PROLACTIN LEVELS IN THE INSULIN TOLERANCE TEST WITH AND WITHOUT PRE-TREATMENT WITH DEXAMETHASONE

By

P. O. Osterman, J. Fagius and L. Wide

ABSTRACT

The serum prolactin response to insulin-induced hypoglycaemia was studied in 20 healthy volunteers. The results of insulin tolerance tests performed after pre-treatment with dexamethasone in a dose of 0.5 mg (0.5-DEX-ITT) and 1.0 mg (1.0-DEX-ITT) were compared with those of a similar control insulin tolerance test (ITT). The serum prolactin levels increased in all but 3 ITT's. In the ITT and 0.5-DEX-ITT the prolactin responses were significantly greater in women than in men. After pre-treatment with dexamethasone the basal serum prolactin levels and the prolactin response to hypoglycaemia were significantly decreased compared with those in the ITT. The prolactin response both to insulin-induced hypoglycaemia and to dexamethasone showed such a large individual variation that this type of response seems unsuitable for evaluation of the hypothalamo-pituitary function.

Numerous studies on human prolactin have been performed during recent years and several factors which stimulate or inhibit prolactin secretion have been reported (Frantz et al. 1972). Insulin-induced hypoglycaemia has been observed to promote an increase in prolactin secretion (Copinschi et al. 1972; Noel et al. 1972), although this finding is not unanimous (Cohen & Gala 1975).

The somatotrophin (GH) response to hypoglycaemia is well established and is used as a test of hypothalamo-pituitary function (Frantz & Rabkin 1964; Greenwood et al. 1966). The purpose of the present investigation was to study the prolactin response to insulin-induced hypoglycaemia in order to see whether
the prolactin response might also be used as a test of this function. In a previous investigation of the 11-hydroxycorticosteroid and GH responses to hypoglycaemia it was found to be advantageous to pre-treat with dexamethasone the evening before an insulin tolerance test (Osterman & Wide 1976). The effect of pre-treatment with 0.5 mg and 1 mg dexamethasone on the prolactin response to hypoglycaemia was therefore also assessed.

MATERIALS AND METHODS

Twenty healthy, non-obese, ambulatory volunteers, 11 women and 9 men, were studied. Their mean age was 24 years (range 19–36 years). None of them were receiving any drugs or oral contraceptives. All the women had normal menstrual cycles. The tests were not performed on any particular day of the menstrual cycle. The subjects fasted from 9 p.m. the day before the test. They arrived at the Department of Neurology at 7 a.m. and rested on a bed until the test was finished. A venous cannula was inserted into a cubital vein and during the test it was kept patent with heparinized isotonic saline. Before each blood specimen was taken, a 1 ml sample was discarded to avoid saline contamination. Blood samples were taken at 7.50, 8.00, 8.30, 8.45, 9.00 and 9.30 a.m. Soluble insulin (0.15 U/kg b. w.) was injected intravenously at 8.00 a.m. Sera for the estimation of prolactin were stored at −20°C until assayed. In the same experiment the GH and 11-hydroxycorticosteroid responses to insulin-induced hypoglycaemia were studied in these 20 volunteers and in an additional 5 healthy subjects. Further details about the experimental design are given in a recent report on that part of the study (Osterman & Wide 1976).

Three insulin tolerance tests were performed on each subject. At 11 p.m. on the day before the test the subject took dexamethasone (Dexacortal®) in a dose of 1.0 mg (1.0-dexamethasone-insulin tolerance test = 1.0-DEX-ITT) or 0.5 mg (0.5-dexamethasone-insulin tolerance test = 0.5-DEX-ITT) or no dexamethasone (control insulin tolerance test = ITT). The 3 tests were usually performed within 1 week on the same subject. In 12 subjects the ITT was carried out first, in 3 subjects the 0.5-DEX-ITT and in 5 subjects the 1.0-DEX-ITT.

The basal levels of serum prolactin (GH and blood glucose) were determined as the mean of the levels at 7.50 and 8.00 a.m.

The measured maximal increase in prolactin (and GH) varied considerably between different subjects (prolactin: range < 0–41 μg/l; GH: range 1.4–38 μg/l) as also did the time at which the maximum occurred. The increase in prolactin (and GH) secretion was therefore estimated planimetrically and expressed in area units (1 area unit = \( \frac{1 \text{ μg} \times \text{min}}{1} \)). An example of the area which was calculated is shown in Fig. 1.

The results were assessed by Student’s t-test for unpaired and paired observations.

Prolactin in serum was measured radioimmunologically by the use of \(^{125}\text{I}\)-labelled human prolactin and rabbit anti-human prolactin antibodies coupled to CNBr-activated ultrafine Sephadex particles (Wide et al. 1967; Wide 1969). The prolactin and anti-prolactin preparations were supplied by the National Institute of Arthritis, Metabolism and Digestive Diseases, National Institute of Health, Bethesda, and the ultrafine Sephadex particles by Pharmacia AB, Uppsala. The sensitivity of the assay was 0.5–1 μg of prolactin per l and it showed no cross-reaction with either follitrophin,
The increase in prolactin secretion in each test was estimated planimetrically and expressed in area units. The figure shows an example of the calculated area.

Mean blood glucose levels (combined values for women and men) in the control-insulin tolerance test and 0.5-dexamethasone-insulin tolerance test (□——□) and in the 1.0-dexamethasone-insulin tolerance test (▽——▽). Range of standard errors of the mean (SEM): 0.09–0.19 mmol/l).
lutrophin, thyrotrophin or somatotrophin in physiological concentrations. The serum samples were assayed in duplicate in 100 μl aliquots, and for each subject all samples were run in the same assay.

The values for blood glucose and serum GH in the 20 subjects were obtained from the previous investigation in which these volunteers were included (Osterman & Wide 1976), and are given below.

Blood glucose was determined by a glucose oxidase method (Hjelm & de Verdier 1963). The blood glucose fell to less than 2.2 mmol/l in all tests but 3 (range 0.7–2.6 mmol/l) and the fall in the basal level exceeded 50 per cent in all tests. The mean levels in each type of test are shown in Fig. 2. There was no difference (<3% at all points of time) between the blood glucose levels in the ITT and the 0.5-DEX-ITT. The levels in the 1.0-DEX-ITT were somewhat higher than in the other 2 tests, the difference being significant for the basal values and the values at 9.00 a.m. (P < 0.02 and P < 0.05, respectively), but the mean nadir blood glucose levels and the mean fall in blood glucose did not differ between the different types of test.

The GH concentration in the serum was assayed by the radioimmunosorbent technique described by Wide et al. (1967), in a recently reported modification in which the antibodies are indirectly coupled to the solid phase (Wide et al. 1973). Data on GH preparations used as reagents and standards in the assay, and the precision and sensitivity of the assay, have been given previously (Osterman & Wide 1976). The mean basal serum GH levels and the mean increases in GH for women and men are

Fig. 3.

Mean serum prolactin levels (combined values for women and men) in the control-insulin tolerance test (■——■), 0.5-dexamethasone-insulin tolerance test (▼……▼) and 1.0-dexamethasone-insulin tolerance test (●——●). The vertical lines represent ± 1 SEM.
Table 1.
The results of the control insulin tolerance test (ITT), 0.5-dexamethasone-insulin tolerance test (0.5-DEX-ITT) and 1.0-dexamethasone-insulin tolerance test (1.0-DEX-ITT) in 20 healthy volunteers.

<table>
<thead>
<tr>
<th>Test</th>
<th>GH</th>
<th>Prolactin</th>
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<tbody>
<tr>
<td></td>
<td>Basal level (µg/l)</td>
<td>Increase (area units)</td>
</tr>
<tr>
<td></td>
<td>Women</td>
<td>Men</td>
</tr>
<tr>
<td>ITT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
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<td>Confidence limits</td>
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<td>0.06–1.3</td>
</tr>
<tr>
<td>Range</td>
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<td>0.14–2.4</td>
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<tr>
<td>0.5-DEX-ITT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>1.9</td>
<td>0.65</td>
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<tr>
<td>Confidence limits</td>
<td>0.91–2.9</td>
<td>0–1.6</td>
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<tr>
<td>Range</td>
<td>0.32–4.5</td>
<td>1.19–3.9</td>
</tr>
<tr>
<td>1.0-DEX-ITT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
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<td>0.31</td>
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<tr>
<td>Confidence limits</td>
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<tr>
<td>Range</td>
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<td>0.15–0.83</td>
</tr>
</tbody>
</table>

Abbreviations: M = arithmetic mean value. Confidence limits = 95 per cent confidence limits of the mean.
shown in Table 1. In all the tests the basal serum GH level was low and there was a normal GH response to the insulin-induced hypoglycaemia. The mean basal serum GH levels were lower in men than in women in all 3 tests, the difference being significant in the ITT \((P < 0.05)\) and 1.0-DEX-ITT \((P < 0.01)\). The GH increase also differed between the sexes. The mean increase in GH secretion was greater in men than in women – this difference being significant in the ITT \((P < 0.01)\) and the 0.5-DEX-ITT \((P < 0.001)\). In men the increase in GH secretion was significantly lower in the 1.0-DEX-ITT than in the ITT \((P < 0.05)\) and the 0.5-DEX-ITT \((P < 0.02)\); in women there were no such differences.

**RESULTS**

The mean prolactin levels in the 3 tests are presented in Fig. 3. The mean basal serum prolactin levels and the mean increases in prolactin for women and men are given in Table 1.

The basal levels were somewhat higher in women than in men in all 3 tests, but none of these differences was significant. There were no significant differences between the basal prolactin levels in the first, second and third test in succession \(\text{women}: 5.8, 4.9\) and \(5.0\ \mu\text{g/l}; \text{men}: 3.3, 2.3\) and \(2.8\ \mu\text{g/l}; \text{women plus men}: 4.6, 3.9\) and \(4.2\ \mu\text{g/l}, \text{respectively}\). The mean basal prolactin level \(\text{combined value for women and men}\) was significantly higher in the ITT than in the 0.5-DEX-ITT and the 1.0-DEX-ITT \((P < 0.02\) and \(P < 0.01,\) respectively), and the basal level in the 0.5-DEX-ITT was significantly higher than that in the 1.0-DEX-ITT \((P < 0.05)\). There was a sex difference in prolactin increase in all 3 tests, women showing greater responses. The difference was significant in the ITT \((P < 0.02)\) and the 0.5-DEX-ITT \((P < 0.02)\) but not in the 1.0-DEX-ITT. The mean increase in serum prolactin level \(\text{combined value for women and men}\) was significantly lower after pre-treatment with 0.5 mg \((P < 0.05)\) and 1.0 mg \((P < 0.01)\) dexamethasone. However, the effect of dexamethasone pre-treatment varied considerably between different subjects – in some the increase in secretion was even greater in the 1.0-DEX-ITT than in the ITT. The responses in the 0.5-DEX-ITT and the 1.0-DEX-ITT showed no significant differences.

Some of the subjects exhibited no increase in prolactin secretion at all in one or more of the tests, although the hypoglycaemia was adequate. In the men 2 showed no response in the ITT, 2 in the 0.5-DEX-ITT and 1 in the 1.0-DEX-ITT. In the women one failed to respond in the ITT, 3 in the 0.5-DEX-ITT and 3 in the 1.0-DEX-ITT. One woman exhibited no increase in any of the 3 tests and 2 men and 1 woman showed no response in 2 tests. The basal prolactin levels of those who failed to respond did not differ from those who did respond.

There was no correlation between the increase in serum prolactin and GH.
DISCUSSION

It is generally considered that a fall in blood glucose to less than 50 per cent of the basal fasting level and to less than 2.2 mmol/l (40 mg/100 ml) is sufficient to promote an ACTH and GH response. In the present study such hypoglycaemia and enhancement of the serum GH levels was obtained in all tests.

The basal serum prolactin levels in men and women in this study are well in accordance with those previously reported in radioimmunoassay investigations (L'Hermite 1973; Aubert et al. 1974). The serum prolactin levels increased in most of the tests. However, the response varied considerably between different subjects, and in 3 control insulin tolerance tests there was no increase at all. Previous reports on the effect of insulin-induced hypoglycaemia on serum prolactin levels have been contradictory. A significant prolactin response has been reported in healthy women (Frantz et al. 1972; Noel et al. 1972). A hypoglycaemia-induced prolactin increase was also found in 15 out of 16 subjects by Copinschi et al. (1975) and the investigators suggested that the insulin tolerance test might be useful as a test for the clinical investigation of prolactin secretion. In contrast, Cohen & Gala (1975) found an increase in the prolactin level in only 2 out of 7 subjects and they attributed the responses in these 2 subjects to stress reactions. The results of the present study show that hypoglycaemia usually promotes an increase in the prolactin level, but the response shows a large individual variation and is sometimes absent. The determination of the prolactin response in the insulin tolerance test therefore seems unsuitable as a diagnostic tool.

The daytime serum prolactin levels in normal subjects have been reported to be higher in women than in men (Jacobs et al. 1972; Guyda & Friesen 1973; Aubert et al. 1974; Adler et al. 1975). The present study also revealed a similar, but non-significant, sex difference in the basal levels. The effect of physical and emotional stress on the basal serum GH level appears to be greater in women than in men (Frantz & Rabkin 1965; Osterman & Wide 1976). The higher basal prolactin levels in women might also be explainable by a greater effect of stress in this sex. The lack of differences in prolactin levels between the first, second and third test in succession in the women indicates that the effect of emotional stress on the prolactin secretion was not as pronounced as on the GH secretion, and that any sex differences in basal serum prolactin levels probably were not due to emotional stress.

The increase in serum prolactin was significantly greater in women than in men. To our knowledge a sex difference in the prolactin response to insulin-induced hypoglycaemia has not been reported previously, although the prolactin responses to thyroliberin (Snyder et al. 1973) and surgical stress (Noel et al. 1972) have been found to be higher in women than in men. The
greater prolactin response to hypoglycaemia in women is in contrast to the findings concerning GH, for which the reverse was noted.

After pre-treatment with dexamethasone there was a decrease in the basal prolactin levels and also in the prolactin responses compared with those in the control tests. The greater prolactin response in the ITT might conceivably be explained by greater emotional stress in that test -- in 12 subjects the ITT was performed first of the 3 tests, whereas the 0.5-DEX-ITT was the first one in only 3 subjects and the 1.0-DEX-ITT was the first in only 5 subjects. The lack of differences in basal prolactin levels between the first, second and third test in succession indicates that any differences in emotional stress between the tests had little effect on the prolactin secretion. The blood glucose levels were slightly higher in the 1.0-DEX-ITT than in the other 2 tests, which might have contributed to the lower prolactin response in the 1.0-DEX-ITT. The mean nadir values and the mean fall in blood glucose levels, however, did not differ in the 3 tests. Neither were there any differences in the blood glucose levels between the ITT and 0.5-DEX-ITT, while the prolactin level increased to a significantly greater extent in the ITT than in the 0.5-DEX-ITT. The results of the present study therefore indicate, in accordance with previous findings (Copinschi et al. 1975), that dexamethasone per se exerts a suppressive effect on the prolactin response to hypoglycaemia and on the basal prolactin secretion, and that this effect is dose-dependent. However, the suppressive effect of dexamethasone appears to be somewhat capricious -- in the present study there were some individuals in whom the response was not suppressed at all, and in some subjects the response was in fact most marked in the 1.0-DEX-ITT.

To summarize, the present results show that insulin-induced hypoglycaemia usually elicits an increase in the serum prolactin level, but that not infrequently the response is absent in healthy individuals. The prolactin increase is greater in women than in men. Pre-treatment with dexamethasone suppresses the prolactin response to hypoglycaemia. The prolactin response both to insulin-induced hypoglycaemia and to dexamethasone shows such a large individual variation that this response seems unsuitable for evaluation of the hypothalamo-pituitary function.

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