NOTE

Dexamethasone Suppressibility of Plasma Pregnenolone (3β-Hydroxy-5-pregnen-20-one) in Normal Men

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Synopsis

In order to examine the dexamethasone suppressibility of plasma pregnenolone, 9 a.m. and overnight suppression tests were performed in normal adult subjects and plasma pregnenolone levels were radioimmunoassayed. The results were as follows: 1) In the 9 a.m. test, plasma pregnenolone was suppressed to the lowest level at the time between 30 min and 2 hr after dexamethasone; 2) there was no significant difference in dexamethasone suppressibility of plasma pregnenolone between the 9 a.m. test and overnight test; 3) there was no significant difference from each other among the plasma pregnenolone levels after dexamethasone administration (0.5 mg to 3 mg) in both tests; 4) after dexamethasone administration, plasma pregnenolone was not suppressed below 40% of the basal level in both tests; 5) discussions were made about the results, comparing with those of the suppressibility of cortisol which were previously reported from this laboratory.

Pregnenolone is the first steroid formed from cholesterol in the biosynthesis of the steroid hormones of the human adrenal cortex and gonads. However, little is known about its physiological behavior in the steroid biosynthesis.

On the other hand, pregnenolone is shown to be converted to dehydroepiandrosterone (DHA, the major adrenal androgen), via 17-hydroxypregnenolone. The previous report from this laboratory (Nishida et al., 1977b) has demonstrated that the dexamethasone suppressibility of plasma DHA is poorer than that of plasma cortisol in normal men. The present study was undertaken to measure plasma pregnenolone after dexamethasone administration in normal men to examine the dexamethasone suppressibility of the hormone. Overnight (Nugent et al., 1965) and 9 a.m. (Nishida et al., 1976; Nishida et al., 1977b) dexamethasone suppression tests were employed for this purpose.

Materials and Methods

9 a.m. test

A single dose of dexamethasone (0.5 mg, 1 mg, 2 mg, 3 mg) was administered orally at 9 a.m. to 5 to 9 normal male or female adult volunteers for each group. In two normals, 4 mg test was performed. Blood specimens were collected before and 30 min, 1 hr and 2 hr after dexamethasone administration.

Overnight test

The test was performed by administrating dexamethasone (0.5 mg, 1 mg, 2 mg, 3 mg) between 11 and 12 p.m. and drawing blood at 9 a.m. the next morning in 5 to 10 normal male or female adult volunteers for each group. Plasma pregnenolone was determined by a radioimmunoassay using an antiserum raised against pregnenolone-3-hemisuccinate BSA (Nishida et al., 1977a). The antiserum crossreacted with progesterone.
The steroids known to be present near the pregnenolone fraction on the chromatography had minor or no detectable crossreactivities. The antiserum showed the crossreactivity below 0.1% with dexamethasone. Plasma (0.2 ml) was extracted with 1 ml of ether and the extract was transferred to a Sephadex LH-20 microcolumn (8 mm × 60 mm) with 0.2 ml of developing solvent (benzene : methanol 85:15, v/v). The fraction containing pregnenolone was collected from 1.4 ml to 2.2 ml. And the fraction was incubated with 10,000 dpm of $^3$H-pregnenolone and 0.25 ml of the antiserum solution, diluted to 1:45,000 with 0.05 M borate buffer, pH 8, for 30 min in room temperature. Separation of free hormone from bound one was made with 0.2 ml of saturated ammonium sulfate.

Results

9 a.m. suppression test

In a preliminary study, it was confirmed that plasma pregnenolone reached its lowest level at the time between 30 min and 2 hr after dexamethasone administration at 9 a.m.

As shown in Table 1, plasma pregnenolone levels after dexamethasone (0.5 mg to 3 mg) were significantly different from the control pregnenolone level, which was measured in the plasma obtained at 9:30 a.m. 10 a.m. and 11 a.m. The decreased ratio of 9 a.m. level in the control test (80.4±4.0%) was not significantly different from that of 0.5 mg test (57.4±4.4%) or 1 mg test (50.0±5.4%), but was different from that of 2 mg test (p<0.05) or 3 mg test (p<0.02).

However, there was no significant difference from each other among the plasma pregnenolone levels or suppressed ratios of the basal level after dexamethasone (0.5 mg to 3 mg).

No further suppression was obtained after 4 mg dexamethasone.

Overnight suppression test

As shown in Table 2, the plasma pregnenolone levels did not differ significantly each other after 0.5 mg to 3 mg of dexamethasone administration. The suppressed ratio of the basal level in 0.5 mg test was not different from that of the 0.5 mg test at the p<0.05 level.

<table>
<thead>
<tr>
<th>Table 1. 9 a.m. dexamethasone suppression test in normal subjects.</th>
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<tbody>
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<td><strong>Before</strong></td>
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<tr>
<td>Control test (n=5)</td>
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<tr>
<td>0.5 mg test (n=5)</td>
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<tr>
<td>1 mg test (n=6)</td>
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<tr>
<td>2 mg test (n=9)</td>
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<tr>
<td>3 mg test (n=7)</td>
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<td>4 mg test (n=2)</td>
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</table>

Plasma pregnenolone; ng/ml, means±S.E.M.
1) The lowest one of plasma pregnenolone levels among 30 min, 1 hr and 2 hr-level after dexamethasone.
2) No dexamethasone was administered in the test.
3) These pregnenolone levels were different (P<0.01) from that of the control test.
4) These pregnenolone levels were not different from that of the 0.5 mg test at the P<0.05 level.

<table>
<thead>
<tr>
<th>Table 2. Overnight dexamethasone suppression test in normal subjects.</th>
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<td><strong>Before</strong></td>
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<tr>
<td>0.5 mg test (n=10)</td>
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<tr>
<td>1 mg test (n=9)</td>
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<td>2 mg test (n=6)</td>
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<td>3 mg test (n=5)</td>
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</table>

Plasma pregnenolone; ng/ml, means±S.E.M.
1) These pregnenolone levels were not different from that of the 0.5 mg test at the p<0.05 level.
not different from that of 1 mg test, but was different from that of 2 mg test (p<0.01) or 3 mg test (p<0.001).

There was no significantly different dexamethasone suppressibility of plasma pregnenolone between the 3 mg 9 a.m. test and 3 mg overnight test.

**Discussion**

In the previous paper about the dexamethasone suppressibility of plasma cortisol (Nishida et al., 1976), we reported that in the 9 a.m. test the basal cortisol level (128.5±42.0 ng/ml) decreased to the limits of the method (13.9±4.9 ng/ml, 11.8±4.7% of basal level) 4 hr after 1 mg of dexamethasone in 20 normal subjects including 6 subjects of the present study, and that no further suppression of plasma cortisol was obtained (26.0±8.0 ng/ml, 17.4±5.8% of basal level) after 2 mg dexamethasone in 20 normal subjects including 9 subjects of the present study.

In the 9 a.m. test of the present study, plasma pregnenolone was suppressed to the lowest level at the time between 30 min and 2 hr after dexamethasone, whereas the maximum plasma cortisol suppression was obtained at the time between 2 hr and 4 hr after dexamethasone (Nishida et al., 1976) and the maximum plasma DHA suppression was between 4 hr and 6 hr after dexamethasone (Nishida et al., 1977b). The difference in the time to induce the maximum dexamethasone suppression between pregnenolone and cortisol seems reasonable, since mean metabolic clearance rates for pregnenolone and cortisol are reported to be 1,050 l plasma/day and 200 l plasma/day, respectively (Gower, 1975).

However, the conflicting differences in dexamethasone suppressibility between plasma pregnenolone and plasma cortisol were revealed in the present study. Three mg of dexamethasone induced the maximum plasma pregnenolone suppression, whereas plasma cortisol needed 1 mg for its maximum suppression (Nishida et al., 1976). And after dexamethasone administration, plasma pregnenolone was not suppressed below 40% of the basal level, whereas the plasma cortisol level was suppressed to 10% of the basal level and reached the limits of the method (Nishida et al., 1976). Although the contribution to pregnenolone by hydrolysis of the relatively abundant pregnenolone sulfate (Gower, 1975) or the contribution to pregnenolone by gonadal secretion as free or sulfated pregnenolone (Laatikainen et al., 1971) is suggested to be involved, the mechanism of the poor dexamethasone suppressibility of plasma pregnenolone remains unclear.

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**References**


