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High Serum Allopregnanolone Levels in Girls with Precocious Puberty

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Allopregnanolone, a circulating neuroactive steroid hormone, is involved in the modulation of behavioral functions, stress, and the neuroendocrine axis. The aim of this study was to evaluate serum allopregnanolone concentrations in girls with central precocious puberty (n = 12), girls with normal pubertal development at the same pubertal stage (n = 17), and prepubertal girls (age-matched; n = 16). Gonadotropin and steroid hormones (allopregnanolone, cortisol, dehydroepiandrosterone sulfate, and E2) were assessed in all patients. GnRH and ACTH stimulation tests were performed in all girls with central precocious puberty and in some pubertal controls. Basal allopregnanolone levels in girls with central precocious puberty were significantly higher than in normal controls ($P < 0.01$). Allopregnanolone levels increased significantly after GnRH and ACTH stimulation tests ($P < 0.05$) both in girls with central precocious puberty and in those with normal pubertal development. There was no difference found between the peak values.

In conclusion, our study shows that allopregnanolone is hypersecreted in central precocious puberty, confirming a pubertal stage-related increase in its levels during normal pubertal development. The increase in serum allopregnanolone after GnRH and ACTH stimulation tests demonstrates that both adrenal cortex and gonads are sources of this neuroactive steroid. (*J Clin Endocrinol Metab* 87: 2262–2265, 2002)

RECENT STUDIES demonstrated that some steroid hormones are synthesized and act within the brain. As these hormones have a role in the central nervous system, they have been called neuroactive steroids (1). The most extensively studied are the sedative-hypnotic 3α-hydroxy ring A-reduced pregnane steroids, including 3α-hydroxy-5α-pregnan-20-one or allopregnanolone. Allopregnanolone is a ligand of γ-aminobutyric $acid_A$ receptors in the central nervous system, producing anxiolytic-sedative effects under stress conditions or pregnancy (2, 3) and modulating the hypothalamic-pituitary-gonadal axis (4, 5). Ovarian and adrenal origins were postulated as sources of these hormones, as demonstrated by the higher concentrations observed in female rats, the decrease in plasma levels after ovariectomy, and their disappearance after combined adrenalectomy and ovariectomy (6). The highest allopregnanolone levels in women have been found during the luteal phase, and its concentrations did not change significantly after menopause. As the increase in allopregnanolone levels was demonstrated after GnRH and ACTH tests, both ovarian and adrenal origins are suggested (7, 8). Serum allopregnanolone levels remain unchanged during the first 2 yr of life, whereas they significantly increase throughout puberty in relation to age and Tanner's stage, suggesting a possible role of this neurosteroid in the maturational process of hypothalamic-pituitary-gonadal and hypothalamic-pituitary-adrenal axes (9).

Abbreviations: A, Androstenedione; CPP, central precocious puberty; DHEAS, dehydroepiandrosterone sulfate; 17-OHP, 17-hydroxyprogesterone.

The aim of this study was to evaluate the difference in allopregnanolone concentrations among girls with central precocious puberty (CPP), girls in early puberty, and the prepubertal population.

Subjects and Methods

Subjects

The study was approved by the local ethical committee of University of Modena, and informed consent was obtained from the children and/or their parents. Forty-five girls with normal body weight were studied and separated into three groups: group A, girls with CPP (n = 12) at Tanner pubic hair and breast stages 2–3 and 8.1 ± 0.8 yr of age (mean ± SD); group B, girls in early puberty (n = 17) at Tanner pubic hair and breast stages 2–3 and 10.9 ± 1.8 yr of age (mean ± SD); and group C, prepubertal girls (n = 16), 8.4 ± 1.6 yr of age (mean ± SD). The clinical characteristics of the subjects are reported in Tables 1 and 2. The subjects in group A were comparable to prepubertal girls for chronological age and to girls with early puberty for pubertal stage. Patients with CPP had bone age advanced by 1.99 ± 0.91 SD. No difference in the bone age between this group and group B was present (Table 1). No subject had acute or chronic disease or had entered menarche. Patients with secondary precocious puberty and polycystic ovary syndrome were excluded from the study.

Methods

All girls underwent a blood sample drawn between 0700–0900 h for measurement of basal serum allopregnanolone, dehydroepiandrosterone sulfate (DHEAS), 17-hydroxyprogesterone (17-OHP), androstenedione (A), cortisol, ACTH, LH, FSH, and E2. In patients with signs of early pubertal development, a GnRH test (LH-releasing hormone, Ferring Pharmaceuticals Ltd., Kiel, Germany; iv bolus injection of 75 $\mu g/m^2$) and an ACTH test (Synacten, Ciba-Geigy, Huningue, France; iv bolus injection of 0.25 mg) were performed. An indwelling venous

TABLE 1. Clinical characteristics of the patients

	Group A (n = 12)	Group B (n = 17)	Group C (n = 16)
CA (yr ± SD)	8.1 ± 0.8	10.9 ± 1.8	8.4 ± 1.6
BA (yr ± SD)	10.2 ± 1.15	10.5 ± 1.2 (n = 9)	7.4 ± 1.1 (n = 7)
Weight (kg ± SD)	31.6 ± 8.31	34.0 ± 7.21	24.4 ± 6.84
Height (cm ± SD)	134.6 ± 10.9	136.1 ± 8.44	121.2 ± 9.83
Pubertal stage	PH and B 2-3	PH and B 2-3	PH and B 1

CA, Chronological age; BA, bone age.

TABLE 2. Clinical details of patients with CPP

Patient	CA (yr)	First signs of puberty (yr)	BA (yr)	Weight (kg)	Height (cm)
1	8.8	7.5	10.5	37.5	139.9
2	9.1	7.8	11	32.2	143.2
3	7.9	7.3	7.9	19.2	115
4	8.4	7.5	10.5	27.2	133.2
5	7.2	7.0	11	35	134.8
6	6.2	5.9	8	21.8	125.8
7	8.5	7.8	10	25.7	120.2
8	7.6	7.0	10	26.4	131.6
9	7.9	7.3	11	30.9	133.5
10	9.3	7.8	11	43.6	146.6
11	8.9	7.8	10.5	33	137.7
12	8.5	7.0	11.5	47	154

CA, Chronological age; BA, bone age.

catheter was inserted into an antecubital vein and kept patent by a slow saline infusion. During the stimulation tests, blood samples were obtained between 0900–1100 h. These were taken at 15, 30, 45, 60, and 90 min after LH-releasing hormone injection for measurement of allopregnanolone, LH, FSH, and E2 concentrations and 60 and 120 min after administration of ACTH for measurement of allopregnanolone, DHEAS, 17-OHP, A, and cortisol levels. Blood samples for allopregnanolone and E2 evaluation were centrifuged, and serum was stored at −20 C until assayed. Commercial kits were used for the estimation of serum concentrations of LH, FSH, 17-OHP, and A.

All girls with CPP underwent a cerebral-pituitary nuclear magnetic resonance (NMR) and a left hand x-ray for bone age determination (calculated using the Greulich and Pyle method).

Assay for allopregnanolone, E2, cortisol, and DHEAS

Analytical grade solvents were purchased from Merck & Co., Inc. (Darmstadt, Germany); the C_{18} Sep-Pak cartridge was obtained from Waters Corp. (Milford, MA). Standard allopregnanolone was purchased from Sigma (St. Louis, MO), and 5α-[9,11,12-N-^{3}H]pregnan-3α-ol-20-one (45Ci/nmol) was purchased from Amersham Pharmacia Biotech (London, UK). The polyclonal antiserum, raised in sheep against allopregnanolone carboxymethyl ether coupled to BSA, was provided by Dr. R. H. Purdy (VA Medical Center, San Diego, CA). Serum samples after extraction and chromatographic partition were assayed using a previously described RIA method (8). The sensitivity of the assay, expressed as the minimal amount of allopregnanolone distinguishable from the zero sample with 95% probability, was 15 pg/tube. The intra- and interassay coefficients of variation were 6.8% and 8.1%, respectively.

Serum E2 and cortisol were determined, after extraction and chromatographic partition on a C_{18} Sep-Pak cartridge, by RIA using a commercially available kit (Radim, Pomezia, Italy). The sensitivity of the assay was 10 pg/ml, and the intra- and interassay coefficients of variations were 3.7% and 5.8%, respectively.

Serum samples for the determination of DHEAS were extracted with ether, purified through a C_{18} Sep-Pak cartridge, and then assayed by RIA using a trade kit (Diagnostic Systems Laboratories, Inc., Webster, TX). The sensitivity was 15 pg/ml, and the intra- and interassay coefficients of variation were 3.1% and 6.9%, respectively. The extraction and chromatography step was also used for these steroids to increase the reliability criteria of the specific methods.

Statistical analysis

All results are presented as the mean ± SD. Statistical analysis (SPSS software, SPSS, Inc., Chicago, IL) was performed using ANOVA for comparison of hormone levels among the groups and Scheffé's F test to localize significant differences in mean values. A *t* test for independent samples was used to evaluate statistical differences between basal and peak concentrations. Significance was set at $P < 0.05$.

Results

Among groups A, B, and C no differences in basal serum levels of LH (1.17 ± 1.26, 0.66 ± 1.14, and 0.16 ± 0.17 mIU/ml, respectively; $P = 0.125$), FSH (3.17 ± 1.60, 2.84 ± 1.18, and 2.05 ± 1.54 mIU/ml, respectively; $P = 0.344$), E2 (32.0 ± 28.3, 24.2 ± 13.7, and 31.7 ± 18.3 pg/ml, respectively; $P = 0.693$), DHEAS (0.62 ± 0.30, 0.79 ± 0.60, and 0.30 ± 0.19 μg/ml, respectively; $P = 0.096$), 17-OHP (1.26 ± 0.48, 0.90 ± 0.56, and 0.97 ± 0.75 ng/ml, respectively; $P = 0.459$), cortisol (10.1 ± 4.70, 7.46 ± 3.16, and 11.6 ± 6.90 μg/ml, respectively; $P = 0.375$), A (54.0 ± 25.4, 58.3 ± 19.1, and 37.1 ± 14.2 ng/ml, respectively; $P = 0.146$), and ACTH (30.3 ± 23.1, 11.9 ± 3.95, and 22.3 ± 10.4 pg/ml, respectively; $P = 0.208$) were found. However, allopregnanolone levels in group A were significantly higher than those in groups B and C (0.91 ± 0.35, 0.47 ± 0.29, and 0.42 ± 0.14 nmol/liter, respectively; $P = 0.000$).

After the GnRH stimulation test, LH levels increased significantly in all CPP girls with values above 5 mIU/ml, confirming the central origin of precocious pubertal development. A significant increase in allopregnanolone levels was found in response to GnRH administration in both group A (0.91 ± 0.35 *vs.* 1.28 ± 0.41 nmol/liter; $P = 0.038$) and group B (0.47 ± 0.29 *vs.* 1.50 ± 0.59 nmol/liter; $P = 0.000$; Fig. 1, *upper panel*). There was no statistical difference in peak allopregnanolone values between the two groups (1.50 ± 0.39 *vs.* 1.50 ± 0.59 nmol/liter, respectively; $P = 0.713$).

After ACTH administration, significant increases in 17-OHP, cortisol, and A levels were found, but no alteration of adrenal function was demonstrated in either group A or group B. Allopregnanolone levels significantly increased in both group A (0.72 ± 0.28 *vs.* 1.29 ± 0.31 nmol/liter; $P = 0.034$) and group B (0.49 ± 0.20 *vs.* 1.17 ± 0.09 nmol/liter; $P = 0.006$; Fig. 1, *lower panel*), but no differences were found in peak values (1.36 ± 0.26 *vs.* 1.20 ± 0.09 nmol/liter, respectively; $P = 0.363$).

No difference was found in peak allopregnanolone values obtained after GnRH and ACTH administration between the two groups [group A, 1.50 ± 0.39 *vs.* 1.36 ± 0.26 nmol/liter ($P = 0.530$; group B, 1.50 ± 0.59 *vs.* 1.20 ± 0.09 nmol/liter ($P = 0.425$)], suggesting that both gonads and adrenal cortex are important sources for the secretion of this neuroactive steroid.

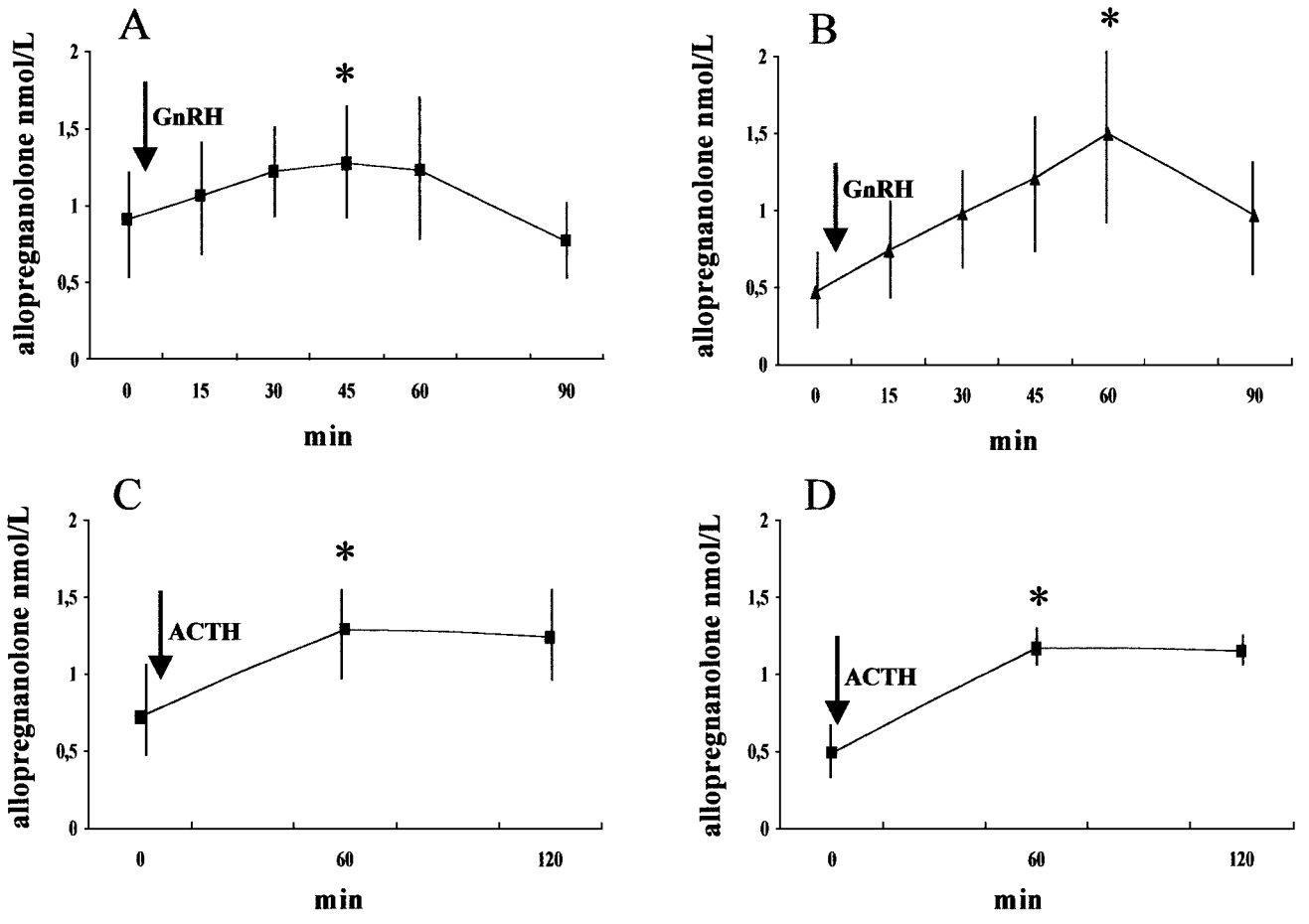

FIG. 1. The *upper panel* shows the mean ± SD allopregnanolone levels in response to the GnRH test in girls with CPP (A) and in girls at early pubertal stages (B). The *lower panel* shows the mean ± SD allopregnanolone levels in response to an ACTH test in girls with CPP (C) and in girls at early pubertal stages (D). *, $P < 0.05$ *vs.* 0 min.

Discussion

The present study shows that girls with CPP have higher basal allopregnanolone levels. The onset of puberty represents an event modulated by complex interactions between neuroendocrine and hormonal factors, inducing activation of the hypothalamic-pituitary-gonadal axis (10–12). During the prepubertal period the hypothalamic-pituitary-gonadal axis is inactive, as demonstrated by undetectable serum concentrations of LH and sex hormones. Probably in this period the hypothalamic and pituitary activities are suppressed by neuronal restraint pathways and negative feedback exerted by low amounts of circulating gonadal steroids. During clinical puberty, gonadotropin secretion becomes pulsatile. This is responsible for the enlargement and maturation of the gonads and for sex hormone secretion. However, this pulsatile secretion as well as the synergism of FSH and LH action in promoting gonadal maturation make the interpretation of their precise roles difficult. Adrenal cortical androgens also play a role in pubertal maturation. Serum levels of DHEA and DHEAS begin to rise at approximately 6–8 yr of age before any increase in LH or sex hormones and before the earliest physical changes of puberty appear. A single measurement of DHEAS is commonly used as a marker of adrenal androgen secretion.

The appearance of secondary sexual characteristics before 8 yr of age in girls and 9 yr in boys is generally considered precocious puberty. CPP includes hypothalamic-pituitary-gonadal axis activation, which, in turn, is responsible for secondary sexual signs and a gonadotropin-mediated increase in the size and activity of the gonads. The importance of adrenal androgen in the early pubertal changes has been postulated for a long time. The present study suggests the role of allopregnanolone. Several studies have demonstrated poor mental health, increased behavioral problems, and a lower intelligence quotient in children with premature adrenarche. It is very important to understand whether psychological stress triggers premature adrenarche or whether it is the early adrenal hormone secretion that leads to psychosocial problems.

Girls with CPP have allopregnanolone concentrations higher than controls. Allopregnanolone suppresses, in a concentration-dependent manner, the release of hypothalamic GnRH *in vitro*. This has been demonstrated when an intracerebroventricular injection led to a decrease in the number of oocytes retrieved on the day of estrus in rats (4, 5). In contrast, other studies show allopregnanolone to stimulate GnRH release from hypothalamic neurons (13), and its administration to estrogen-primed rats produces a

dose-related stimulatory effect on LH and FSH secretion (14).

The significant increase in allopregnanolone we found after both GnRH and ACTH stimulation tests confirm that both the ovary and the adrenal cortex are sources of this neurosteroid. The lack of differences in the peaks, after the tests, underlines that these sites of production might contribute to circulating concentrations in the same manner. Previous studies in fertile subjects demonstrated that serum allopregnanolone levels increased in response to CRH and ACTH tests, suggesting that the adrenals were the most important source of circulating allopregnanolone. However, the variation in its concentrations demonstrated in rats and humans during the menstrual cycle (higher levels during the luteal phase than the follicular phase) also suggested a gonadal contribution (8). This hypothesis was confirmed by the presence of allopregnanolone in rat ovarian tissue (6).

It is likely that girls with CPP associated with early pubertal maturation or emotional and behavioral lability were more prone to psychological disorders (11). It is possible that the stress caused by precocious puberty in a younger girl's life may be responsible for an increase in allopregnanolone levels.

In conclusion, girls with precocious puberty show higher levels of allopregnanolone, which may have an impact on neuroendocrine and behavioral functions.

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