Adrenarche: A Survey of Rodents, Domestic Animals, and Primates

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ABSTRACT. The concentrations of the adrenal steroids dehydroepiandrosterone (DHA), dehydroepiandrosterone sulfate (DHAS), and Δ⁴-androstenedione (Δ⁴-A) have been measured by RIA before and after sexual maturation in plasma of rodents, domestic animals, and primates to determine whether these species exhibit an adrenarchal process comparable to man. The average concentrations of DHA and DHAS were less than 60 ng/dl and 5 μg/dl, respectively, in plasma of sexually mature rodents and domestic animals, and a significant increase in the plasma DHA level after sexual maturation was seen only in the rabbit and dog. The concentrations of DHA, DHAS, and Δ⁴-A in 21 rhesus monkeys from 0-3 yr of age were 2021 ± 235 ng/dl (mean ± SE), 357 ± 60 μg/dl, and 107 ± 9 ng/dl, respectively, and did not increase during sexual maturation. By contrast, DHA, DHAS, and Δ⁴-A levels in plasma of chimpanzees were 5.9-fold, 3.3-fold, and 4.8-fold greater, respectively, in 7- to 22- compared to 0- to 3-yr-old animals. Temporally, the increase in DHA levels in the chimpanzee is apparent at 5 yr and this precedes the increase in gonadal steroids, as is characteristic of human adrenarche. It is apparent that adrenal androgen levels and their developmental patterns differ markedly among species, and that among the species examined, only the chimpanzee exhibits an adrenarche comparable to that of man. (Endocrinology 103: 2112, 1978)

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ketamine anesthesia were obtained from the National Zoo, Washington, DC. All blood samples were collected on ice between 0800-1200 h, centrifuged within 2 h, and kept at 20°C until assay.

**Measurement of plasma DHA**

DHA was measured by RIA using a rabbit antiserum to DHA-3-hemisuccinate-bovine serum albumin (BSA). The cross-reactivity of the antiserum to other compounds (compared to DHA) was: 71% to DHAS, 1.6% to Δ4-A, 0.15% to Δ3-androsten-3β,17β-diol, and less than 0.1% to progesterone, 17-hydroxyprogesterone, pregnenolone, 17-hydroxyprogrenolone, testosterone, cortisol, estradiol, and cholesterol (all unlabeled steroids were obtained from Sigma). Ether extracts of 0.5 ml serum were dried under air, redissolved in cyclohexane-benzene-methanol (80:15:5), and chromatographed on Sephadex LH-20 columns (Pharmacia). Dried eluates were dissolved in 1 ml buffer [0.5 mg/ml bovine γ-globulin fraction II (Pentex) plus 0.5 mg/ml BSA fraction V (Pentex) in Dulbecco’s phosphate-buffered saline]. Each sample was monitored for recovery and assayed in a total volume of 1 ml containing antiserum at a final dilution of 1:10,000 and 32,000 dpm [3H]DHA as trace. Ethanol extracts of 50 μl serum were centrifuged to remove precipitated protein, dried under air, and assayed, as described above for DHA, using DHAS as standard. The assay had no detectable blank using plasma from a postmenopausal Addisonian patient receiving dexamethasone replacement. The intraassay variability was 7%; the interassay variability was 9%. The slope of the logit-log dose-response curve was −0.920 ± 0.021; the ED50 was 367 ± 8 pg; and the least detectable dose was 13 ± 1 pg (mean ± SE; n = 6). We employed 5 ng/dl as the detection limit of the assay.

**Measurement of plasma DHAS**

DHAS was measured by RIA using the antiserum to DHA described above and 32,000 dpm [3H]DHAS as trace. Ethanol extracts of 50 μl serum were centrifuged to remove precipitated protein, dried under air, and assayed, as described above for DHA, using DHAS as standard. The assay had no detectable blank using plasma from a postmenopausal Addisonian patient receiving dexamethasone replacement. The intraassay variability was 7%; the interassay variability was 9%. The slope of the logit-log dose-response curve was −0.920 ± 0.021; the ED50 was 367 ± 8 pg; and the least detectable dose was 13 ± 1 pg (mean ± SE; n = 6). We employed 5 ng/dl as the detection limit of the assay.

**Measurement of plasma Δ4-A**

Δ4-A was measured by RIA using rabbit antiserum to Δ4-A-6-hemisuccinate-BSA. The cross-reactivity of the antiserum to other compounds (compared to Δ4-A) was: 2.29% to DHA, 1.0% to testosterone, 0.064% to 11-deoxycortisol, 0.027% to 17-hydroxyprogesterone, 0.007% to estrone, and less than 0.001% to cortisol and estradiol. Serum aliquots (0.5 ml) were extracted with ether and chromatographed on Sephadex LH-20 columns, as described above for DHA. The assay utilized antiserum at a final dilution of 1:10,000 and 32,000 dpm [3H]Δ4-A (New England Nuclear; 40-60 Ci/mmol) as tracer. The remainder of the assay

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**Table 1. Species, sex, age, type of anesthesia, and method of blood collection for rodents and domestic animals**

<table>
<thead>
<tr>
<th>Species</th>
<th>Sex</th>
<th>Age</th>
<th>Type of anesthe-sia</th>
<th>Method of blood collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat (Holtzman)</td>
<td>M</td>
<td>21 Days</td>
<td>None</td>
<td>Decapitation</td>
</tr>
<tr>
<td>Guinea pig (Hartley)</td>
<td>M</td>
<td>21 Days</td>
<td>None</td>
<td>Decapitation</td>
</tr>
<tr>
<td>Hamster (Syrian golden)</td>
<td>M</td>
<td>35 Days</td>
<td>Ether</td>
<td>Cardiac puncture</td>
</tr>
<tr>
<td>Dog</td>
<td>M</td>
<td>77 Days</td>
<td>None</td>
<td>Peripheral vein</td>
</tr>
<tr>
<td>Sheep</td>
<td>M</td>
<td>2 Months</td>
<td>None</td>
<td>Peripheral vein</td>
</tr>
<tr>
<td>Pig</td>
<td>M</td>
<td>3 Months</td>
<td>None</td>
<td>Peripheral vein</td>
</tr>
<tr>
<td>Goat</td>
<td>M</td>
<td>2 Months</td>
<td>None</td>
<td>Peripheral vein</td>
</tr>
<tr>
<td>Horse</td>
<td>M</td>
<td>7-22 Yr</td>
<td>None</td>
<td>Peripheral vein</td>
</tr>
<tr>
<td>Cow</td>
<td>F</td>
<td>2-4 Yr</td>
<td>None</td>
<td>Peripheral vein</td>
</tr>
<tr>
<td>Chicken</td>
<td>F</td>
<td>8 Months</td>
<td>Ether</td>
<td>Cardiac puncture</td>
</tr>
</tbody>
</table>
procedure is identical to that of DHA. The assay blank was 5 ng/dl using plasma from a postmenopausal Addisonian patient receiving dexamethasone replacement. The within-assay variability was 7%; the between-assay variability was 12%. The slope of the logit-log dose response curve was \(-1.111 \pm 0.012\); the ED\(_{50}\) was 145 \(\pm\) 4 pg; and the least detectable dose was 9 \(\pm\) 2 pg (mean \(\pm\) SE; \(n = 6\)). The measurement of known quantities of \(\Delta^4\)-A added to blank plasma at multiple doses gave results within \(\pm 7\%\) of the true value. We employ 15 ng/dl as the detection limit of the assay.

**Measurement of plasma cortisol**

Plasma cortisol was measured by RIA, as previously described (18).

**Calculation of results**

Results of the RIA were calculated using the computer program of Rodbard (19). Comparison of plasma steroid levels was made using Student’s \(t\) test.

**Results**

**Adrenal androgens in rodents and domestic animals (Fig. 1)**

The concentration of DHA before sexual maturation did not significantly exceed the assay detection limit of 25 ng/dl in any of the species examined. After sexual maturation, significant increases in DHA were seen in the rabbit (\(P < 0.001\)) and dog (\(P < 0.01\)). DHAS concentrations were below the detection limit of 5 \(\mu\)g/dl in all of the species examined. The concentration of \(\Delta^4\)-A before sexual maturation significantly exceeded the assay detection limit of 15 ng/dl in the rat (\(P < 0.001\)), guinea pig (\(P < 0.02\)), and hamster (\(P < 0.03\)). After sexual maturation, \(\Delta^4\)-A concentrations were not significantly altered in the rat and guinea pig, were decreased in the hamster (\(P < 0.05\)), and were increased in the dog, although not reaching statistical significance (\(P < 0.06\)). \(\Delta^4\)-A concentration also exceeded the assay detection limit in the sexually mature hen (\(P < 0.03\)).

**Adrenal androgens in the rhesus monkey (Fig. 2)**

The concentrations of DHA, DHAS, and \(\Delta^4\)-A did not increase with age. The DHAS level at 4-6 months was lower than at 2-4 months (\(P < 0.05\)), and at 6-36 months was less than in all three preceding age groups (\(P < 0.002\)). Plasma cortisol concentrations in the four groups were, in order of increasing age, 19.8 \(\pm\) 1.4, 22.4 \(\pm\) 1.0, 25.0 \(\pm\) 7.2, and 17.9 \(\pm\) 2.8 \(\mu\)g/dl (mean \(\pm\) SE). There was no significant

**Fig. 1.** Concentration of DHA, DHAS, and \(\Delta^4\)-A in plasma of rodents and domestic animals before and after sexual maturation. Details of the age and sex of animals used, the type of anesthesia, and the method of blood collection are presented in Table 1. Each value is the mean \(\pm\) SE of determinations in duplicate for six plasma samples. For the rat, guinea pig, and hamster, each sample was pooled from 2-12 animals. For the other species, individual plasma samples were used. Detection limit for each assay. In calculating the mean \(\pm\) SE, undetectable values were set equal to the detection limit.

**Fig. 2.** Concentration of DHA, DHAS, and \(\Delta^4\)-A in plasma of rhesus monkeys with increasing age. Each value is the mean \(\pm\) SE of determinations in duplicate for individual plasma samples from all animals in each age range. The number and sex of animals in each group were: 0-2 months, three males and three females; 2-4 months, three males and four females; 4-6 months, one male and two females; 6-36 months, five females. The ages of the five animals in the 6- to 36-month group were 8, 19, 28, 32, and 36 months.
difference in DHA, DHAS, Δ⁴-A or cortisol concentrations between male and female animals.

Adrenal androgens in the chimpanzee (Fig. 3)

A progressive rise in DHA, DHAS, and Δ⁴-A was observed with increasing age. The DHA level was significantly increased at 3–7 yr compared to 0–3 yr \((P < 0.02)\), and at 7–11 yr compared to 3–7 yr \((P < 0.002)\). Similar increases were seen in the DHAS concentration, with significantly increased levels at 7–11 yr and >11 yr compared to 0–3 yr \((P < 0.005)\). Δ⁴-A concentration also increased with age and was closely correlated with the DHA concentration in the same sample \((r = 0.91; P < 0.01)\). Plasma cortisol concentrations in the four groups were, in order of increasing age, 18.3 ± 2.5, 23.2 ± 2.8, 26.5 ± 1.7, and 23.7 ± 2.5 \(\mu\)g/dl (mean ± SE). There was no significant difference in the concentration of DHA, DHAS, Δ⁴-A, or cortisol between males and females in any age group. Above age 7, female chimpanzees had higher Δ⁴-A levels \([132 ± 18 \text{ ng/dl (mean ± SE)}]\) than males \([92 ± 9 \text{ ng/dl}]\), but this did not reach statistical significance \((P < 0.08)\).

Adrenal androgens in other primate species

The concentrations of DHA, DHAS, and Δ⁴-A in plasma of several species of primates from the National Zoo, Washington, DC, are presented in Table 2.

Discussion

The plasma concentration of DHA in sexually mature rodents and domestic animals is an order of magnitude lower than the normal adult values in man (normal range, 200–800 ng/dl) \((4, 5)\). A rise in DHA levels after sexual maturation, as is characteristic of adrenarche in man, is observed only in the rabbit and dog, and only in the dog is there an accompanying rise in Δ⁴-A as in man. Since DHA levels were undetectable in many of the species examined, it is not possible to rule out increased DHA levels in these species after sexual maturation.

It would seem, however, that none of these nonprimate species provides a satisfactory model for the study of adrenarche as it occurs in man.

The DHA levels in the current study are lower than the previously reported values of 109 ± 56 and 138 ± 70 ng/dl (mean ± SE) for the sexually immature and mature rat, and 76 ± 41 and 313 ± 85 ng/dl for the sexually immature and mature guinea pig \((15)\). The differences between these observations and ours are likely due to the use of a RIA in the current study. The double isotope dilution method was employed in the previous report.

The plasma concentrations of DHA \([2132 ± 503 \text{ ng/dl (mean + SE)}]\) and Δ⁴-A \((106 ± 10 \text{ ng/dl})\) in male rhesus monkeys in this study are similar to the values of 2120 ± 335 and 170 ± 37 ng/dl, respectively, observed previously by Snipes et al. \((17)\). These investigators also found no difference in DHA and Δ⁴-A concentrations between mature and immature monkeys. The rhesus monkey is thus similar to man in having high levels of adrenal androgens in comparison to rodents and domestic animals, but differs from man in possessing high levels continuously from birth to sexual maturity with no adrenarchal process.

The increase of adrenal androgen levels with age in the chimpanzee differs markedly
from the pattern in rhesus monkey and closely resembles adrenarche in man. The concentration of DHA is higher than in man at comparable stages of development: approximately 4 times greater in the 0- to 3-yr group compared to children <6 yr old, and 2 times greater in the >11-yr-old group compared to adult human values (4, 5). These differences may be largely due to differences in stress during blood collection, since plasma cortisol values in the chimpanzee samples were 2–3 times greater than those in the previously reported human samples (5) and DHA is known to be secreted synchronously with cortisol (20). Differences in stress do not seem to contribute, however, to the age-related increase in adrenal androgens in the chimpanzee or to the marked difference in DHA concentration between the chimpanzee and rhesus monkey, since the cortisol levels in each age group and in each species were very similar. The timing of the rise in DHA levels in the chimpanzee in relation to gonadal maturation is also comparable to that in man; DHA levels have begun to rise by 5 yr of age, 2 yr before testosterone levels begin to increase (21). In man, DHA levels are significantly elevated by age 7, several years before the pubertal rise in gonadal steroids. Thus, the chimpanzee seems to undergo an adrenarchal process similar to man, and provides an animal species in which further studies of the mechanism and physiological importance of adrenarche can be pursued.

It is apparent from Figs. 1-3 and Table 2 that there is considerable species variation in the plasma concentrations of DHAS in relation to plasma DHA. There is also a significant age-related decrease in the ratio of DHAS to DHA in both the chimpanzee and rhesus monkey. Therefore, it seems that DHAS concentration cannot be assumed to provide a reliable indication of adrenal androgen secretion in other species.

The phylogenetical relationship of the primate species examined in this study is shown in Fig. 4. The number of samples obtained from primate species other than the rhesus monkey and chimpanzee was not sufficient to determine whether these species undergo adrenarche.

The fact that adrenarche occurs in certain primate species (man and chimpanzee) but
not in others (rhesus monkey) raises the question of whether this difference in adrenal androgens may play a role in differences in sexual maturation among these species. Since androgens of adrenal origin have been postulated to initiate activation of the hypothalamic-pituitary-gonadal axis at puberty (23), an interesting speculation is that the persistent high levels of adrenal androgens in the rhesus monkey may play a role in its early sexual maturation (24), whereas the low levels of adrenal androgens before adrenarche in man and chimpanzee may be a factor in the relatively delayed onset of puberty in these species.

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References


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