0031-3998/87/2201-0041$02.00/0
PEDIATRIC RESEARCH

Vol. 22, No. 1, 1987
Printed in U.S.A.

Plasma Adrenocorticotropin, Cortisol, and Dehydroepiandrosterone Response to Corticotropin-Releasing Factor in Normal Children during Pubertal Development

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ABSTRACT. The adrenocorticotropin (ACTH), cortisol, and dehydroepiandrosterone responses to synthetic human corticotropin-releasing factor (CRF) were studied in 28 endocrinologically healthy children (age 1–16 yr) and in six adult volunteers (age 24–42 yr). CRF was given as an intravenous bolus (1 μg/kg body weight) between 0900 and 1000 hr. Significant increments in ACTH and cortisol levels after CRF were observed in all subjects, with an ACTH peak value of 48.2 ± 3.4 pg/ml at 10 min ($p < 0.001$). The ACTH and cortisol response patterns after CRF did not change with age or pubertal maturation and did not differ in children and in adults. In contrast, the dehydroepiandrosterone response to CRF clearly was related to the stage of pubertal development. The peak value after CRF significantly increased from puberty stage 1 to puberty stage 5 (164 ± 18 *versus* 779 ± 86 ng/100 ml, $p < 0.001$). In adults, the mean dehydroepiandrosterone peak value after CRF did not differ from that of P5 children. These results show that CRF can be given safely to children. The absence of age-dependent ACTH and cortisol responses and a dehydroepiandrosterone response changing with pubertal maturation points to the existence of factors involved in the control of adrenal androgen production other than ACTH. (*Pediatr Res* 22: 41–44, 1987)

Abbreviations

ACTH, adrenocorticotropin
DHEA, dehydroepiandrosterone
CRF, corticotropin-releasing factor

Since the isolation and characterization of CRF (1–3) several studies of its use in humans have been published (4–11). Synthetic CRF 1–41 has been reported to be highly potent in stimulating the release of ACTH- and proopiomelanocortin-derived peptides from the pituitary of rodents and of humans (12, 13). Furthermore, the CRF-induced ACTH increase stimulates the concomitant release of cortisol and aldosterone from the adrenals (4–11, 14). Because of these effects and of its relative safety during clinical studies, CRF provides a powerful tool for studying ACTH secretion in normal as well as pathological situations. Several recent studies describe use of the CRF test in the evaluation of Cushing syndrome (15–17), Nelson syndrome (19, isolated pituitary ACTH deficiency and adrenal insufficiency (19, 20). Data on the administration of CRF to children are limited so far to two reports (21, 22). In one study, Ross *et al.* (21) could find no age-dependent changes in the ACTH or cortisol responses to CRF in a group of normal children.

In the present study, CRF was administered to hospitalized but endocrinologically healthy children, and the CRF-induced activation of the pituitary-adrenal axis was studied in relation to pubertal maturation. DHEA responses as well as ACTH and cortisol responses to CRF were assessed since the maturation of adrenal androgen production is associated with pubertal development (23–26).

SUBJECTS AND METHODS

Subjects. Approval for studies was obtained from the ethical committees of the three pediatric clinics in Tübingen, Bonn, and Parma. The parents of all hospitalized but endocrinologically healthy children gave informed consent before the test was done. The normal children had no physical abnormalities except short stature. According to the pubic hair stages of Tanner, nine children, all males, were in puberty stage 1, nine (six males and three females) were in puberty stage 2, and 10 (six males and four females) were in puberty stage 4 to 5. Additionally, six normal adult volunteer subjects (age 24–42 yr) were included.

CRF stimulations test. Human synthetic CRF 1–41 was obtained from Bissendorf Peptide (Wedemark, FRG). The peptide was dissolved in saline and given at a dose of 1 μg/kg body weight as an intravenous bolus between 0900 and 1000 h. Blood samples for ACTH, cortisol, and DHEA measurements were obtained 15 min before, immediately before, and 5, 10, 15, 30, 60, 90, 120, and 180 min after CRF administration. The samples were collected in prechilled polystyrene tubes containing EDTA, placed on ice, and centrifuged 10 min later. Aliquots for measurements of ACTH, cortisol, and DHEA were separately frozen at −30° C until assay.

Hormone assays. ACTH was measured in unextracted plasma by the RIAmat Kit of Byk Mallinckrodt (Dietzenbach, FRG). The antibody used in the RIA is specific for the 11–24 sequence of ACTH. Intra- and interassay coefficients of variation were 7 and 8.5% respectively; the sensitivity was 5 pg/ml. The cortisol radioimmunoassay was performed with a MAIA kit provided by Serono (Freiburg, FRG). Intra- and interassay coefficients of variation of this method were 4.6 and 5.6%. DHEA was measured by a kit manufactured by Wien Laboratories (Succasunna, NJ) and supplied by DRG Instruments (Marburg, FRG). The coefficients of variation of this method were 6 and 11%.

Received July 17, 1986; accepted February 2, 1987.
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C.T. is a recipient of a research fellowship from the University of Parma, Parma, Italy.

Statistical analysis. Results are expressed as mean ± SEM. For undetectable hormone concentrations the value of the detection limit of the method was taken (*i.e.* 5 pg/ml for ACTH, 0.3 μg/ml for cortisol, and 50 ng/ml for DHEA). For statistical analysis, paired (within group differences) and unpaired (between group differences) Student's *t* tests were applied, as the samples were found to be normally distributed.

RESULTS

At the dose of 1 μg/kg body weight CRF produced no side effects in most children and was well tolerated by all children. A flush lasting about 20 min was observed once in a 7-year-old boy. The ACTH and cortisol responses in the 28 children are given in Figure 1. ACTH control levels showed considerable variation (range: at −15 mm 5.0–58.0, at 0 time 5.0–39.4 pg/ml), and although in most subjects the −15 min value was higher than the 0 value, the mean values did not differ significantly (22.5 ± 3.4 *versus* 19.7 ± 2.1 pg/ml, NS). After CRF the mean ACTH levels significantly ($p < 0.01$) rose in all subjects to a peak value of 48.2 ± 3.4 pg/ml at 10 min and decreased to 32.8 ± 3.2 and to 30.0 ± 3.5 pg/ml at 15 and 30 min. These values were significantly ($p < 0.05$) higher than the control values.

The cortisol levels showed less variation. As with ACTH, the −15 min value was higher than the 0 value in almost all subjects, but the mean values did not differ significantly (13.9 ± 1.2 *versus* 11.3 ± 0.8 μg/100 ml, NS). After CRF, cortisol levels rose to a peak value of 20.2 ± 0.8 μg/100 ml ($p < 0.001$) at 30 min and progressively declined thereafter. The ACTH and cortisol response patterns in the six adult volunteers did not differ from those in children (data not shown). When the ACTH and cortisol peak levels after CRF were related to the pubertal stage of the subjects, no differences were observed between prepubertal, pubertal, and adult subjects (Fig. 2).

In contrast, significant differences emerged in the DHEA response to CRF. In all subjects CRF elicited a clear increase in DHEA levels, the peak value occurring at 30 to 60 min postinjection. While DHEA control levels significantly increased throughout puberty (from 88.7 ± 12 at P1 to 594 ± 48 ng/100 ml at P5, $p < 0.001$), the peak DHEA levels after CRF significantly increased from P1 to P2 (164 ± 18 *versus* 392 ± 39 ng/100 ml, $p < 0.001$) and from P2 to P4–5 (779 ± 86, $p < 001$) (Table 1 and Fig. 2). The DHEA peak value seen in adults after CRF (723 ± 78 ng/100 ml) did not differ from that seen in P4–5 subjects.

DISCUSSION

CRF stimulation in children induces an ACTH increase which is comparable to that seen in adults, as shown in a recent report of Ross *et al.* (21). These authors measured ACTH and cortisol levels every 30 min after CRF stimulation and found that the peak value of these two response parameters occurred at 30 min. In the present study, taking the blood samples at shorter intervals after CRF, it becomes evident that the ACTH peak occurs earlier and that the response pattern is biphasic. There is a rapid and steep ACTH increase immediately after CRF followed by a prompt decrease. At 15 or 30 min after CRF, ACTH mean levels are already 30% lower than at 10 min although significantly elevated when compared to the mean basal level. This pattern of response, which was not age dependent, suggests the existence of two pools of pituitary ACTH, one which is very quickly releasable. Although many studies have already been performed in different clinical entities, this finding has not been stressed previously by other authors. In our opinion, knowledge of such an ACTH response pattern might be of importance in the interpretation of CRF tests in pathological subjects in which a discrepancy exists between the ACTH and cortisol responses (18). In

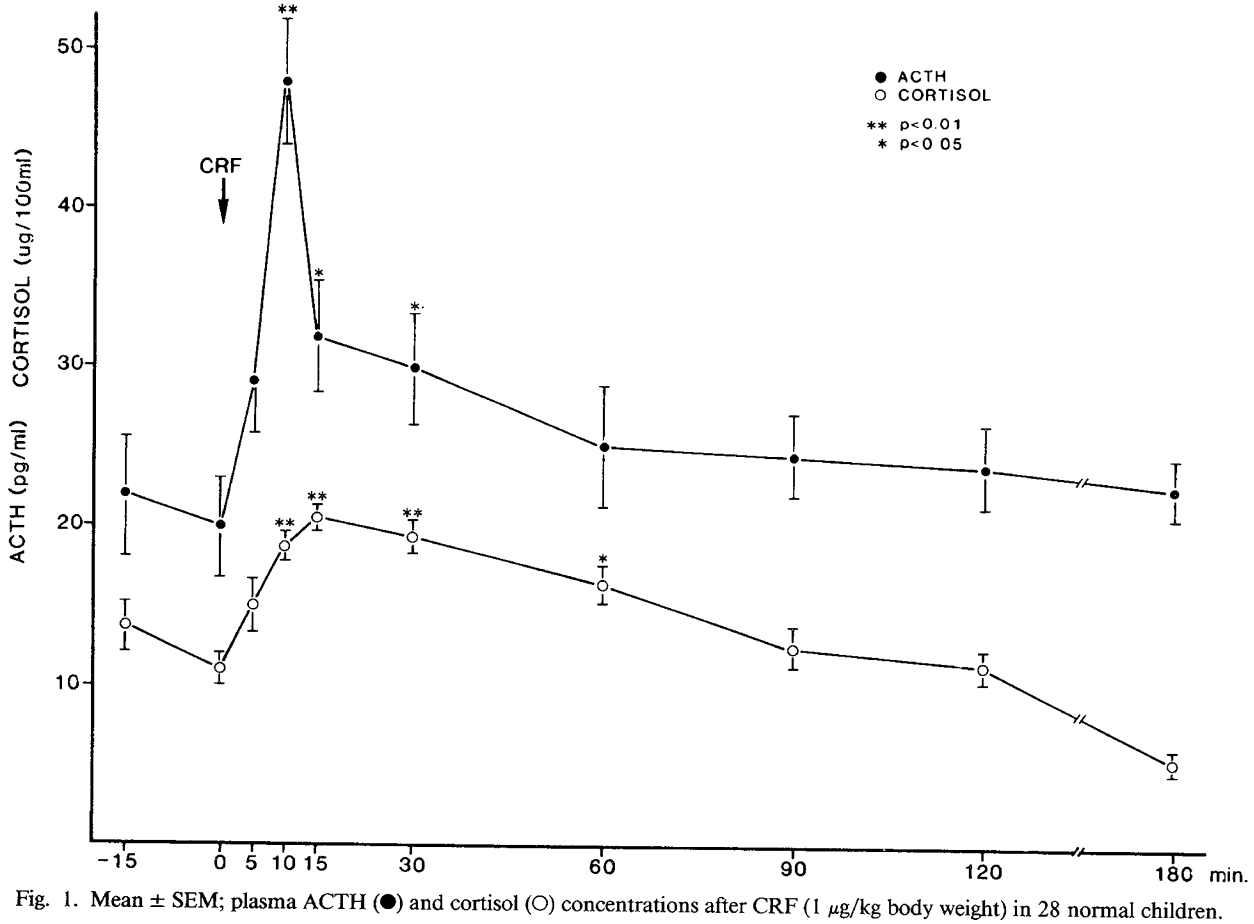

Fig. 1. Mean ± SEM; plasma ACTH (●) and cortisol (○) concentrations after CRF (1 μg/kg body weight) in 28 normal children.

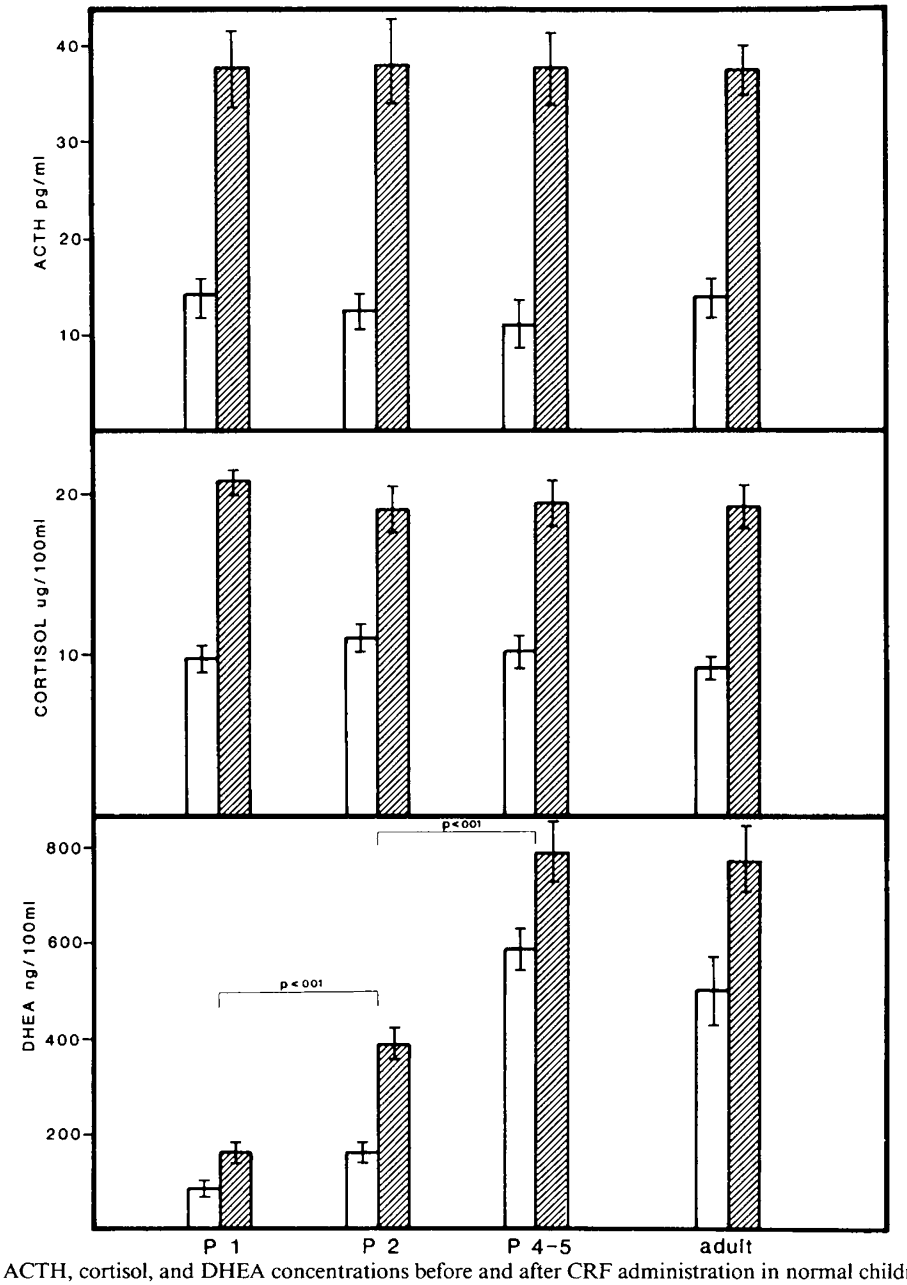

Fig. 2. Mean ± SEM; ACTH, cortisol, and DHEA concentrations before and after CRF administration in normal children and adult volunteers. *Open bars*, control values; *hatched bars*, peak values. The groups are the same as in Table 1.

Table 1. *Mean ± SEM plasma DHEA concentrations (ng/100 ml) before and after CRF stimulation (1 μg/kg body wt)*

Min	0	15	30	60	90
P_1 ($n = 9$)	87 ± 12	152 ± 19*	164 ± 18*	121 ± 17	118 ± 8
P_2 ($n = 9$)	158 ± 16	281 ± 27*	392 ± 39†	365 ± 31*	172 ± 18
P_4 ($n = 10$)	594 ± 48	647 ± 48	779 ± 86*	701 ± 37*	611 ± 15
Adult ($n = 6$)	502 ± 39	632 ± 45*	711 ± 52*	723 ± 78*	545 ± 31

* $p < 0.05$ over the 0 value.
† $p < 0.001$ over the 0 value.

most children, ACTH and cortisol control levels (−15 value) are markedly elevated, sometimes in the range of the post-CRF levels. This observation is due to stressful stimuli (venipuncture) which are probably much stronger in children than in adults.

Although there are no changes in ACTH and cortisol responses to CRF related to pubertal maturation, as previously stressed by Ross *et al.* (21), the DHEA response is clearly related to pubertal developmental stage. This response is similar to that in children when the adrenals are stimulated with exogenous ACTH (23–26). The DHEA peak value under CRF stimulation progressively increases from puberty stage 1 to puberty stage 4–5. As we observed in a previous study done with exogenous ACTH stimulation (23), DHEA responsiveness to CRF also is highest at puberty stage 2 (Fig. 2). Since the ACTH and cortisol responses

to CRF do not change with age, it may be concluded that the observed DHEA response is due to some other, yet unknown, factor(s) directly involved with adrenal androgen production. The results of the present study show that CRF can be used to test the pituitary adrenal axis in children as in adults. It activates the pituitary-adrenal axis stimulating release of ACTH, cortisol, and DHEA. Therefore its diagnostic use might be extended to disturbances of pubertal development related to adrenal function.

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