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Contribution of the Adrenal Gland to the Production of Androstenedione and Testosterone during the First Two Years of Life*

FRANK BIDLINGMAIER, HELMUTH G. DÖRR, WOLFGANG EISENMENGER, URSULA KUHNLE, AND DIETRICH KNORR

University of Munich, Children's Hospital and Institute for Forensic Pathology (W.E.), D-8000 Munich 2, West Germany

ABSTRACT. Androstenedione and testosterone were measured in whole adrenal glands of 56 previously healthy boys who died suddenly between birth and 2 yr of age. In each adrenal gland, the concentration of androstenedione considerably exceeded that of testosterone. The highest concentrations were found during the first week of life (median, 295 ng/g; range, 98–320 ng/g). Thereafter, values decreased rapidly until the end of the first year of life (median, 10 ng/g; range, 4.4–22.7 ng/g). Adrenal testosterone concentrations averaged 15% of those of androstenedione in the same gland and similarly decreased until the end of the first year. The decrease of adrenal androgen concentrations paralleled the involution of the fetal adrenal zone. A close correlation existed between the concentration of androstenedione in adrenal tissue and plasma. However, no correlation existed between adrenal and plasma testosterone. When the adrenals and testes of the same infant were compared, there was 10 times more androstenedione in the adrenals than in the testes during the first 2 yr of life. The testes contained more testosterone than the adrenals only during the first 4 months. Thus, in infant boys the adrenals are the main source of androstenedione during the first 2 yr. After the sixth month of life, they also are the main source of testosterone. (*J Clin Endocrinol Metab* **62:** 331, 1986)

IN INFANT boys, a transient increase of gonadotropins, especially LH, occurs during the first months of life (1). A striking elevation of plasma androgens is associated with this increase (2). In the same age group we also found enhanced testicular activity (3). Testicular testosterone concentrations were maximal in boys 1–3 months of age, with peak values similar to those in pubertal or even adult testes. Thereafter, testicular testosterone declined sharply and reached the low normal range for older prepubertal boys by 6 months of age. Plasma and testicular testosterone concentrations were closely correlated, which indicates the testes are the source of the early plasma testosterone surge. In contrast, testicular androstenedione concentrations were low, and there was no correlation between plasma and testicular androstenedione concentrations. These results suggested that the infantile testes contribute little to circulating androstenedione concentrations.

To evaluate the significance of the adrenal cortex as a source of circulating androgens in male infancy, we measured androstenedione and testosterone in whole adrenals of previously healthy infant boys who died suddenly. Many of the subjects were identical to those from whom testes were taken for a previous investigation of testicular androgens (3). Thus, we compared the androgen content of adrenals and testes and estimated the relative contribution of both glands to the circulating levels of androstenedione and testosterone during infancy.

Subjects and Methods

Specimens

Adrenals were obtained post-mortem from 56 infant boys between 0 and 2 yr who died of sudden infant death syndrome or accident. Body weight was within the normal range in all infants. The time interval between death and autopsy was less than 36 h. In each subject both adrenals were excised and cleaned of adhering tissue, then separately weighed, minced, and stored at −80 C. In each infant, heart blood was obtained for the determination of plasma steroids.

Although the possibility of post-mortem changes in tissue steroid content must be taken into account, the use of autopsy material is justified. Ruokonen *et al.* (4) found no differences in steroid composition between testes of cadavers and those removed surgically. Dickerman *et al.* (5) found no change in the cortisol content of bovine adrenals kept, after death, for 30 min to 21 h at 20 C before freezing. In our study, the testoster-

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Address correspondence and requests for reprints to: Dr. F. Bidlingmaier, Children's Hospital, University of Munich, Lindwurmstrasse 4, D-8000 Munich 2, West Germany.
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one and androstenedione contents showed no change in an adrenal homogenate maintained at room temperature from 12–36 h after death.

Steroid determinations

The minced and frozen adrenal tissue was homogenized with four parts (wt/vol) ice-cold distilled water. Homogenization was performed with a glass-teflon homogenizer (Potter S, Braun, Melsungen, West Germany). Androstenedione and testosterone levels in both plasma and tissue homogenates were determined by RIA after chromatography on Sephadex LH-20 (3). This system separated testosterone and androstenedione. However, dihydrotestosterone and dehydroepiandrosterone partially eluted with testosterone. The antiserum used for testosterone determinations had 6.4% cross-reactivity with dihydrotestosterone, 4% with androstenedione, and less than 0.1% with dehydroepiandrosterone. The antiserum used for androstenedione determinations cross-reacted 1.2% with testosterone and less than 0.1% with dihydrotestosterone and dehydroepiandrosterone. Plasma cortisol was measured by the method of Pham-Huu-Trung *et al.* (6). The interassay variability for a pooled plasma sample was 7.8% for testosterone (mean, 89 ng/dl), 8.2% for androstenedione (mean, 85 ng/dl), and 6.4% for cortisol (mean, 11.2 μg/dl). Intraassay coefficients of variance for determination of androstenedione and testosterone from adrenal homogenates were 12.8% and 15.6%, respectively. Interassay coefficients of variance were 12.3% and 14.6%, respectively.

Statistics

Data were expressed as median and range for each age group. Differences between age groups were compared by the Kruskal and Wallis nonparametric ranked analysis of variance (7). Interrelationships between corresponding steroid levels were evaluated by linear regression analysis. In two-sided tests, P values less than 0.05 were considered significant.

Results

As in our previous study (3), elevated cortisol levels in the heart plasma of approximately 50% of the deceased infants indicated preterminal stress and enhanced adrenal activity. The plasma of the same subjects also had elevated androstenedione. Accordingly, there was a close correlation ($P < 0.001$) between cortisol and androstenedione in the same plasma sample. In contrast, the plasma testosterone levels of deceased boys were the same as those of healthy boys and were not correlated with plasma cortisol or androstenedione.

Adrenal size changed markedly during the first 2 yr of life. Figure 1 shows the combined weights of both adrenals for each subject. The values were highest during the first week after birth (median, 7.99 g; range, 7.25–9.5 g). They were significantly ($P < 0.005$) lower in 1- to 3-month old boys (median, 4.95 g; range, 3.1–9.1 g) and even lower in the 3- to 6-month age group (median, 4.2 g; range 3.1–6.25 g). After the age of 6 months, adrenal weights increased. Adrenal weights of boys 6–12 months old (median, 5.2 g; range, 2.85–7.2 g) were higher than those of the preceding group and were even higher from 12–24 months (median, 6.85 g; range, 5.9–7.65 g) ($P < 0.05$), without reaching the weight of neonatal adrenals.

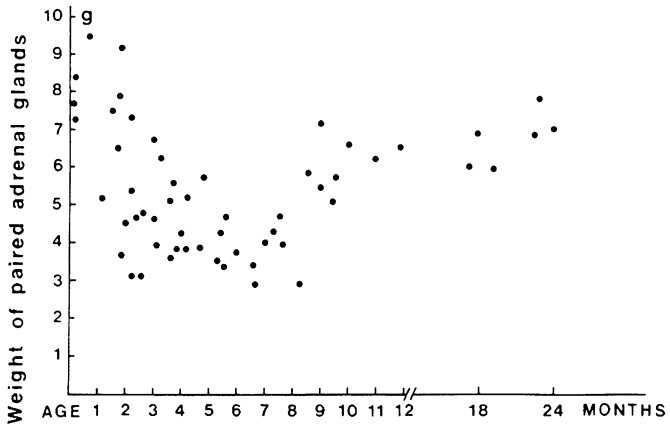

FIG. 1. Combined weight of paired adrenal glands during the first 2 yr of life.

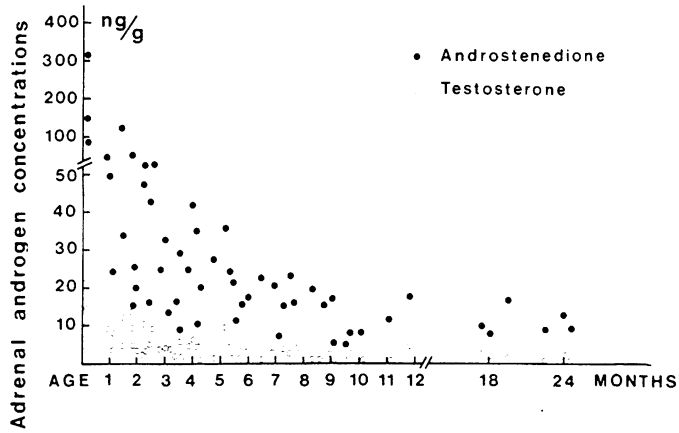

FIG. 2. Concentrations of androstenedione (●) and testosterone (○) in adrenal glands of infant boys (nanogram per g wet tissue).

The tissue concentrations of androstenedione and testosterone are shown in Fig. 2. The adrenal concentrations of androstenedione significantly exceeded those of testosterone at all times. The highest androstenedione concentrations were found in infants who died immediately after birth. During the first week of life, the median concentration of androstenedione in adrenal tissue was 295 ng/g (range, 98–320 ng/g). In infant boys 1–3 months of age, the androstenedione concentrations (median, 33.1 ng/g; range, 12.3–124 ng/g) were significantly lower than those of the neonates ($P < 0.0001$), but they were significantly higher ($P < 0.05$) than those of 3- to 6-month old boys (median, 21.2 ng/g; range, 7.9–42 ng/g). After the end of the sixth month, the androstenedione concentrations were lower than those of the preceding group ($P < 0.05$) and remained low until the end of the second year

(median, 10 ng/g; range, 4.4–22.7 ng/g). The androstenedione concentrations in adrenal tissue and plasma closely correlated ($P < 0.005$). On an average, 1 g adrenal tissue contained 50 times more androstenedione than 1 ml of plasma.

The adrenal testosterone averaged 15% of adrenal androstenedione. The adrenal concentrations of both androgens were significantly correlated ($P < 0.05$). During the first week of life, adrenal testosterone concentrations varied between 15 and 45 ng/g. These values were significantly ($P < 0.001$) higher than those of infants 1–3 months of age (median, 4.6 ng/g; range, 2.3–24 ng/g). Adrenal testosterone concentrations in 3- to 6-month old boys (median, 2.7 ng/g; range, 0.4–9.9 ng/g) were significantly ($P < 0.05$) lower than those of the preceding group. After the age of 6 months, the values continued to decrease (median, 1.8 ng/g; range, 0.34–4.25 ng/g), however, this decrease was not statistically significant. On an average, the concentrations of testosterone were 7.7 times higher in the adrenals than in plasma. There was no correlation between adrenal and plasma testosterone concentrations. The correlation of plasma cortisol and adrenal androstenedione in the same subjects was not significant ($0.1 > P > 0.05$). However, when age was considered (Fig. 3) in groups of infants with similar plasma cortisol levels, the youngest infants had the highest androstenedione concentrations.

The total content of the organ is more important than the tissue concentration in evaluating the contribution of a gland to circulating hormone. Therefore, the total androstenedione and testosterone contents of both adrenals of each subject were calculated from organ weights and tissue concentrations and were then compared with the androstenedione and testosterone contents in the testes of the same deceased infants reported previously (3). During the first 2 yr of life (Fig. 4), the adrenals contained at least 10 times more androstenedione than the testes. Additionally, more androstenedione was stored in the adrenals than testosterone in the testes. The testes contained significantly ($P < 0.001$) more testosterone than the adrenals only during the first 4 months of life. Thereafter, this difference was no longer significant. After the age of 6 months, the testosterone content in the adrenals was higher than in the testes.

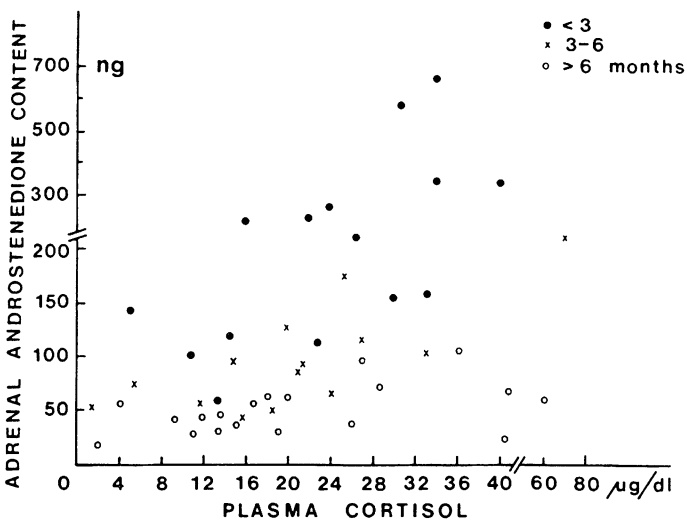

FIG. 3. Relationship between plasma cortisol concentrations and adrenal androstenedione content of infant boys of different ages.

Discussion

Whereas there is a large body of evidence indicating that the high concentrations of testosterone in the plasma of 1- to 3-month old boys are produced by transiently activated testes (2, 3), the origin of androstenedione is not clearly identified. After comparing plasma steroids in male and female infants in the basal state with those after ACTH stimulation, various authors suggested that during early infancy androstenedione originates, at least in part, from the adrenal cortex (2, 8, 9). In the present study, plasma and adrenal concentrations of androstenedione and testosterone in infant boys were investigated to obtain a more direct parameter of the importance of the adrenals in androgen production. By comparing these data with our previous results on testicular androgens in the same infants, the different roles of the adrenals and testes in the production of both androgens in the male infant could be evaluated.

In comparison to results in the testes, the pattern of androstenedione and testosterone in adrenals was reversed. The major adrenal androgen was androstenedione which correlated significantly with plasma andro-

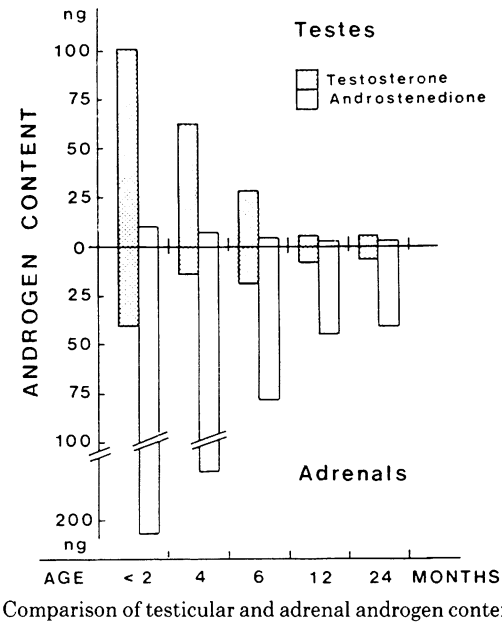

FIG. 4. Comparison of testicular and adrenal androgen content in male infants during the first 24 months of life. The *bars* represent the median values of the various age groups.

stenedione in all age groups. These findings demonstrate the importance of the adrenals as a source of androstenedione in male infancy. In contrast, adrenal testosterone concentrations were low and did not correlate with plasma concentrations. By considering not only tissue concentrations but also organ weights and comparing the total content of androgens in the adrenals and testes, the different roles of these glands become more apparent. Thus, circulating androstenedione in the male infant originates almost exclusively from the adrenals whereas the characteristic testosterone surge during the first 4 months of life originates from the testes. However, significant amounts of testosterone in the adrenals suggest that, during infancy, the adrenals are also involved in testosterone secretion. After the age of 6 months, the adrenals contain more testosterone than the testes and probably are the main source of testosterone until puberty.

Whereas the transient activation of testicular testosterone production probably depends on the rapid increase of plasma LH after birth, it is unlikely that the high androstenedione concentrations in the adrenals of young infants are a consequence of high circulating gonadotropins such as hCG or LH. hCG is thought to be a tropic hormone for the fetal adrenal cortex during the first half of pregnancy (10, 11). However, later in pregnancy, hCG is replaced by ACTH. After birth, there is no evidence that hCG or pituitary LH regulate adrenal activity. It is postulated that a non-ACTH pituitary hormone may be responsible for enhanced adrenal androgen production in a number of physiological situations (12). However, the existence of such a hormone is still controversial (13). Forest (8) reported that young infants are especially sensitive to ACTH, the response to ACTH stimulation in infancy yielding higher increments of plasma cortisol and androstenedione than later in childhood. It seems unlikely, however, that an elevation of ACTH is responsible for the high androstenedione concentrations in adrenal tissue during infancy. Although plasma ACTH levels are elevated only during the first few days of life, and equal those of older children by 1 week of life (14), adrenal androstenedione concentrations gradually decline from birth until the end of the first year.

The age dependence of adrenal androstenedione content in infancy may be explained by the fundamental morphological changes of the adrenal cortex which are reflected in the marked changes in adrenal weight during the first year of life. At birth, about 75% of the adrenal cortex consists of the fetal zone. This fetal zone involutes rapidly during the first month of life and more slowly thereafter (15). By the end of the first year, only the stroma of the degenerated fetal zone is left, with the definitive zone of the cortex increasing (16).

During pregnancy, the main products of the fetal adrenal cortex are pregnenolone, dehydroepiandrosterone, and its sulfate which serve as important precursors for estrogen synthesis in the placenta (17). Testosterone and androstenedione are only produced in small amounts by human fetal adrenals since the activity of the adrenal 3β-hydroxysteroid dehydrogenase enzyme system of the fetus is low (18, 19). The low enzyme activity may be caused by inhibitors originating from the placenta, possibly estrogens (18, 20). After birth, rapid activation of the 3β-hydroxysteroid dehydrogenase enzyme system leads to large amounts of androstenedione and testosterone in the adrenals and plasma. Parallel involution of the postnatal fetal zone, decreasing concentrations of adrenal androstenedione, and close correlation between adrenal and plasma concentrations of androstenedione in infancy suggest that, during the first year of life, adrenal androstenedione content and circulating androstenedione levels depend mainly on the cell mass of the fetal adrenal cortex.

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Fourth International Forum of Andrology

The Fourth International Forum of Andrology will be held June 19 and 20, 1986, at the Hotel Intercontinental, Paris, France. The program will include lectures on acute and chronic prostatitis, male contraception and hormonal causes of male infertility, and poster presentations on new research in andrology.

For further information please contact:

Professor G. Arvis
Department of Andrology-Urology
Hopital Saint-Antoine
184 rue du Faubourg Saint-Antoine
F-75012 Paris, France

Telephone: (1) 43 43 73 40