EVALUATION OF PROPRANOLOL-GLUCAGON TEST

By
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ABSTRACT

A propranolol-glucagon test was evaluated in 24 control normal children, 21 pituitary dwarfs, 15 patients with constitutional short stature, 2 with chromosomal aberration and 4 with miscellaneous diseases. The dose of glucagon enough for the stimulation of human growth hormone (HGH) secretion is more than 20 μg/kg of body weight. During the test in the control subjects the serum HGH level increased from 2.3 ± 1.2 ng/ml to a maximum level of 30.0 ± 15.1 ng/ml, when 10 mg propranolol, regardless of body weight and 30 μg glucagon per kg of body weight are given. The dose of propranolol administered ranged from 0.2 to 1.0 mg/kg of body weight in normal children studied. Serum 11-OHCS also increased significantly from 14.5 ± 11.2 μg/100 ml to 30.1 ± 15.5 μg/100 ml (P < 0.01). There was no difference in the maximum level of urinary total catecholamines in propranolol-glucagon test between 7 pituitary dwarfs and 7 control subjects. The mechanism of HGH response to propranolol-glucagon administration is unknown, but propranolol-glucagon administration is a sensitive and reliable provocative test for HGH secretion, since false negative responses of HGH are not observed in patients with non-pituitary disease.

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It has been widely accepted that the propranolol-glucagon test (P-G test) is useful for the evaluation of human growth hormone (HGH) secretion (Mitchell et al. 1971; Parks et al. 1973). This provocative test has a merit that the procedure is simpler than that of insulin-induced hypoglycaemia (ITT) and arginine infusion (A test), since propranolol can be administered orally and glucagon is given intramuscularly or subcutaneously. However, precise evaluation of this test has not been previously reported. This paper describes endocrinological change during the test in normal subjects and in patients with abnormal stature.

SUBJECTS AND METHODS

Subjects

Twenty-one cases of pituitary dwarf aged from 7 to 25 years were studied. Diagnostic criteria are as follows: 1) well-proportioned dwarf, height is less than -3.0 sd, 2) height increase is less than 12 cm per 3 years when the patients are over 6 years, 3) ratio of bone age to chronological age is less than 0.75, 4) the maximum HGH level in responses to both ITT and A test is less than 5 ng/ml. In this series, patients with short stature, who had no response to the above mentioned two provocative tests but significant or normal response to P-G test were excluded from the group of pituitary dwarfs. Twenty-four normal subjects and 15 non-pituitary dwarfs aged from 4 to 18 years were also studied as control subjects. Patients with simple obesity (1 case), eunuchoid gigantism (1 case), chromosomal aberration (2 cases) and short stature of unknown origin (2 cases) were also investigated for comparison. The serum HGH was measured by radioimmunoassay, serum 11-OHCS by the method of DeMoor et al. (1962) and urinary catecholamine by fluorimetry according to von Euler & Flooding (1956) after absorption and elution on a commercially available alumina column (Oxford Laboratory Inc., Foster City, CA). The recovery by this method ranged from 60 to 80 per cent in our laboratory and the values were corrected in each assessment. Bone age was evaluated according to the standard of Greulich and Pyle.

Procedure

In insulin-induced hypoglycaemia (ITT), regular insulin was injected iv at a dose of 0.1 unit per kg of body weight and in an arginine test (A test), arginine chloride was injected iv at a dose of 0.5 g per kg of body weight over a period of 30 min. Blood specimens were obtained every 30 min for 2 h. In the propranolol-glucagon test (P-G test), glucagon was injected im at a dose of 30 \( \mu g/kg \) of body weight, and 10 mg of propranolol was simultaneously given orally. Blood was drawn every 30 min for 3 h. Subjects whose blood glucose did not decrease to less than 50% of initial value in ITT were excluded from this study.

The sera were stored at -20°C for analysis of HGH and 11-OHCS. The doses of propranolol and glucagon were changed in some of the experiments as indicated in Table 1. All these provocative tests were undertaken after over night fasting. In one of the experiments urine was collected every 2 h before and after the beginning of the P-G test, for a period of 6 h, and kept for assessment of catecholamines. In this experiment, 7 control subjects and 7 pituitary dwarfs who showed normal urinary 17-OHCS following SU 4885 administration were studied. The number of subjects in each experiment is summarized in Table 1.
Table 1.
The purpose of each experiment and number of subjects studied.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purpose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of Subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>3</td>
<td>3</td>
<td>10</td>
<td>7</td>
<td>(5)</td>
</tr>
<tr>
<td>F</td>
<td>1</td>
<td>2</td>
<td>5</td>
<td>5</td>
<td>(2)</td>
</tr>
<tr>
<td>Total</td>
<td>4</td>
<td>5</td>
<td>15</td>
<td>12</td>
<td>(7)</td>
</tr>
<tr>
<td>Propranolol (po)</td>
<td>20 mg</td>
<td>10 mg</td>
<td>10 mg</td>
<td>10 mg</td>
<td>10 mg</td>
</tr>
<tr>
<td>Glucagon (im)</td>
<td>1 mg</td>
<td>10, 20, 30 μg/kg of body weight</td>
<td></td>
<td>30 μg/kg of body weight</td>
<td></td>
</tr>
</tbody>
</table>

1) N: normal subjects.
2) P. D.: pituitary dwarf.
3) C. S. S.: constitutional short stature.
4) ( ): included in experiment 3.
RESULTS

1) Effect of propranolol on HGH secretion following glucagon (Experiment 1)

In order to assess the effect of propranolol on HGH secretion to glucagon stimulation, 1 mg of glucagon was given to 4 normal subjects and 1 pituitary dwarf and, at intervals of 1 week, 1 mg of glucagon was given to the same subjects simultaneous oral administration of 20 mg propranolol. The average maximum level of serum HGH in the P-G test (37.5 ± 6.6 ng/ml) was significantly ($P < 0.05$) higher than that following glucagon administration only (22.9 ± 16.9 ng/ml).

The increase of serum 11-OHCS ranged from 3.0 to 10.5 µg/100 ml (7.4 ± 2.9) in the glucagon test and from 15.0 to 30 (20.5 ± 6.8) µg/100 ml in P-G test. The latter was significantly ($P < 0.01$) higher than the former.

2) Effect of dose of glucagon on HGH secretion (Experiment 2)

In order to determine an appropriate dose of glucagon for stimulating HGH secretion, 5 normal children were given glucagon at the dose of 10, 20, and 30 µg per kg of body weight at interval of 2 days and simultaneously given 10 mg of propranolol regardless of the body weight. Fig. 1 shows that HGH did not increase to more than 10 ng/ml in 2 of 4 cases when glucagon was given at a dose of 10 µg/kg of body weight, but the maximum level of HGH was more than 10 ng/ml in all cases following a dose of 20 and 30 µg/kg of body weight. The pituitary dwarf showed no response of HGH to any dose of glucagon administered.

![Fig. 1. HGH response to propranolol-glucagon test. Ten mg of propranolol regardless of body weight and glucagon at the dose of 10, 20 and 30 µg/kg were given to 5 normal children and 1 pituitary dwarf. HGH responded to propranolol (10 mg) and glucagon (10 µg/kg) in 2 of 5 normal subjects and to propranolol (10 mg) and glucagon (20–30) µg/kg) in all patients. No response of HGH was observed in a pituitary dwarf (○—○).](image-url)
Serum HGH (●—●) and 11-OHCS (●...●) responses to P-G test in 12 normal subjects and serum HGH response to P-G test in 12 pituitary dwarfs (O——O). The mean maximum level of HGH and the mean increment of 11-OHCS during the test was 30.0 ± 15.1 ng/ml and 20.3 ± 6.4 µg/100 ml, respectively. In the pituitary dwarfs, HGH levels before and at 120 min after the test were 1.6 ± 0.31 and 2.2 ± 1.32 ng/ml, respectively.

3) Normal range of HGH secretion in response to propranolol-glucagon stimulation (Experiment 3)

Ten mg of propranolol, regardless of body weight and 30 µg glucagon per kg of body weight were given simultaneously to 15 control subjects. The HGH level increased from 2.3 ± 1.2 ng/ml to more than 10 ng/ml in all cases with mean and sd of maximum levels, 30.0 ± 15.1 ng/ml (Fig. 2). Hence, HGH response to P-G test was considered to be normal when the maximum level of HGH was more than 10 ng/ml. The serum cortisol increased from 14.5 ± 11.2 µg/100 ml to 30.1 ± 15.5 µg/100 ml at 180 min (P < 0.01), and its increment was 20.3 ± 6.4 µg/100 ml. In 12 pituitary dwarfs, HGH levels before and at 90, 120, 180 min after the test were 1.6 ± 0.31, 2.2 ± 1.32, 1.8 ± 1.41, 1.8 ± 0.92 ng/ml, respectively. The maximum levels were all less than 3.8 ng/ml. Although 10 mg of propranolol was given to each subject regardless of body weight, this dose was in the range of 0.2 to 1.0 mg per kg of body weight as observed in those control subjects who showed normal HGH response to P-G test.

The blood glucose levels of control and patients were 82.0 ± 10.0; 137.5 ± 25.5 and 75.6 ± 14.1 mg/100 ml at 0, 30 and 150 min, respectively, while those of pituitary dwarfs were 79.7 ± 12.4, 127.6 ± 17.6 and 66.2 ± 17.6 mg/100 ml at 0, 30 and 150 min, respectively. There was no significant correlation between the maximum HGH levels and the changes in the falling blood glucose levels in the control subjects.
Table 2.
Change of urinary total catecholamines level (μg/2 h) in propranolol-glucagon test for 7 normal children and 7 pituitary dwarfs. Total urinary catecholamines increased significantly in both groups.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Before P-G test</th>
<th>After P-G test</th>
<th>Maximum level during the test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time h</td>
<td>2 h (-2 ~ 0)</td>
<td>2 h (0 ~ +2)</td>
</tr>
<tr>
<td>Pituitary dwarfs</td>
<td></td>
<td>2 h (-2 ~ 0)</td>
<td>2 h (0 ~ +2)</td>
</tr>
<tr>
<td></td>
<td>µg</td>
<td>2 h (-2 ~ 0)</td>
<td>2 h (0 ~ +2)</td>
</tr>
<tr>
<td></td>
<td>µg</td>
<td>2 h (-2 ~ 0)</td>
<td>2 h (0 ~ +2)</td>
</tr>
<tr>
<td>Pituitary dwarfs</td>
<td>A</td>
<td>0.71 ± 0.32</td>
<td>0.94 ± 0.62</td>
</tr>
<tr>
<td></td>
<td>NA</td>
<td>1.43 ± 0.96</td>
<td>2.62 ± 2.75</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>2.15 ± 1.09</td>
<td>3.85 ± 3.42</td>
</tr>
<tr>
<td>Normal children</td>
<td>A</td>
<td>0.57 ± 1.58</td>
<td>2.36 ± 1.59</td>
</tr>
<tr>
<td></td>
<td>NA</td>
<td>1.12 ± 0.63</td>
<td>1.57 ± 1.19</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>1.69 ± 1.10</td>
<td>3.90 ± 2.54</td>
</tr>
</tbody>
</table>

A: Adrenaline, N: Noradrenaline, T: Total catecholamines.

1) significantly higher (P < 0.05) than the initial values.
2) significantly higher (P < 0.025) than the initial values.

4) Catecholamine levels in propranolol-glucagon test (Experiment 4)

Urinary catecholamines were assayed for the specimen obtained in -2 ~ 0, 0 ~ +2, and +2 ~ 4 h of the PG test in 7 control subjects and 7 pituitary dwarfs who showed a normal response of urinary 17-OHCS to SU 4885 as mentioned before. Total urinary catecholamines increased significantly from 1.69 ± 1.10 to the maximum level of 4.39 ± 2.59 μg/2 h (P < 0.05) in control subjects, and 2.15 ± 1.09 to 6.55 ± 3.03 μg/ml in pituitary dwarfs (P < 0.025) as shown in Table 2. There was no significant difference, however, in the maximum levels between pituitary dwarfs and control subjects.

5) Comparison of P-G test with other provocative tests (Experiment 5)

The maximum levels of HGH in ITT and P-G test were compared in patients with pituitary dwarfs (12 cases) and non-pituitary dwarfs (12 cases). None of pituitary dwarfs responded to either test. In 4 of the 12 non-pituitary dwarfs,
the HGH level did not increase to more than 10 ng/ml in ITT, despite a normal response to the P-G test. In one case of eunuchoidal gigantism (pure gonadal dysgenesis) the maximum HGH level in ITT and A test was less than 5 ng/ml but a significant increase of HGH from 0.9 to 14.2 ng/ml was obtained only in the P-G test. In 6 cases with miscellaneous diseases, the serum HGH did not increase to more than 10 ng/ml in ITT and/or A test, but this occurred in P-G test. In 2 patients with chromosomal aberration, no HGH responses to both ITT and A test were observed, but the response was normal in the P-G test.

**DISCUSSION**

Mitchell et al. (1971) reported that propranolol enhanced the HGH response to glucagon while Parks et al. (1973) also compared to the HGH responses to P-G stimulation with other provocative tests. It has been confirmed that this test provides a potent stimulus to HGH secretion in children but the actual dose of glucagon and propranolol necessary has not been established. According to our limited data, HGH secretion is consistently stimulated when glucagon is given at a dose of more than 20 μg/kg of body weight. The dose of propranolol probably does not need to be more than 1 mg per kg of body weight, when glucagon is administrated at the dose of 30 μg/kg of body weight.

The mechanism of HGH response to propranolol and glucagon administration is unknown. Serum glucagon level reaches a peak at 15 min and the highest level is sustained until 120 min when exogenous glucagon is given sc (Sugase et al. 1976), although no data are available when this is given im. HGH and 11-OHCS start to increase at 90 and 120 min, and the average peak of serum 11-OHCS is observed at 180 min of the test. This time lag suggests that there must be some intermediate substance which occurs between exogenous glucagon circulating in the blood and the HGH response. It seems that this HGH response is caused by a stress since a significant increase of serum 11-OHCS and urinary catecholamines is associated with this. Further investigation, however, will be needed to confirm how such a stress is induced by P-G test.

HGH secretion in the ITT and the A test is functionally impaired in patients with hypogonadism (Hashimoto et al. 1971) and in prepubertal normal subjects (Parker et al. 1967) and it returns to normal when treated with sex hormones. The P-G test is a sensitive and reliable provocative test for HGH secretion since the HGH secretion in some patients with miscellaneous diseases showed no or sub-normal response to other tests though normal to P-G. It is natural, however, that the normal HGH secretion in response to P-G test does not always mean that there is a normal HGH secretion in response to the physiological stimuli of daily life.

249
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REFERENCES


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