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Remarkable Changes in the Plasma Levels of Pituitary Protein "7B2" during Childhood

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ABSTRACT. We measured plasma immunoreactive (IR)-7B2 concentrations in 96 children (57 males and 39 females) from the newborn period to 20 yr of age. Plasma IR-7B2 concentrations in infants less than 2 yr of age (range 175–580 pg/ml, $n = 19$) were much higher than those in adults (range 20–138 pg/ml). Plasma levels of IR-7B2 decreased with age during childhood to reach the adult level at 15–20 yr. Significant negative correlations were found between plasma levels of IR-7B2 and dehydroepiandrosterone sulfate ($r = -0.4154$, $p < 0.05$, $n = 27$), luteinizing hormone ($r = -0.4948$, $p < 0.05$, $n = 20$) and follicle-stimulating hormone ($r = -0.4682$, $p < 0.05$, $n = 20$). The possibility of a relationship between the reduction of plasma IR-7B2 levels and pubertal development warrants attention. (*Pediatr Res* 24: 194–196, 1988)

Abbreviations

IR, immunoreactive
LHRH, luteinizing hormone releasing hormone
RIA, radioimmunoassay
GH, growth hormone
LH, luteinizing hormone
FSH, follicle-stimulating hormone
T, testosterone
E_2, 17-β-estradiol
DHEA-S, dehydroepiandrosterone sulfate

7B2 is a protein recently isolated from porcine and human pituitary glands (1, 2). 7B2 release from the pituitary gland and adrenal medulla of several species was demonstrated *in vitro*; hence, 7B2 may be a secretory protein (3–7). IR-7B2 is present in human plasma and the level is elevated in patients with chronic renal failure and patients with various endocrine tumors (8–10). We observed an age-related increase of plasma IR-7B2 levels in adults (20–87 yr of age) and very high levels in the cord blood (8, 11). In light of these observations, it seemed important to study plasma IR-7B2 levels during childhood. Thus, we measured plasma IR-7B2 concentrations in infants and children and partially characterized the molecular form, using gel permeation chromatography. In addition, we compared the IR-7B2 content in the pituitary glands of young and older persons.

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MATERIALS AND METHODS

Blood samples were taken from 96 subjects (57 males and 39 females, newborn to 20 yr of age) into chilled glass tubes containing EDTA-2Na between 0800–1000 h after an overnight fast. After centrifugation, the plasma was stored at −20° C until assay. Plasma levels of creatinine, blood urea nitrogen, bilirubin, glutamic oxaloacetic acid transaminase and glutamic pyruvic acid transaminase were within normal limits in all subjects. The stature and body weight of the subjects were within the mean ± 2 SD for age and struma was not found in all subjects. Pubertal stages of the subjects during puberty were corresponding to age.

LHRH testing was performed on six children with stature less than −2 SD of the mean height for age (four males and two females, 5–13 yr of age). Plasma GH levels were normal in these children. Blood samples were collected at 0, 30, 60, 90, and 120 min after an intravenous infusion of 2 μg/kg LHRH (Tanabe Pharmaceutical Co., LTD, Osaka, Japan) through an indwelling catheter placed into a forearm vein. Plasma 7B2, LH, and FSH were measured in each sample by radioimmunoassay.

For measurements of pituitary tissue 7B2 concentrations, half pituitary glands were obtained at autopsy 1–4 h after death and stored at −80° C until extraction. Three pituitary glands were obtained from children (less than 1 yr of age); 2 died of the respiratory distress syndrome and one of 18-trisomy. Four pituitary glands were obtained from adults (35–91 yr of age); two died of lung cancer and two of cardiac desease. Pituitary metastasis was excluded in the cancer patients. The frozen pituitary glands were homogenized in 1 M acetic acid containing 20 mM HCl with a Polytron. After boiling in a water bath for 10 min, the homogenates were centrifuged at 1000 × g for 20 min at 4° C. An aliquot of the supernatant was stored at −20° C.

Gel permeation chromatography was performed on a Sephadex G-100 column (95 × 1.5 cm) equilibrated with 1 M acetic acid. A total of 2 ml of pooled plasma from each of the two groups [younger group: 2.5 ± 0.5 yr old (mean ± SD), $n = 3$; older group: 11.3 ± 1.5 yr old, $n = 3$] was applied to the column and eluted with 1 M acetic acid at a rate of 7 ml/h at 4° C. Fractions (1.3 ml) were collected, dried with a centrifugal concentrator (Taiyo VC-36, Tokyo, Japan), and reconstituted with 0.3 ml of RIA buffer before assay.

Plasma IR-7B2 concentrations were measured using a specific RIA as described by Iguchi *et al.* (3). The 7B2-antiserum used was raised in rabbits against a synthetic fragment of 7B2 corresponding to residues 23–39 (a gift from Dr. S. R. Bloom, Hammersmith Hospital, London, England). The antiserum cross-reacted 33%, on a molar basis, with authentic porcine 7B2 but showed no cross-reaction with proopiomelanocortin-related peptides, arginine vasopressin, oxytocin, growth hormone, insulin, glucagon, somatostatin, pancreatic polypeptide, or secretin. The

intra- and interassay coefficients of variation were 7.5 and 11.2%, respectively ($n = 5$). A dilution curve of pooled plasma showed a parallel displacement with an RIA-standard curve (data not shown).

Plasma levels of GH, LH, FSH, T, E_2, and DHEA-S were measured using RIA-kits (Eiken ICL, Daiichi RI Co., Tokyo, Japan; Diagnostic Products Corporation, Los Angeles, CA). Protein concentration was measured using a Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, Richmond, CA) with bovine serum albumin as a standard.

Statistics. Correlations between plasma IR-7B2 concentrations and age and between plasma concentrations of IR-7B2 and DHEA-S, T, E_2, LH, and FSH were determined by linear regression analysis, and the significance of variance in the calculated regression coefficient was assessed using Student's *t*-test. The difference of plasma IR-7B2 concentrations between the sexes was assessed using Wilcoxon's rank sum test applied to 2-yr age groupings of the children. Statistical analysis of the elevation of plasma IR-7B2 after LHRH infusion was performed using Student's *t* test.

RESULTS

The mean (±SD) concentration of IR-7B2 in infants less than 2 yr of age was 394 ± 96 pg/ml (range 175–580, $n = 19$), a value significantly higher than that in adults aged 20–87 yr (20–138 pg/ml, $n = 674$). As shown in Figure 1, a significant reduction of plasma IR-7B2 concentration was observed during childhood (male: $r = -0.7854$, $p < 0.01$, $n = 57$; female: $r = -0.6903$, $p < 0.01$, $n = 39$) and the mean level of plasma IR-7B2 approximated the adult value at the age of 15–20 yr. There was no significant difference in plasma IR-7B2 concentrations between the sexes.

The responses of plasma IR-7B2 after the LHRH administration in six individuals are shown in Figure 2. We defined a 10% increase in plasma IR-7B2 concentrations (peak *versus* basal value) as a positive response, and by this criterion four of six children were responders. The mean (±SD) percent increases of plasma IR-7B2 concentrations at 30, 60, 90, and 120 min after the LHRH administration in four responders were 117 ± 2.5, 128 ± 14.3, 122 ± 24.4, and 110 ± 15.6%, respectively ($p <$

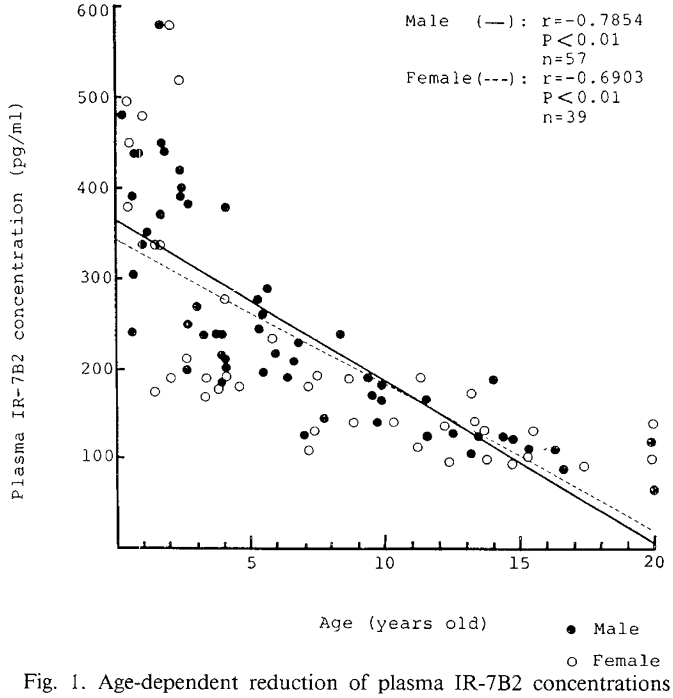

Fig. 1. Age-dependent reduction of plasma IR-7B2 concentrations during childhood.

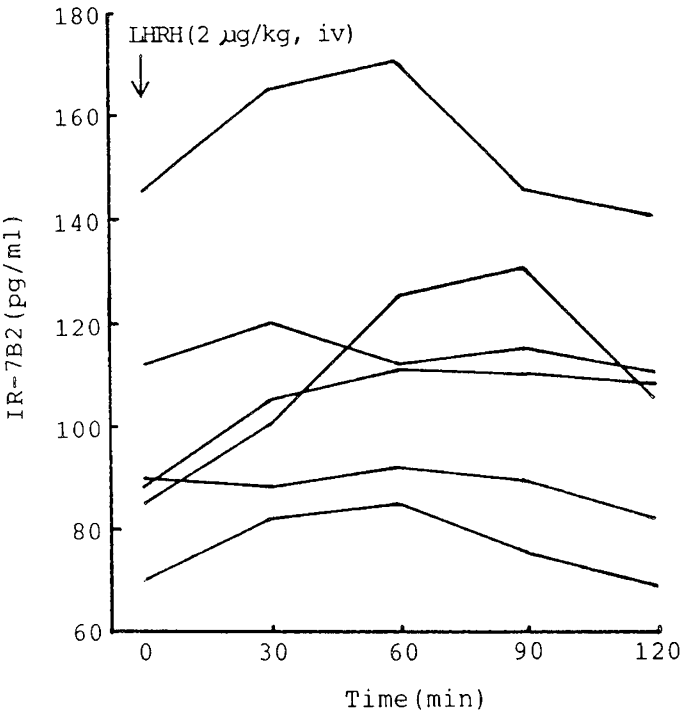

Fig. 2. Changes in plasma IR-7B2 concentrations after an intravenous infusion of LHRH (2 μg/kg) in six children.

0.01 at 30 and 60 min). The average percent increase (peak *versus* basal value) of plasma IR-7B2 concentrations was less than the increase of plasma LH and FSH concentrations (data not shown).

The mean (±SD) concentrations of IR-7B2 in the pituitary glands from children and from adults were 75.0 ± 13.5 and 58.3 ± 19.6 ng/mg protein, respectively. There was no significant difference between the pituitary IR-7B2 content in the children and in the adults.

Significant negative correlations were found between plasma levels of IR-7B2 and those of DHEA-S ($r = -0.4154$, $p < 0.05$, $n = 27$), LH ($r = -0.4948$, $p < 0.05$, $n = 20$) and FSH ($r = -0.4682$, $p < 0.05$, $n = 20$) (Fig. 3). There were no significant correlations between plasma levels of IR-7B2 and those of T and E_2.

Figure 4 depicts the elution profiles on Sephadex G-100 of pooled plasma obtained from two groups of children. Most of the IR-7B2 eluted at an apparent mol. wt. of 20,000 in both the younger and older groups.

DISCUSSION

We noted an age-dependent reduction of plasma IR-7B2 concentrations during childhood. Natori *et al.* (8) reported an age-dependent elevation of plasma IR-7B2 in normal human subjects. However, these data were obtained from subjects more than 20 yr old. However, a high level of plasma IR-7B2 was noted in the cord blood of term infants at the time of normal delivery (11). These data suggested that the plasma IR-7B2 level decreases from a high value at birth to a lower adult level by 15–20 yr of age. Thereafter values increase slowly. During puberty we found significant negative correlations between the plasma levels of IR-7B2 and those of DHEA-S, LH, and FSH. 7B2 is localized in gonadotrophs of the anterior pituitary gland in the rat and an LHRH-induced release of IR-7B2 has been observed in cultured cells of the rat anterior pituitary gland (5, 12). We also noted an elevation of plasma IR-7B2 after LHRH administration in children. These findings suggest that 7B2 may be

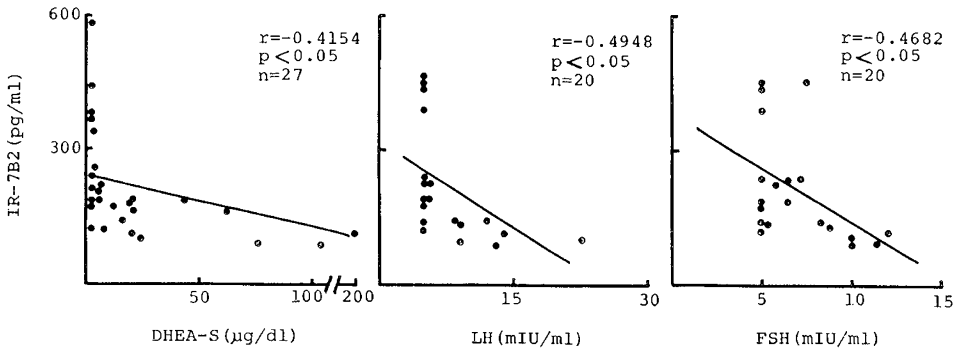

Fig. 3. Correlations between plasma levels of IR-7B2 and DHEA-S, LH, and FSH in children.

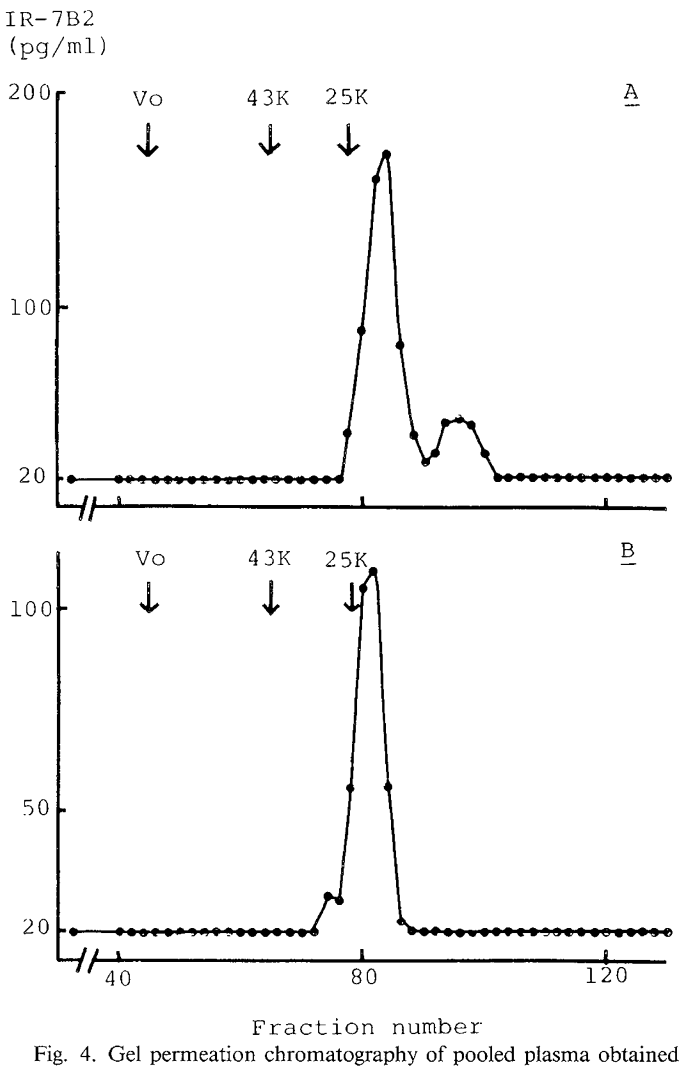

Fig. 4. Gel permeation chromatography of pooled plasma obtained from children on a Sephadex G-100 column (95 × 1.5 cm), *A*, mean (± SD) age 2.5 ± 0.5, $n = 3$; *B*, mean age 11.3 ± 1.5, $n = 3$. Molecular markers are indicated by *arrows* (*Vo*, catalase; *43K*, ovalbumin; *25K*, chymotrypsinogen A).

involved in pituitary-gonadal axis functions and may play a role in events related to puberty.

The origin of plasma IR-7B2 is unknown. The highest concentration of IR-7B2 was observed in the pituitary gland in the rat (3), and secretory characteristics of IR-7B2 were demonstrated in cultured cells of the rat anterior pituitary gland (3, 5). These observations led to the hypothesis that the majority of plasma IR-7B2 originates from the anterior pituitary gland. Marcinkiewicz *et al.* (13) found in the anterior pituitary gland that the immunocytochemical staining of IR-7B2 was more intense in neonatal and young rats than in adult animals. The age-dependent reduction of plasma IR-7B2 may relate to a decrease in IR-7B2 content in the anterior pituitary gland. However, we did not find a significant difference between the pituitary IR-7B2 content in children and adults.

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