THE EFFECT OF EXOGENOUS PRECURSORS ON ETIOCHOLANOLONE/ANDROSTERONE RATIOS IN MAN

By

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ABSTRACT

The effect of the ingestion of large amounts of precursors of urinary androsterone (A) and etiocholanolone (E) on the E/A ratio was explored. Dehydroepiandrosterone (DHEA) in doses of 1000 mg daily resulted in elevation of the E/A ratios from 0.8 to 2.8 by the third day. The E/A ratio remained near 1.0 after the same amount of testosterone and androstenedione. Triiodothyronine administration blocked the E/A changes seen after DHEA. Significant differences were also noted in the modes of conjugation of A and E and in epiaandrosterone excretion after DHEA, androstenedione and testosterone. The data suggest that metabolism of the three precursors does not proceed entirely via the presumed intermediate, androstenedione. The role of enteric metabolism of ingested precursor was examined and found to be of minor importance.

In the normal subject, the urinary etiocholanolone/androsterone (E/A) ratio of approximately one, suggests that the hepatic C19O2-5α and 5β reductases are equally active. The studies of Hellman et al. (1959) and McGuire & Tomkins (1959) demonstrated that the activity of these enzymes could be altered by thyroid hormone. Wilson & Schenker (1964) have noted that glucocorticoids can influence the proportion of 5α and 5β metabolites, leading to increased E/A ratios. Kirschner et al. (1963) noted recently that the ingestion of large doses of DHEA by normal subjects resulted in elevated E/A ratios. It seemed possible then, that DHEA, or one of its metabolic products, was another factor

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capable of directly altering the activity of one of the C19O2-reductases. In this study, the phenomenon has been studied further and an alternate hypothesis has been proposed.

Methods

One tenth of a 24-hour urine collection was treated with β-glucuronidase, extracted with ether, and solvolysis performed as described previously (Kirschner & Lipsett 1963). The β-glucuronidase and solvolysis extracts were treated independently. These fractions will be referred to as glucuronides and sulfates respectively. A1, E and DHEA were measured in each extract after thin-layer and gas-liquid chromatography (GLC) as their trimethyl silyl ethers (TMSi) (Kirschner & Lipsett 1963). The GLC analyses were performed using the stationary phase, 2% SE-60 or 1% NGS. When separation of epiaandrosterone from DHEA was necessary, 1% QF-1 was used. Urinary testosterone was measured directly on 1% SE-30.

The C19O3-17-ketosteroids were measured by GLC after preliminary thin-layer chromatography in the system, benzene:ethyl acetate (40:60) and formation of the TMSi ethers (Kirschner & Lipsett 1964).

Experimental

Six normal men and two normal women ingested 1000 mg DHEA daily for 5 days. Six of the 8 subjects received 1000 mg of testosterone by mouth daily for 5 days and 3 of the subjects ingested 1000 mg of androstenedione daily for 5 days. Complete 24-hour urine collections were obtained and the pertinent metabolites were measured daily.

In a separate study, 3 other subjects received 200 µg of triiodothyronine (T3) orally for the 4 days before and during a 5 day period of ingestion of 1000 mg of DHEA. The urinary metabolites were measured during the period of T3 administration, throughout the DHEA period, and during a control period 2 months later when the subjects were not receiving drugs.

Two experiments with labeled precursors were performed as follows: a normal subject ingested 1000 mg androstenedione daily for 11 days. On the fourth day 5 µc of androstenedione-1,2-3H (New England Nuclear Corp.) was given orally. Urine was collected for 48 hours and the specific activity of urinary epiaandrosterone was determined. On the ninth day, 5 µc of the same steroid was given intravenously, and

1) The following trivial names are used in this report:

Dehydroepiandrosterone, DHEA – 3β-hydroxy-androst-5-en-17-one
Androsterone, A – 3α-hydroxy-5α-androstan-17-one
Etocholanolone, E – 3α-hydroxy-5β-androstan-17-one
Epiandrosterone – 3β-hydroxy-5α-androstan-17-one
Testosterone – 17β-hydroxy-androst-4-en-3-one
Androstenedione – Androst-4-ene-3,17-dione
11-Hydroxyetiocholanolone – 3α,11β-dihydroxy-5β-androstan-17-one
11-Ketoetiocholanolone – 3α-hydroxy-5β-androstane-11,17-dione
11-Hydroxyandrosterone – 3α,11β-dihydroxy-5α-androstan-17-one

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the specific activity of urinary \( \varepsilon \)-androstosterone was again measured. \( \varepsilon \)-Androstosterone was isolated from the solvolysis extract by precipitation with digitonin and its specific activity determined after successive paper chromatography, thin-layer chromatography, acetylation and thin-layer chromatography. Radioactivity was measured in the Packard Tri-carb liquid scintillation spectrometer at 17% efficiency for tritium. Mass was measured by GLC of the \( \varepsilon \)-androstosterone acetate. Using the same protocol, DHEA-7\( \alpha \)-\(^{3}H\) was given both by mouth and intravenously during the administration of 1000 mg of DHEA daily. The specific activities of urinary A and E were determined in similar fashion.

**RESULTS**

In each of the subjects receiving DHEA, the E/A ratio increased when compared to control levels (Fig. 1). The mean E/A ratio increased from 0.8 to 2.8 with a range of 0.7 to 1.0 during control period and 1.9 to 5.8 after DHEA ingestion. In 7 of the 8 subjects, the ratio was at least doubled.

When the E/A ratios were measured daily during DHEA ingestion, the mean value increased to 2.8 by the third day (Fig. 2) and remained at about the same level. By contrast, the E/A ratios observed with testosterone increased transiently to 1.5, and with androstenedione remained unchanged.

The average excretion of E and A during each period is plotted in Fig. 3. The values obtained during the first day of administration of steroid were not included since these were intermediate between the initial and final levels. Roughly similar amounts of etiocholanolone were excreted following administration of each precursor, but the excretion of A was much lower during the DHEA period. The respective values for the excretion of A were: DHEA period, 55 mg daily; testosterone period, 150 mg daily, androstenedione period, 195 mg per day.
ANDROSTENEDIONE

Daily E/A ratios after 1000 mg of each precursor. The number of studies for each steroid is given in parenthesis.

In Fig. 3 the amounts of A and E excreted as sulfate and glucuronide are presented. The relative amounts of A and E conjugated with sulfate and glucuronide varied with the steroid precursor. Approximately 18% of the androsterone and 8% of the etiocholanolone were excreted as sulfates during both the androstenedione and testosterone studies. When DHEA was given,
Table 1.
Effect of Triiodothyronine on E and A Excretion Following DHEA, 1000 µg Daily.

<table>
<thead>
<tr>
<th></th>
<th>Subject J. W.</th>
<th>T3 200 µg daily</th>
<th>Group Averages</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>T3 (3)</td>
<td>Control (8)*</td>
</tr>
<tr>
<td>A mg/24 h</td>
<td>48</td>
<td>80</td>
<td>55</td>
</tr>
<tr>
<td>E mg/24 h</td>
<td>128</td>
<td>94</td>
<td>120</td>
</tr>
<tr>
<td>E/A</td>
<td>2.7</td>
<td>1.2</td>
<td>2.2</td>
</tr>
</tbody>
</table>

* Number of studies.

however, the A and E sulfates accounted for only 2% of the total A and 1% of the total E, respectively.

Since triiodothyronine (T3) has been shown to decrease E/A ratios by increasing the activity of the 5α reductase (McGuire & Tomkins 1959), it was of interest to examine the effect of T3 on the increased E/A ratios resulting from DHEA administration. During the first 4 days on T3 alone, no changes in endogenous E/A ratios were noted; however, when DHEA was then given (Fig. 4) the expected rise in E/A ratio did not occur. Table 1 lists the individual metabolites in one patient who received DHEA with and without T3, as well as the group averages. The changes in excretion of A and E demonstrate that under the influence of T3, more 5α metabolite is produced at the expense of the 5β isomer resulting in equal amounts of A and E.

During the study, the amounts of urinary testosterone, androstenedione, and DHEA were measured. Less than 5 mg/24 hours² of androstenedione were excreted in any period. Testosterone excretion averaged 30 mg/day during the testosterone period but was not measurable during the other periods. The excretion of DHEA after DHEA administration had been measured in 3 patients previously (Kirschner et al. 1963) and with these additional studies, has averaged 60 mg/24 hours or 6% of the ingested dose.

Representative GLC tracings of the β-glucuronide and solvolysis fractions during the testosterone and androstenedione periods are shown in Fig. 5 a and 5 b. The solvolysis fractions consistently demonstrated a large peak with a retention time equal to both that of epiandrosterone TMSi ether and DHEA TMSi ether on XE-60 (Fig. 5 b). To establish the identity of this unknown

² This level depends on the dilution of the urine sample and the sensitivity of GLC. It is quite possible to measure the excretion of much smaller amounts of these steroids.
Representative GLC tracings of urine extracts following 1000 mg of androstenedione. a) β-glucuronide fraction, 1/50 000 dilution. A = Androsterone TMSi ether, E = etiocholanolone TMSi ether. Shaded area represents 5α-androstan-17-one marker. Stationary phase is 2 %XE-60, 195°C 20 psi, 850 volts. b) TMSi ethers of solvolysis extract on the stationary phase XE-60, 1/10 000 dilution. Retention times of epietiocholanolone TMSi and androsterone TMSi are identical as are those of DHEA, TMSi and epiandrosterone TMSi. c) Non-precipitating fractions following digitonin, 1/10 000 dilution, stationary phase is 1 %QF-1, 187°C, 22 psi. d) Precipitating fraction, on the stationary phase QF-1, 1/10 000 dilution. Only one peak, representing epiandrosterone TMSi is seen. DHEA TMSi and epietiocholanolone TMSi are not seen in the dilution used.

peak, we performed digitonin precipitation of the solvolysis extract. The TMSi ether of the fraction contained peaks corresponding to A-TMSi and E-TMSi ether. The β-fraction contained a single peak with a retention time equal to epiandrosterone TMSi ether on QF-1 (Fig. 5 d). On this phase, the TMSi ethers of epiandrosterone and DHEA separate. Epiandrosterone was further characterized by paper chromatography, acetylation and GLC of the derivatives. Epietiocholanolone (3β-hydroxy-5β-androstan-17-one) could not be identified in the GLC tracings following digitonin precipitation.
Table 2.
Excretion of Epiandrosterone.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Precursor*</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Androstenedione</td>
<td>Testosterone</td>
<td>DHEA</td>
</tr>
<tr>
<td>M. K.</td>
<td>29.5†</td>
<td>8.0</td>
<td>&lt; 3.0††</td>
</tr>
<tr>
<td>S. K.</td>
<td>31.5</td>
<td>11.5</td>
<td>&lt; 3.0</td>
</tr>
<tr>
<td>M. L.</td>
<td>28.5</td>
<td>11.0</td>
<td>&lt; 3.0</td>
</tr>
</tbody>
</table>

* Each subject received 1000 mg of each precursor daily for 5 days.
† Mg/24 hours.
†† Limit of detection in presence of large preceding DHEA peak.

Table 3.
Specific Activities of Urinary Metabolites.

<table>
<thead>
<tr>
<th>Androstenedione-1,2-³H</th>
<th>DHEA-7α-³H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral*</td>
<td>IV†</td>
</tr>
<tr>
<td>Epiandrosterone</td>
<td>A</td>
</tr>
<tr>
<td>1.5**</td>
<td>1.2</td>
</tr>
</tbody>
</table>

* 1000 mg and 5 µc labeled precursor given orally.
† 1000 mg given orally and 5 µc labeled precursor given IV.
** All values are cpm/µg.

In Table 2 the excretion of epiandrosterone is compared among the 3 subjects who received all 3 steroid precursors. Epiandrosterone excretion averaged 30 mg/24 hours during the testosterone period. An accurate measurement of epiandrosterone was not possible during the DHEA period since the preceding large DHEA peak made the measurement of the area of the epiandrosterone peak difficult. The recorded values of 3 mg or less are maximal values.

In order to determine whether the effect of DHEA on the E/A ratio was limited to the C₁₉O₂-reductases or was more general, the excretion of the C₁₉O₃-17-ketosteroids was measured in 3 subjects when the E/A ratio was at its peak. The excretion of 11-ketoetiocholanolone was 0.13 to 0.60 mg/24 hours, of 11β-hydroxyetiocholanolone 0.18 to 0.33 mg/24 hours, and of 11β-hydroxyandrosterone, 0.13 to 0.37 mg/24 hours. The resulting ratios of 5β/5α meta-
DHEA metabolites were 2.5, 2.4, and 2.3. As these ratios and the amounts excreted are within the normal range for this laboratory, there was apparently no effect of DHEA on C₁₉O₃-reductases.

The specific activities of urinary metabolites after administration of labeled precursors are given in Table 3. When labeled androstenedione was given orally with 1000 mg of unlabeled precursor, the specific activity of epi-androsterone was 1.5 cpm/µg compared to 1.2 cpm/µg when the labeled androstenedione was given intravenously. The specific activities of A and E were 1.35 and 1.2 when labeled DHEA was given orally, and 1.35 and 1.1 respectively after intravenously administered DHEA-7α-3H.

**DISCUSSION**

It has been generally assumed that androstenedione is the substrate for the C₁₅O₂-reductases and that both testosterone and DHEA are metabolized via androstenedione. This hypothesis has been supported by several studies of the in vitro conversion of DHEA and testosterone to androstenedione (Dorfman & Shipley 1956 a). The approximately equal excretion for A and E after administration of testosterone, DHEA and androstenedione (Dorfman & Shipley 1956 b) was also in accord with the idea of a common precursor of A and E.

Recently, however, Baulieu & Mauvais-Jarvis (1963) showed that an appreciable fraction of testosterone is metabolized via a «diol» bypassing androstenedione, and resulting in a higher E/A ratio. Since 5-androstene-3β,17β-diol, a metabolite of DHEA can be directly converted to testosterone (Baulieu et al. 1963), one route of metabolism of DHEA, as well, may not be through androstenedione.

If androstenedione were the common precursor of epi-androsterone, A, and E, then the excretion and conjugation of these metabolites should be the same after administration of testosterone, DHEA and androstenedione. It is clear from the data of Fig. 3 that androstenedione is a more efficient precursor of epi-androsterone than is testosterone and that DHEA was metabolized to epi-androsterone to only a small extent. This strongly suggests that a fraction of DHEA and testosterone is not metabolized via androstenedione. Examination of the conjugates of A and E after each precursor further supports this conclusion since differences were demonstrated.

We have shown that the increase in E/A ratio resulting from administration of DHEA is due to a decreased conversion to A. This phenomenon is susceptible to several explanations, one of which is that DHEA or one of its metabolic products inhibits a C₁₉O₂-reductase. However, this finding is equally compatible with the metabolism of DHEA in part through an alternate intermediate that is converted preferentially to E. Fukushima et al. (1962) demonstrated the conversion of androst-5-ene-3,17-dione, a metabolite of DHEA to

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3α-hydroxy-androst-5-en-17-one (3α-DHEA). Saturation of the \( \Delta^{5,6} \) double bond in this compound would yield A and E directly. Although this route has little significance at physiological levels it is perhaps pertinent that the administration of a massive dose of 3α-DHEA led to an E/A ratio of 4.5/1 (Fukushima et al. 1963).

Since it was necessary to give these large amounts of steroids by mouth, the possible role of enteric metabolism was considered. It has been shown (Kreek et al. 1963) that in the everted gut sac of the rat, transport of testosterone and androstenedione is dependent on glucuronide conjugation of one or several metabolites. In order to test the possibility that the large excretion of epiandrosterone following administration of androstenedione was due to enteric production of epiandrosterone or one of its precursors, tritium labeled androstenedione was given both intravenously and by mouth during the ingestion of 1000 mg of androstenedione. When the labeled androstenedione was given by mouth, all of the resultant epiandrosterone should have been labeled. On the other hand if epiandrosterone was produced by enteric metabolism, then the epiandrosterone derived from intravenously administered labeled androstenedione would be diluted by the large unlabeled pool of epiandrosterone with a consequent lowering in specific activity. The small differences in specific activity tend to rule out a significant role for enteric metabolism in the production of the major differences in epiandrosterone excretion.

Similarly, if the differences in E/A ratios depended on enteric metabolism, then the specific activities of these metabolites of labeled DHEA should differ following oral and intravenous administration. Since the specific activity of androsterone was the same after oral and intravenous administration of DHEA and the specific activities of the etiocholanolone differed by less than 10%, it is unlikely that enteric metabolism was responsible for the large differences in E/A ratio. We are at present unable to explain why the specific activities of A and E were not the same after oral administration of DHEA-7α-3H.

REFERENCES


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