Transient impairment or delay of urinary trihydroxy-4-pregnanone (THS) response to metyrapone in boys with delayed adolescence and in patients with isolated growth hormone deficiency

M. Zachmann, D. Tassinari, W. Sorgo, G. U. Exner,
B. Kempken and A. Prader

Department of Paediatrics University of Zurich, Kinderspital, 8032 Zurich, Switzerland

Abstract. Twentythree boys with delayed adolescence (age 15.7 ± 2.0, bone age 12.4 ± 2.1 years) were studied. Their cortisol response to insulin was normal. After oral metyrapone (500 mg/m² by mouth) one to three consecutive 12 h urine samples were collected for analysis of THS. Thirtyseven tests with 37 first, 21 second, and 11 third samples were carried out. The results could be divided into two main groups: 25 tests (group A) were subnormal in the first sample, 12 of them with a very weak (40 ± 8 µg/m²/12 h) and 13 with an insufficient (191 ± 16 µg/m²/12 h) THS response. Values in the second and third sample were higher, indicating a delayed response. In 12 tests (group B), the results were normal (1016 ± 143 µg/m²/12 h) in the first and lower in the second and third samples. In three patients with repeated tests, there was improvement with increasing bone age.

The THS-responses to metyrapone did not correlate with those of growth hormone, gonadotrophins, and TSH to stimuli. It is concluded that the THS-response to a single dose of metyrapone may be temporarily insufficient or delayed in delayed adolescence. We interpret this finding as showing transiently reduced or slow hypothalamic responsiveness.

Delayed adolescence is a common problem in paediatric endocrine practice. In mild cases, with familial occurrence, the diagnosis is easy and the distinction from endocrine disorders can be made clinically without extensive laboratory investigations. In more severe cases with marked retardation of growth and bone maturation, the differentiation from GH-deficiency (IGHD or combined with Gn-deficiency) may be difficult, even if laboratory tests are carried out. Transient GH-deficiency has been shown to exist in some of these patients.
(Trygstad 1977; Prader et al. 1980). Originally, we carried out metyrapone tests in uncertain cases to confirm or exclude ACTH-deficiency, which might be present in addition to GH-deficiency in patients with true persistent hypothalamic or hypopituitary dwarfism. However, from the results, we got the impression that an insufficient response to metyrapone could be found even in 'benign' DA, where ACTH-deficiency was excluded by other tests. We have subsequently performed metyrapone tests in boys with confirmed diagnosis of 'benign' DA. In some of them, we did indeed find an impaired or delayed response.

**Patients and Methods**

The original series consisted of 30 boys with DA. Seven of them had to be excluded, because they showed evidence suggesting true persistent GH-deficiency. In the remaining 23 patients, whose results are presented, it is reasonably certain that they do not suffer from any persistent hormone deficiencies. Their clinical and laboratory data are shown in Tables 1 and 2.

Besides a normal subsequent growth pattern, the following reasons make persisting hypothalamic or pituitary hormone deficiencies unlikely: 1) in 19 patients, where information could be obtained from the parents, the father continued to grow after the age of 18 years and/or menarche in the mother occurred above age 14.5 years; 2) 5 patients were of normal stature for CA (Prader et al. 1981) during prepuberty and throughout the observation period. Among those whose stature was normal during prepuberty, but below the third centile at a pubertal CA, none showed any evidence of acquired organic GH-deficiency (normal skull radiography, no diabetes insipidus, no neurological symptoms); 3) 5 other patients, whose GH-stimulation tests gave intermediate results, received hGH temporarily (before the present study was performed). It had some effect on the growth rate (Prader et al. 1980), but did not induce the catch-up spurt characteristic of true GH-deficiency; 4) in 3 patients, who were given a long-acting testosterone preparation intramuscularly or oxandrolone by mouth (psychosocial indication), androgen alone induced a marked acceleration of the growth rate (Hopwood et al. 1979) as would not be seen in true GH-deficiency (Aynsley-Green et al. 1976); 5) in 5 patients, the GH-response to arginine and in 9 that to insulin was normal. In the remaining patients, intermediate GH-results were found. None of the patients had an absent GH-response to both insulin and arginine; 6) in 12 patients, in whom it was analyzed, T₄ was normal; 7) the LH- and FSH-response to LRH was normal in 11 patients, in whom it was studied; none had anosmia; 8) ACTH-deficiency was excluded in 16 of the 23 patients by a normal plasma cortisol response to insulin. In the others, there was no clinical evidence suggesting ACTH-deficiency (normal physical performance, no history of hypoglycaemia).

The mean CA of the 25 patients was 15.6 ± 0.46 years, their mean BA (Greulich & Pyle 1959) 12.4 ± 0.45 years (Table 1). The mean BA-retardation was thus 3.2 ± 0.13 years (range 2.1 to 4.9 years).

Height for CA was retarded by a mean of 2.61 sd (Table 1). For BA, it was normal (mean =–0.45 sd). Pubic hair stages ranged from 1 to 5 and were mostly 2 or 3 (Tanner 1962). Some of the patients with stage 5 had been pre-treated with androgens (see below).

Testicular volume ranged from 1 to 20 ml (mean 7.2 ± 0.8 ml) (Zachmann et al. 1974b). Only one patient had a TV of 20 ml (CA 18.7, BA 14 years). Within 1.7 years, his TV increased from 3.5 to 20 ml.

In the 23 patients, 37 metyrapone tests (single dose, 500 mg/m² by mouth) with collection of 12 h urine samples were carried out as reported (Zachmann et al. 1974a). The test was subsequently expanded by collecting, if possible, a second and third consecutive 12 h sample. In this way, 21 second and 11 third samples were collected in addition.

In one patient 7, in one 4, in one 3, and in three patients 2 metyrapone tests were performed at different stages of maturation.

Seven patients had received androgens before the test (longacting testosterone ester preparation (Triolandren® CIBA) 100 mg monthly im, discontinued at least 3 months before the test). One patient received oxandrolone (0.15 mg/kg daily) by mouth and this was also discontinued before the test. Two tests were repeated while the patients were on androgen treatment. Six tests were done in 3 patients who had received hGH (6 IU/m² im twice weekly), which was discontinued at least 1 month before the test. Three other tests were performed while the patients were on hGH treatment. In one patient, a full dose classic metyrapone test (2000 mg/m², divided into 4 doses every 6 h with 24 h urine collection on the following day) was done in addition to the single dose test. The results of the patients with DA were compared with those in 18 patients (12 boys, 6 girls) with true, persistent IGHD, all of whom had responded to subsequent hGH treatment with a characteristic catch-up growth spurt. In all these patients, the cortisol response to insulin was normal (basal 13.8 ± 9.2, maximum after insulin 31.4 ± 3.2 µg/100 ml), excluding concomitant ACTH-deficiency.

Since metyrapone tests in these patients had been carried out before the study in boys with DA, only a first 12 h sample has been collected. It is therefore not possible to draw any conclusions with respect to the THS dynamics during the second and third 12 h period after ingestion of metyrapone in patients with IGHD as in the boys with DA. For analysis, standard statistical procedures were used. Unless stated otherwise, mean values
Clinical data ($\bar{x} \pm$ SEM) in patients with delayed adolescence. Group A$_1$ = absent, A$_2$ = insufficient, B = normal THS response to metyrapone in first 12 h urine sample (see text).

<table>
<thead>
<tr>
<th></th>
<th>CA (years)</th>
<th>BA (years)</th>
<th>Height SDS for CA</th>
<th>Height SDS for BA</th>
<th>TV (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group A$_1$</strong></td>
<td>15.65 ± 0.6</td>
<td>12.0 ± 0.7</td>
<td>-2.72 ± 0.52</td>
<td>-0.29 ± 0.36</td>
<td>8.0 ± 1.7</td>
</tr>
<tr>
<td>(n = 12)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td><strong>Group A$_2$</strong></td>
<td>15.60 ± 0.5</td>
<td>12.4 ± 0.6</td>
<td>-2.41 ± 0.42</td>
<td>-0.31 ± 0.38</td>
<td>7.5 ± 1.2</td>
</tr>
<tr>
<td>(n = 13)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Group B</strong></td>
<td>15.59 ± 0.6</td>
<td>12.8 ± 0.5</td>
<td>-2.72 ± 0.24</td>
<td>-0.76 ± 0.23</td>
<td>6.1 ± 1.2</td>
</tr>
<tr>
<td>(n = 12)</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

plus minus standard error of the mean are given. For the calculations, THS-values below the detection limit of gas chromatography (about 10 µg/12 h) were considered as 10 µg/12 h.

Results

The results are shown in Fig. 1 and Table 2.

1) Results in delayed adolescence

a) First 12 h sample

According to the THS-response in the first 12 h sample, the test results could be divided into insufficient (group A) and normal (above 300 µg/m²/24 h, Zachmann et al. 1974a, group B). Group A was further divided into two subgroups: in group A$_1$, there was almost no THS-response (below 100, mean 40 ± 8 µg/m²/12 h). In group A$_2$, there was a definite, but insufficient response (above 100, below 300 µg/m²/12 h, mean 191 ± 16 µg/m²/12 h). In group B, by contrast, the response was normal (1016 ± 143 µg/m²/12 h). The response during the first 12 h was thus insufficient in about two thirds of the tests in our patients, while in endocrinologically normal subjects of the earlier study (Zachmann et al. 1974a), no insufficient response was observed. Neither the clinical data (Table 1), nor the other hormone results (Table 2) were significantly different in the subgroups A$_1$ and A$_2$ and in group B.

b) Second and third 12 h samples

In subgroup A$_1$, the second (151 ± 83 µg/m²/12 h) and third (30 ± 35 µg/m²/12 h) samples also contained small quantities of THS. However, unlike in normal subjects, THS in the second sample was higher than in the first one. THS of the first and second samples combined (181 ± 84 µg/m²/24 h) was also considerably lower than in 24 h urine of normal children (1368 ± 128 µg/m²/24 h, lower normal limit 500 µg/m²/24 h, Zachmann et al. 1974a). This indicates that, in subgroup A$_1$, THS excretion was not only delayed, but also quantitatively insufficient over a period of 24 to 36 h.

In subgroup A$_2$, THS in the second 12 h sample was considerably higher (672 ± 323 µg/m²/12 h) than in the first sample and also than in the second sample of subgroup A$_1$. THS of the first and second samples combined (863 ± 312 µg/m²/24 h) was normal. This indicates that, in this subgroup, THS excretion after metyrapone was delayed, but quantitatively normal over a period of 24 h.

In group B, the second and third samples contained considerably less THS than the first one (724 ± 201 and 227 ± 74 µg/m²/12 h respectively), as is the case in normal subjects. The combined first and second samples (1740 ± 373 µg/m²/24 h) gave similar values to those found in the normal children previously studied (Zachmann et al. 1974a).

c) Repeated tests

In the 6 patients, in whom 2 or more tests were performed, different patterns were observed. Among the patients who had two tests, no clear trend could be recognized: in one, THS in the first sample decreased from 230 to 20 µg/m²/12 h, in the two others, it increased from 40 and 150 to 332 and 850 µg/m²/12 h, respectively.
Table 2.

Pituitary hormones before and after stimulation and T4 (± SEM) in patients with delayed adolescence. Groups see Table 1 and text. Because of variation with bone age, normal values are not included.

<table>
<thead>
<tr>
<th></th>
<th>T4 (ng/100 ml)</th>
<th>TSH (µU/ml)</th>
<th>GH (µU/l)</th>
<th>LH (ng/ml)</th>
<th>FSH (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0'</td>
<td>20'</td>
<td>60'</td>
<td>b</td>
</tr>
<tr>
<td>Group A1</td>
<td>8.7 ± 1.2</td>
<td>1.3</td>
<td>9.7</td>
<td>7.2</td>
<td>12.5</td>
</tr>
<tr>
<td>(n = 5)</td>
<td>(n = 5)</td>
<td>(n = 5)</td>
<td>(n = 5)</td>
<td>(n = 6)</td>
<td>(n = 5)</td>
</tr>
<tr>
<td>Group A2</td>
<td>7.0 ± 0.8</td>
<td>1.7</td>
<td>12.2</td>
<td>9.8</td>
<td>5.8</td>
</tr>
<tr>
<td>(n = 5)</td>
<td>(n = 3)</td>
<td>(n = 4)</td>
<td>(n = 4)</td>
<td>(n = 2)</td>
<td>(n = 2)</td>
</tr>
<tr>
<td>Group B</td>
<td>8.2 ± 1.1</td>
<td>1.1</td>
<td>18.1</td>
<td>12.2</td>
<td>25.6</td>
</tr>
<tr>
<td>(n = 2)</td>
<td>(n = 2)</td>
<td>(n = 5)</td>
<td>(n = 5)</td>
<td>(n = 6)</td>
<td>(n = 4)</td>
</tr>
</tbody>
</table>

b = basal.
m = maximum after stimulation.
Urinary THS-response ($\bar{x} \pm$ SEM) to metyrapone in 23 boys with delayed adolescence. n = number of tests, sample 1,2,3 = first, second and third consecutive 12 h urine sample after a single dose of metyrapone (500 μg/m² by mouth).

Normal: maximum in first 12 h sample and above 300 μg/m²/12 h.

By contrast, in the three patients with 3 or more consecutive tests, a tendency became evident for THS first to decrease and then increase with progressing bone maturation. Interestingly, in all these three boys, the minimum THS in the first sample was seen at a BA close to 14 years with higher values at lower and higher BA.

One of these three patients had had previous androgen treatment and two previous hGH treatment (see below).

From this limited experience, it appears that the reduced responsiveness to metyrapone is of transient nature and is most marked around a pubertal BA of about 14 years.

d) Effect of androgens

Among the tests done in patients who had been pre-treated with androgens, but did not receive androgens three months before and during the test, 3 showed an absent response as in group A₁, 2 an insufficient one as in group A₂. One had a normal response as in group B. In one patient who had two tests, one before and one after a period of androgen treatment, the test result after treatment was slightly better (203 μg/m²/12 h) than before (144 μg/m²/12 h, first sample), but still insufficient.

Androgen treatment during a repeated metyrapone test had a small effect in two patients (288 and 150 versus 215 and <10 μg/m²/12 h respectively before androgen).

Previous androgen treatment, discontinued for three or more months before repetition of the test does therefore not seem to influence the results. Repetition of the test during androgen treatment seems to have a slight effect, which, however, is far smaller than that of androgens on GH-secretion in delayed adolescence (Illig & Prader 1970).

e) Effect of hGH

Eight tests were performed in 3 patients after discontinuation of previous hGH treatment. Two were normal (332 and 1767 μg/m²/12 h, first sample) and 6 insufficient (2 as in group A₁ (10 and 87 μg/m²/12 h), and 4 as in group A₂ (114, 147, 191, and 257 μg/m²/12 h)).

Three tests in two patients were carried out
during hGH treatment. One was normal (1113 µg/m²/12 h, first sample), and two were insufficient as in group A₁ (10 and 54 µg/m²/12 h). hGH treatment, whether given one or more months before or during the test does not therefore seem to have any influence on the test result.

1) High dose metyrapone
In one patient, whose THS in the single dose metyrapone test was 10 µg/m²/12 h in all three samples, a full dose test performed at the same age of maturation gave a normal THS-response (4860 µg/m²/24 h, day after 4 × 500 mg/m² of metyrapone). This single observation is in agreement with the normal cortisol response to insulin and excludes true ACTH-deficiency as a cause of the insufficient response to the single dose in DA.

2) Results in patients with isolated growth hormone deficiency
Among the patients with IGHD, 9 had a normal response (above 300 µg/m²/12 h, first 12 h) with a mean THS value of 593 ± 103 µg/m²/12 h, the 9 others an inadequate response of 127 ± 21 µg/m²/12 h.

Those with a good response to metyrapone were clinically in no way different from those with an inadequate response, and their cortisol response to insulin was not significantly different: the patients with a normal THS-response had a mean basal cortisol of 15.9 ± 3.6 µg/100 ml, those with an inadequate THS-response one of 11.7 ± 1.7 µg/100 ml (ns). The mean maximum cortisol after insulin was 33.4 ± 5.6 µg/100 ml and 29.5 ± 1.8 µg/100 ml respectively.

Discussion
Our results show that the urinary THS-response to a single dose of metyrapone is missing in about one third of patients with so-called 'benign' DA, is retarded in one third, and is normal in another third.

An insufficient adrenal steroid response to metyrapone is known to occur under various circumstances other than ACTH-deficiency. They include untreated hypothyroidism (where the steroid response is quantitatively normal, but delayed as in group A₂ of our series, Havard et al. 1970), oestrogens (Sprunt et al. 1968), antiepileptic drugs and tranquillizers (mainly of the hydantoin and phenothiazine type, Jubiz et al. 1970), and hypoglycaemia per se, regardless of its cause (Zachmann & Zagalak 1974).

In our patients, true ACTH-deficiency was excluded because of the normal cortisol response to insulin in 16 patients and because of the normal response to a full dose metyrapone test in one patient.

Hypothyroidism (primary, secondary or tertiary) was also excluded clinically and by the normal T₄ and TSH values in 12 patients.

Oestrogen levels have not been studied, but there is no reason to suspect that they were increased in our patients during testing. None of the patients had pubertal gynaecomastia. The effect of the antiepileptic and other mentioned drugs is mainly due to their influence on hepatic metabolism and elimination of metyrapone (Jubiz et al. 1970). None of our patients were on any of these drugs and none had symptoms suggesting altered hepatic function.

Hypoglycaemia was also excluded in our patients clinically and by a normal glucose curve during the insulin tolerance test. Although ACTH-levels could not be measured, because the metyrapone tests were done overnight, the most likely explanation of our results seems to be a reduced and/or delayed response of ACTH to the falling cortisol levels after metyrapone, possibly as a consequence of a reduced sensitivity at the hypothalamic receptor site. Hypothetically, reduced hypothalamic serotonin in delayed adolescence might also play a role, since antiserotoninergic drugs have been shown to inhibit the ACTH-response to metyrapone (Ca­vagnini et al. 1975). This functional dysregulation seems to have no consequences under physiological conditions, as illustrated by the absence of clinical signs of secondary adrenal insufficiency and by the normal cortisol. The low metyrapone dose used in our study seems to have the advantage of detecting small alterations of the ACTH secretion pattern: the normal full dose metyrapone test result in the patient with a reduced response to the small dose suggests that this minor dysregulation can probably be overcome by a stronger stimulus.

The longitudinal test results in few patients do not allow farreaching conclusions. However, the data are consistent and suggest that the dysregulation is transient in nature.

This hypothesis is made more probable by analogy with the pattern of GH-secretion in DA. It is well documented that the GH-response to stimuli
is often transiently in an intermediate or low range in patients with DA (Prader et al. 1980), while integrated 24 h GH-concentrations or values during sleep may remain normal (Butenandt et al. 1976) or be low (Bierich & Pothoff 1979). Even transient GH-deficiency with results similar to those in true hypopituitary patients have been reported (Trygstad 1977; Gourmelen et al. 1979). One difference between GH- and ACTH-secretion in DA is their alteration by androgens: while the GH-response to stimuli is greatly and rapidly enhanced by androgens (Martin et al. 1968, 1979; Deller et al. 1966; Illig & Prader 1970), there appears from our results to be only a minimal or no androgen-induced increment of the THS-response to metyrapone.

Whether transiently reduced or delayed secretion of GH and ACTH are signs of a more global hypothalamic insensitivity in DA is unknown. While the hypothalamus-pituitary-thyroid axis is clearly not involved, the situation is less clear with respect to Gn-secretion, mainly because normal values have a wide variation. In boys with DA, normal LRH test results have been reported (Illig et al. 1974), but a reduced response has also been observed quite frequently (De Lange et al. 1978). In these latter cases, it is not clear whether the patients suffer from true Gn-deficiency or from 'benign' DA. At present, only clinical follow-up and repeated LRH-tests at a later maturation stage will prove the transient nature of the dysfunction. Because some patients with normal GH-levels in our series had an insufficient or absent response of THS to metyrapone, and others with intermediate GH-levels had a normal metyrapone test result, the reduced secretion of GH and ACTH does not apparently always occur at the same time in an individual patient. Unfortunately, our longitudinal data are insufficient to accurately evaluate possible interrelations of timing.

Since DA is often familial in occurrence, it is nevertheless conceivable that the same or related genetic factors lead first (at a BA of about 12 years or earlier) to a reduced GH-, and later (at a BA of about 14 years) to a reduced ACTH-secretion. While a stimulating effect of ACTH on GH is well documented (Zahnd et al. 1969) it is not known whether low GH-levels might secondarily cause low ACTH-levels. Our observation that patients with true persistent IGHD may also have a reduced response of THS to metyrapone in the absence of significant ACTH-deficiency suggests that some interactions between GH and ACTH may exist. Our experience in IGHD is, however, too limited to draw clear conclusions. Further studies in patients with IGHD including a second and third 12 h urine sample and repetition of the tests on hGH treatment will be necessary to clarify this point. The mostly normal metyrapone tests in DA at a BA, when adrenarche occurs (about 8–11 years) and the minimal response at a higher pubertal BA of about 14 years suggest that a reduced or delayed ACTH-secretion does not seem to be related to adrenarche in DA, but occurs at a later stage. This is in agreement with the observation of normal DHEA levels for BA in boys with DA (Rolland et al. 1977; Copeland et al. 1977). On the other hand, many patients with DA do already have symptoms (retarded BA and/or small stature) at a very young age before or at the time of expected adrenarche. Whether this might be due to the reduced GH-secretion, which could precede the reduced ACTH-secretion, or to a simultaneously reduced hypothetical adrenarche-inducing factor, is not known. Our experience in patients with IGHD is in favour of the former, and the normal DHEA levels are against the latter hypothesis.

From a practical and diagnostic point of view, a separation of 'benign' DA from true GH-deficiency (with or without concomitant true ACTH-deficiency) can be very difficult. Differentiation seems to be possible by performing GH-stimulation tests before and after androgen priming, a metyrapone test, and cortisol determinations during an insulin tolerance test. The results would have to be interpreted as follows: 1) in 'benign' DA, the GH-response to stimuli and the THS-response to metyrapone may be independently low or normal, but the cortisol response to insulin is always normal. If the GH-response is low, it can be normalized by androgen priming; 2) in persistent IGHD, the GH-response to stimuli (before and after androgen priming) is always low. The THS-response to metyrapone may be low or normal, and the cortisol response to insulin is normal; 3) in organic or idiopathic hypopituitarism with combined GH- and ACTH-deficiency, all test results are low.

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The cooperation of Dr. Ruth Illig (determinations of T₄, TSH, GH, LH, and FSH, Table 2) is gratefully acknowledged.
References


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