Glucagon and Clonidine Testing in the Diagnosis of Pheochromocytoma

Ehud Grossman, David S. Goldstein, Aaron Hoffman, and Harry R. Keiser

We assessed the sensitivity and specificity of glucagon stimulation and clonidine suppression tests in the diagnosis of pheochromocytoma in 113 hypertensive patients, 39 with and 74 without the tumor. In the glucagon stimulation test, blood was sampled 2 minutes after intravenous injection of 0.28 μmol (1 mg) glucagon, and in the clonidine suppression test, blood was sampled 3 hours after administration of oral clonidine, 1.30 μmol (0.3 mg)/70 kg body wt. Baseline levels of catechols in antecubital venous blood were abnormal, with norepinephrine greater than 7.10 nmol/l (1,200 pg/ml), epinephrine greater than 1.51 nmol/l (276 pg/ml), norepinephrine/dihydroxyphenylglycol (DHPG) ratio greater than 1.09, or dopa greater than 35.53 nmol/l (7,000 pg/ml), in 30 of 39 patients with pheochromocytoma (sensitivity 77%) and 1 of 74 patients without pheochromocytoma (specificity 99%). Results of the glucagon test were abnormal (norepinephrine greater than 11.83 nmol/l [2,000 pg/ml] or more than threefold increase from baseline) in 25 of 31 patients with pheochromocytoma (sensitivity 81%) and 0 of 72 patients without pheochromocytoma (specificity 100%). Results of the clonidine test were abnormal (after clonidine norepinephrine greater than 2.96 nmol/l [500 pg/ml] or less than 50% decrease from baseline) in 29 of 30 patients with pheochromocytoma (sensitivity 97%) and in 7 of 30 patients without pheochromocytoma (specificity 67%). Very high baseline levels of catechols therefore indicated the presence of pheochromocytoma, but there were several false-negative results when normal levels were obtained. The glucagon test alone was highly specific but not sensitive, and the clonidine test was highly sensitive but less specific. Of 50 patients undergoing both glucagon stimulation and clonidine suppression tests, the results of at least one test were abnormal in 22 of 22 patients with pheochromocytoma (sensitivity 100%) and in 6 of 28 patients without pheochromocytoma (specificity 79%). When both tests were negative, the diagnosis could be excluded, and the results were conclusive in 80% of the patients. Combined glucagon stimulation and clonidine suppression testing is worthwhile in the diagnosis of pheochromocytoma by blood tests. (Hypertension 1991;17:733-741)
The failure of clonidine to suppress NE levels therefore is a positive, or abnormal, result; however, false-positive and false-negative results have been obtained from clonidine suppression testing since some patients without pheos can fail to suppress plasma NE levels after clonidine administration, and some patients with pheo can have normal levels of NE after clonidine.

Several stimulation tests have been proposed in the diagnostic evaluation of pheo, including pressor responses to sympathomimetic agents and glucagon. Because these can produce dangerous increases in pressure in patients with pheo, stimulation tests involving measurements of pressor responses no longer are done. Pretreatment with the α-adrenergic receptor antagonist phenoxybenzamine prevents or ameliorates hypertensive responses but does not appear to prevent plasma catecholamine responses during glucagon stimulation testing.

Glucagon stimulation is thought to be the safest and most specific provocative test for pheo. In this simple test, blood samples for catecholamines are obtained before and 1–2 minutes after intravenous injection of 1 mg glucagon. Because previous studies have included relatively small numbers of patients, estimations of diagnostic sensitivity and specificity related to glucagon stimulation testing have not been reported. Biochemical assays typically have involved measurements only of NE and epinephrine (EPI), and comparisons have not been made between groups with malignant or benign pheos and between groups with sporadic or familial forms. In general, there are no clear, quantitative limits beyond which the biochemical responses to glucagon can be considered to be diagnostic. The usefulness of combined glucagon stimulation and clonidine suppression testing also has not been evaluated.

Over the past 5 years, we have performed clonidine suppression and glucagon stimulation tests in a large number of hypertensive patients with and without pheo. The present report summarizes this experience and establishes ranges of normal and pathological responses to these tests.

For determinations of plasma levels of catechols, we used an accurate liquid chromatographic-electrochemical method that allows simultaneous assessments of concentrations of DHPG, NE, dihydroxyphenylalanine (dopa), EPI, and dopamine.

We wanted to answer the following questions: 1) What is the frequency of false-positive and false-negative results when plasma levels of catechols are measured under resting conditions in hypertensive patients? 2) Do glucagon stimulation and clonidine suppression tests improve the sensitivity or specificity of plasma NE measurements in the diagnosis of pheo? 3) Is a glucagon stimulation test safe? 4) Does treatment with phenoxybenzamine interfere with the results of a glucagon stimulation test? and 5) Does a combination of a glucagon stimulation and a clonidine suppression test lead to sufficiently sensitive and specific results to be useful in screening patients for pheo during single clinic visits? We were especially interested in determining the proportion of patients where results of combined glucagon stimulation and clonidine suppression tests would be conclusive.

### Methods

#### Patients

Of 113 hypertensive patients referred to the Hypertension-Endocrine Branch of the National Heart, Lung, and Blood Institute, either because of symptoms or signs suggesting pheo (e.g., paroxysmal hypertension, orthostatic hypotension, headache, pallor, sweating, palpitations) or because of a family history of pheo, all consented to take part in the study, which was approved by the Institute's Intramural Research Board. Clinical findings are summarized in Table 1.

Of the 113 patients, 74 did not have pheo, based on diagnostic testing including assays of catecholamines and catecholamine metabolites in plasma and urine, computed tomography scans, nuclear magnetic resonance, or MIBG scanning when needed, and clinical follow-up for at least 1 year. The remaining 39 patients had histologically proven pheochromocytomas, either benign (n=25) or malignant (n=14). All patients had determinations of baseline plasma levels of catechols. In 103 patients, a glucagon stimulation test was done (72 patients without and 31 patients with pheo); 60 patients underwent a clonidine suppression test (30 patients without and 30 patients with pheo); and 78 patients (42 patients without and 36 patients with pheo) had 24-hour urine collections for determinations of vanillylmandelic acid (VMA) and total metanephrines (MN). In five patients, testing was conducted more than once, either before and after removal of the tumor or before and during chemotherapy for malignant pheo. Combined glucagon and clonidine testing was conducted in 28 patients without pheo and 22 patients with pheo.

#### Testing Protocol

Patients were taken off medical treatment for at least 1 week before testing. The only exception was that patients could be treated with phenoxybenzamine (n=16).

The glucagon stimulation test was performed in the morning after the patient had fasted overnight.

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**Table 1: Patient Characteristics**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patients without pheochromocytoma</th>
<th>Patients with pheochromocytoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>74</td>
<td>39</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>46±15 (range 16-79)</td>
<td>41±12 (range 21-77)</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>39/35</td>
<td>18/21</td>
</tr>
<tr>
<td>Race (W/B)</td>
<td>64/10</td>
<td>36/3</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>94±13</td>
<td>100±11</td>
</tr>
</tbody>
</table>

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**Parameter**

- **n**: Number of patients
- **Age (yr)**: Mean ± SD (range)
- **Sex (M/F)**: Male/Female
- **Race (W/B)**: White/Black
- **Mean arterial pressure (mm Hg)**: Mean ± SD
An intravenous needle with heparin lock was inserted in an arm vein. The patient rested in the supine position for 20–30 minutes, and heart rate and blood pressure were recorded every 5 minutes with an automated sphygmomanometer. Glucagon (0.28 μmol, 1 mg) was then injected as an intravenous bolus, and blood pressure and heart rate were recorded every 60 seconds for the next 10 minutes. Venous blood was obtained before and 2 minutes after administration of the drug.

When both glucagon and clonidine tests were done in the same session, a period of 1 hour was allowed to elapse after glucagon administration. A second baseline blood sample was drawn, and then clonidine was administered. For the clonidine suppression test, 1.30 μmol (0.3 mg)/70 kg body wt clonidine was given orally, and blood pressure and heart rate were recorded for 3 hours. Venous blood samples were obtained before and 3 hours after drug administration.

Assays

Each blood sample (5 ml) was placed in a heparinized glass tube and chilled in ice. Samples were centrifuged in a refrigerated centrifuge within 30 minutes, and the plasma was transferred to plastic cryotubes, frozen in dry ice, and stored at -70°C until assayed.

The catechol contents in 1-ml aliquots of plasma were partially purified by batch alumina extraction, separated using liquid chromatography, and quantified by the current produced on exposure of the column effluent to oxidizing and then reducing potentials in series.19 Recovery through the alumina extraction step averaged 70–80% for catecholamines and 45–55% for dopa and DHPG. Concentrations of catechols in each sample were corrected for recovery of the internal standard dihydroxybenzylamine. Levels of dopa and DHPG were further corrected for differences in recovery of the internal standard and of dopa or DHPG in a mixture of external standards. The limit of detection was about 10 pg/ml for each catechol.

Urinary VMA and total MN were measured by methods previously described by Pisano et al.20–21 Normal values for VMA and MN are less than 51.5 μmol and less than 4.7 μmol/24 hr.

Data Analysis

Data are presented as mean±SD. In patients where repeated tests were done, only one test was included unless the patient's condition had changed. Analyses of variance for repeated measurements was done to evaluate the responses to glucagon or clonidine. Student's t test was used to assess the significance of differences between groups. Analysis by χ² was used to compare the frequencies of normal and abnormal values or responses in subgroups. Sensitivity and specificity for each test or combination of tests were calculated according to formulas published by the American College of Physicians.22

Results

Baseline Concentrations of Catechols

The upper limits (mean±3 SD) for patients without pheo were 6.95 nmol/l (1,174 pg/ml) for NE and...
Of the patients with malignant pheo, one half had normal plasma NE levels at rest (Figure 1).

Using these reference values, 13 patients with pheo who had normal baseline plasma NE levels, eight had substantial increases in NE levels in subgroups of patients with pheo (Table 3). After glucagon administration, plasma NE levels exceeded the upper limit of normal (7.85 nmol/l, mean±3 SD) in 24 of 31 patients with pheo and in one other patient, plasma NE levels were increased by at least threefold or to over 200 nmol/l, and in one other patient, the ratio of NE to DHPG was elevated (1.51).

Responses to Glucagon

Glucagon was tolerated well by all the patients. In seven patients, after administration of glucagon, the systolic blood pressure exceeded 200 mm Hg; in most of these, phentolamine (5 mg) was given intravenously, and the blood pressure always returned rapidly to baseline, and symptoms of catecholamine excess were abolished.

Glucagon did not affect plasma levels of DHPG, dihydroxyphenylacetic acid, or dopamine; NE levels increased and dopa levels decreased slightly in patients without pheo (Table 3). After glucagon administration, plasma NE levels exceeded the upper limit of normal (7.85 nmol/l, mean±3 SD) in 24 of 31 patients with pheo and in one of 72 patients without pheo (Figure 2). When the results of the glucagon test were considered to be positive if plasma NE levels were increased by at least threefold or to over 11.83 nmol/l (2,000 pg/ml), then all the patients without pheo had a negative test. Among 13 patients with pheo who had normal baseline plasma NE levels, eight had substantial increases in NE levels that were diagnostic after administration of glucagon.

<table>
<thead>
<tr>
<th>Catechol</th>
<th>Patients without pheochromocytoma</th>
<th>Patients with pheochromocytoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHPG (nmol/l)</td>
<td>6.21±2.29</td>
<td>5.86±2.18</td>
</tr>
<tr>
<td>NE (nmol/l)</td>
<td>2.21±1.36</td>
<td>2.47±1.79*</td>
</tr>
<tr>
<td>Dopa (nmol/l)</td>
<td>12.74±6.29</td>
<td>11.97±6.54†</td>
</tr>
<tr>
<td>EPI (nmol/l)</td>
<td>0.25±0.30</td>
<td>0.60±0.91*</td>
</tr>
<tr>
<td>DOPAC (nmol/l)</td>
<td>14.58±12.05</td>
<td>14.33±11.45</td>
</tr>
<tr>
<td>Dopamine (nmol/l)</td>
<td>0.22±0.49</td>
<td>0.23±0.48</td>
</tr>
<tr>
<td>NE/DHPG</td>
<td>0.38±0.20</td>
<td>0.43±0.25*</td>
</tr>
</tbody>
</table>

DHPG, 3,4-dihydroxyphenylglycol; NE, norepinephrine; Dopa, dihydroxyphenylalanine; EPI, epinephrine; DOPAC, 3,4-dihydroxyphenylacetic acid.

*p<0.01 vs. baseline.
†p<0.05 vs. baseline.

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Of the remaining five patients, two had malignant pheo (one had extremely high dopa levels and the other extremely high EPI levels), two had familial disease (Sipple's syndrome or von Hippel-Lindau syndrome), and one had a sporadic, benign pheo. Of the 18 patients with pheochromocytoma, baseline norepinephrine levels were normal at baseline but abnormally high after glucagon.

Overall, the glucagon test was negative in six of 31 pheo patients (false-negative rate of 19%). Four of the six patients had a clonidine test done, and in all four of these patients the results were positive.

Plasma EPI levels increased significantly after administration of glucagon, regardless of the diagnosis (Table 3). In some patients with pheo, in parallel with the increases in NE levels, large increases in EPI levels also were observed.

Pressor responses to glucagon were not analyzed, since most patients with pheo were treated with an α-blocker that blunted the hypertensive response. Plasma NE responses to glucagon did not differ between patients treated with phenoxybenzamine and those not treated with phenoxybenzamine. Among 13 patients with pheo and normal baseline plasma NE levels, the glucagon test was positive in four of six patients treated with phenoxybenzamine and in four of seven patients not treated with phenoxybenzamine (p = NS).

In three patients with malignant disease who had a positive glucagon test before chemotherapy, clinical remission was associated with conversion to a negative test. A subsequent positive test indicated relapse.

### Responses to Clonidine

Clonidine suppressed DHPG, dopa, and NE levels significantly in patients without pheo (Table 4, Figure 3). The clonidine suppression test results were considered to be normal when plasma norepinephrine levels 3 hours after clonidine decreased to below 2.96 nmol/l (500 pg/ml) (see Reference 1). When this criterion was applied, five patients with pheo had
normal suppression after clonidine (false-negative result), and two patients without pheo had a failure to suppress plasma NE (false-positive result). When the criterion for suppression was a NE concentration after clonidine of less than 2.96 nmol/l and a 50% decrease in the plasma norepinephrine level after clonidine,9 then 29 of the 30 patients with pheo had an abnormal clonidine test (Figure 3B); however, according to these criteria, seven patients without pheo also had an abnormal test.

Sensitivity and Specificity

The sensitivity and specificity of each test are summarized in Table 5. When results of the glucagon and clonidine tests were analyzed in the same patients, the results were conclusive, either excluding or diagnosing pheo, in 80% of the patients. Two of six patients without pheo and with inconclusive plasma results had elevated urinary excretion rates of VMA or MN, and three of five patients with pheo and inconclusive plasma results had normal urinary excretion rates of VMA and MN.

The positive predictive value of the glucagon test was 100%, and the negative predictive value was 96%.

Discussion

Pheo poses a diagnostic challenge for every physician who sees patients with hypertension. Various diagnostic tests have been suggested. None is ideal. Measurement of 24-hour urinary excretion of free NE and DHPG has been recommended as a sensitive and specific marker for pheo3; however, this approach can be inconvenient and sometimes impossible in outpatient settings. There still is a need for sensitive, specific blood tests.

Measurement of plasma levels of catechols is an alternative; however, plasma catecholamine levels can vary from day to day in a pheo patient, and in patients with intermittently secreting pheos, plasma NE levels may not always be increased. In the present study, when plasma NE levels were extremely high—more than 1,500 pg/ml or 8.9 nmol/l—the specificity was 100%, that is, this finding alone appears to be sufficient to diagnose pheo, and no further biochemical testing is needed. This may be true, however, only when the patient is not being treated with medication (except for phenoxybenzamine) and blood is drawn 20-30 minutes after insertion of an indwelling intravenous catheter, with the patient in the supine position.

The present results, and the findings of others3-5,24 indicate, however, that baseline levels of catechols are not sufficiently sensitive, because some patients with pheo have normal levels at rest. In the present study, one third (13 of 39) of the patients with pheo had NE levels that were within the range of patients with essential hypertension. Plasma NE levels can be especially misleading in patients with malignant pheo and in those with familial diseases associated with pheo. In the present study, about one half of such patients (seven of 14 patients with malignant pheo...
and three of eight patients with familial diseases) had normal NE levels at rest. The sensitivity of plasma NE concentrations in pheo has varied in different studies, probably because of marked variability in NE concentrations and different proportions of patients with malignant or familial disease. Our results indicated relatively low sensitivity of plasma NE levels alone in patients with malignant or familial disease.

Among the 13 patients with pheo who had normal plasma NE levels under resting conditions, two had elevated EPI levels; one had an increased NE/DHPG ratio, and one patient with malignant disease had extremely high plasma dopa levels, as has been reported in malignant disease. Thus, in addition to NE levels one should consider obtaining levels of other catechols. In particular, high plasma dopa levels may be a marker of malignant disease. The NE/DHPG ratio has been suggested as a better index than NE alone in the diagnosis of pheo, but in the present study, this index was increased only in one patient with normal NE levels. Even taking into account the patients with normal NE levels and abnormal levels of other catechols at rest, there still was a small group of pheo patients who had normal baseline concentrations of all the catechols.

To improve the sensitivity and specificity of plasma levels of catechols, we conducted glucagon stimulation and clonidine suppression tests, in most cases even before results about the catechol concentrations at rest were known. The interpretation of the glucagon test originally was based on the blood pressure response, but later it was shown that the catecholamine response is much more sensitive and specific. Because glucagon increases the blood pressure in patients with pheo through release of NE and EPI from the tumor, the test has been considered risky and has been limited to patients with a diastolic pressure less than 100 mm Hg. In the present study, glucagon was administered to a large number of pheo patients without any untoward consequences. Treatment with α-adrenergic receptor blockers, which prevented or ameliorated pressor responses to glucagon, did not interfere with the biochemical results, confirming a previous case report.

The sensitivity and specificity of the glucagon stimulation test depend on the criteria for a positive or negative test. Based on a small group of patients, Bravo et al defined a positive test when plasma NE increased to at least three times the baseline value or to over 2,000 pg/ml within 1–3 minutes after glucagon administration. In the present study, these criteria proved to be 100% specific, since there were no false-positive results among the large group of patients without pheo. Kuchel et al reported a high percent of false-positive results after glucagon, but they did not present their criteria for a positive test. In the present study, even when patients had normal baseline levels of NE, a positive glucagon test always was associated with pheo, and there were no false-positive results. Glucagon stimulation testing seems to be worthwhile when the baseline NE levels are within the normal range (i.e., below 6.9 nmol/l).

The frequency of false-negative results with plasma NE levels (i.e., baseline levels within normal limits) has been a difficult problem that clonidine suppression testing would not be expected to solve. The glucagon stimulation test was positive and diagnostic in eight of 13 patients with pheo who had normal resting plasma levels of NE. The test was negative in five patients with pheo; all but one of these were patients with malignant or familial disease. Siqueira-Filho et al described four patients with Sipple’s syndrome and pheo in whom the results of the glucagon stimulation test were negative. It seems that the glucagon test is less sensitive in patients with malignant or familial disease; nevertheless, in three patients with malignant pheo, the glucagon test results reflected disease activity.

The clonidine suppression test, first described in 1981, is accepted as a diagnostic test in patients with elevated baseline NE levels; however, the clonidine suppression test is not sensitive and specific enough when it is used as a single test. Most studies have considered the clonidine test to be positive (abnormal) when the sum of plasma NE and EPI levels fails to decrease below 2.96 nmol/l, regardless of the baseline levels. Using this criterion, we also found that the test was not sensitive enough, since in five of 30 patients with pheo the test was negative. Dupont et al reported a patient with an EPI-secreting pheo in whom plasma NE levels were suppressed normally after clonidine administration. One of our patients with a normal clonidine test also had an EPI-secreting tumor. Plewe et al described normal clonidine test results in five patients with pheochromocytoma and with normal plasma catechols. All patients with pheochromocytoma in the present study who had normal suppression after clonidine (NE levels after clonidine less than 2.96 nmol/l) also had baseline plasma NE levels below 4.4 nmol/l. It seems that the clonidine suppression test is not sensitive in patients with normal baseline plasma NE levels or with EPI-secreting tumors. The use of a more liberal criterion for a positive test—an NE concentration after clonidine of more than 2.96 nmol/l (500 pg/ml), or less than a 50% decrease in plasma NE levels after clonidine—increased the sensitivity to 97%, and even patients with pheo and normal baseline plasma NE levels had an abnormal clonidine test. When this liberal criterion was used, however, the specificity of the test was reduced; by this criterion, seven patients without pheo in the present study had an abnormal clonidine test. Similar results were published by Elliott and Murphy. We therefore suggest that clonidine suppression testing alone not be done if the baseline NE level is below 4.4 nmol/l.

We found it feasible and convenient to conduct glucagon stimulation and clonidine suppression tests in the same outpatient clinic visit, and this markedly improved the accuracy of the results. Pretreatment
study there were no pheo patients who had both negative glucagon and clonidine tests, that is, if both tests are negative, the diagnosis can be excluded.

Alternatively, all the testing can be performed during one outpatient visit, with the clonidine test following the glucagon test. The results will either exclude or diagnose pheo in most patients; however, it may be necessary that the patient be taken off all medications other than phenoxymenzamine for at least 1 week.

In conclusion, the glucagon stimulation test, with measurement of plasma concentrations of catechols, is a safe adjunctive test in the diagnostic evaluation of pheo. The glucagon test increases the specificity of the biochemical testing and is useful in patients with normal baseline levels of catechols. False-positive results are less of a problem than false-negative results when the glucagon test is performed. A clonidine suppression test alone, with liberal criteria for an abnormal result, largely eliminates the problem of false-negative results but at the cost of nonspecificity (false-positive results). Combined glucagon stimulation and clonidine suppression tests can be the basis for a highly sensitive and specific diagnostic strategy and can be conducted easily and safely in a single outpatient visit.

References


FIGURE 4. Proposed diagnostic schema for pheochromocytoma.

with the α-receptor blocker phenoxbenzamine did not alter the catechol responses to either test, as noted by others,18-30 and improved the safety of the tests. When both tests were used, the results were conclusive in 80% of the patients.

The present results lead us to suggest the following diagnostic strategy for hypertensive patients in whom pheo is suspected (Figure 4). First, obtain plasma for catechols at baseline. If plasma levels of catechols are markedly increased (e.g., plasma NE greater than 8.9 nmol/l), pheo can be diagnosed biochemically, since there are no false-positive results, even in patients on α-blockers.

If the catechol levels are normal in patients under resting conditions, and pheo still is suspected clinically, then glucagon stimulation testing is performed. If the test is positive, pheo can be diagnosed biochemically, since there are no false-positive results. If a clonidine test were done instead of a glucagon test at this point, false-positive results could be obtained.

This will still leave a small proportion of pheo patients with normal NE levels at baseline and negative results of the glucagon test. Clonidine suppression testing may then be performed, since in the present
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