman CA, Walker RF: Sex differences in growth hormone secretion by rats administered GH releasing hexapeptide. Endocrinology 1991; 129:2592–2598
29. Devesa J, Arce V, Lois N, Diaz MJ, Tresguerres JAF, Lima L: Alpha-2 adrenergic agonism enhances the GH response to GHRH through an inhibition of hypothalamic somatostatin release in normal men. J Clin Endocrinol Metab 1990; 71:1581–1588
30. Siever LJ, Uhde TW, Silberman EK, Jimerson DC, Aloi JA, Post RM, Murphy DL: The growth hormone response to clonidine as a probe of noradrenergic receptor responsiveness in affective disorder patients and controls. Psychiatry Res 1982; 6:171–183
31. Tulandi T, Lal S, Guyda H: Effect of estrogen on the growth hormone response to the alpha-adrenergic agonist clonidine in women with menopausal flushing. J Clin Endocrinol Metab 1987; 65:6–10
32. Checkley SA, Glas IB, Thomson C, Corn T, Robinson P: The GH response to clonidine in endogenous as compared with reactive depression. Psychiatr Med 1984; 14:773–777
33. Dolan RJ, Calloway SP: The human growth hormone response to clonidine: relationship to clinical and neuroendocrine profile in depression. Am J Psychiatry 1986; 143:772–774
34. Schittecatte M, Charles G, Machowski R, Garcia-Valentin J, Mendlewicz J, Wilmotte J: Reduced clonidine REM sleep suppression in patients with primary major affective illness. Arch Gen Psychiatry 1992; 49:637–642