LONG-TERM EFFECTS OF HUMAN GROWTH HORMONE ON PLASMA AMINO ACIDS TRANSPORT IN HYPOPITUITARY DWARFISM

By

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ABSTRACT

The chronic effects of human growth hormone (hGH) on the transport of plasma amino acids (PAA) induced by glucose administration were studied in 17 patients with GH deficiency at different stages in the course of GH therapy. The study comprised 9 patients before and after 2–3 months of the therapy and 8 patients after prolonged treatment of 2–3 years. Five normal children served as controls. Analysis of 13 neutral and acidic PAA concentrations before and 2 h after glucose loading was carried out, and a decrease in PAA was expressed as a percentage of 2 h value to the initial level (PAA ratio). Fasting levels of several PAA before treatment were significantly lower than those of controls which gradually rose during the course of the therapy. The mean ± sd value of PAA ratio was also reduced before treatment (68 ± 16 %) vs. that of controls (82 ± 16 %, P < 0.05), which rose after 2–3 months of GH therapy to a comparable level of controls (91 ± 26 %). In patients treated for 2–3 years, however, PAA ratio was decreased to the level of pre-treatment (57 ± 17 %, P < 0.01 vs. control). These changes were pronounced in glucogenic, branched-chain and aromatic amino acids. Serum gamma-glutamyltranspeptidase (GGTP) activity was also low before GH therapy and normalized by the treatment. However, no significant correlation was noted between PAA ratio and serum GGTP activity or GH level. These results indicate that PAA transport evoked by endogenous insulin changes considerably according to the duration of GH therapy, and this may reflect a peripheral alteration of responsiveness to exogenous GH in the prolonged course of GH therapy in pituitary dwarfs.
It is well known that human growth hormone (hGH) exerts a stimulatory effect on membrane transport of amino acids in tissues of hypophysectomized animals and man (Kostyo 1968; Zachmann 1969; Zachmann et al. 1972). This insulin-like effect of GH, however, has been reported to be transient and followed by a refractory phase of amino acids uptake in vitro (Hjalmarson 1968; Nutting 1976; Nutting & Coats 1977). Recent observation suggested that the biphasic pattern of responsiveness to GH was a generalized cellular phenomenon to the hormone (Albertsson-Wikland & Isaksson 1978). Clinically, there have been several reports on acute changes in plasma amino acids (PAA) induced by exogenous hGH administration to hypopituitary dwarfs. However, these results were rather contradictory. Following several days of im hGH administration, a significant increase in PAA was found (Zachmann 1969), whereas other investigators reported a decrease in PAA after an iv infusion of hGH (Rabinowitz et al. 1968; Stahnke et al. 1977). Furthermore, little is known about the long-term effects of hGH on PAA transport. It has been shown that glucose ingestion caused a reduction in PAA concentration after 1–2 h (Munro & Thomson 1953; Swendsen et al. 1967), which was mediated by secretion of endogenous insulin (Zinneman et al. 1966). Because the stimulation of PAA transport by glucose loading appears to be more physiological than that by the direct injection of exogenous hGH, and to be more appropriate for the purpose of examining chronic effects of hGH, we studied the actions of hGH on PAA transport mediated by endogenous insulin at the different stage of hGH therapy of hypopituitary dwarfs.

MATERIALS AND METHODS

Seventeen patients with pituitary dwarfism aged 4–19 years were examined. Clinical diagnosis was based on the significant short stature (under 3rd percentile of standard height in Japanese pre-school and school children, published by the Ministry of Education, 1970), retarded bone age without primary hypothyroidism (based on the standard by Greulich & Pyle (1959) and low or absent serum GH responses to both insulin-induced hypoglycaemia and arginine infusion (below 5 ng/ml). They were divided into 3 groups according to the duration of hGH therapy; group I: 9 patients before hGH therapy, group II: the same patients after 2–3 months of hGH treatment, and group III: 8 patients who had received hGH therapy for 2–3 years. hGH (Crescormone, Kabi) was administered im twice a week at a dose of 0.3–0.5 IU/b.w. Five normal children were studied as controls. To these groups of patients, 2 g/kg of glucose was administered orally after 4 to 6 h of a fasting period. Heparinized blood samples were obtained before and 2 h after glucose loading, when a decrease in PAA was found to be most pronounced (Zinneman et al. 1966; Swendsen et al. 1967). The degree of decrease in PAA was expressed as a percentage of 2 h value to the initial level (PAA ratio). Neutral and acidic amino acids concentrations were measured by an automatic amino acids analyzer (Hitachi KLA-3B type) according to the procedure described by Spackman et al. (1958). Basic PAA were excluded because preliminary experiments showed that these concentrations were relatively stable on glucose ad-
administration. The tests were performed 1–4 days after the last hGH injection. In these samples, plasma GH concentration and gamma-glutamyltranspeptidase (GGTP) (E.C.2.3.2.2.) activity were also determined. Plasma GH was estimated by double-antibody radioimmunoassay with commercially available kit (Dainabott RI Lab. Tokyo) and GGTP was measured by the method of Orlowski & Meister (1963) with prolongation of the incubation time for 2 h. Statistical analysis was done by Student's t-test.

RESULTS

1) PAA concentrations before treatment. — Fig. 1 shows the pre-treatment values of fasting PAA in each group. The concentrations of threonine (Thr), serine (Ser), proline (Pro), glycine (Gly), alanine (Ala), valine (Val), methionine (Met), and tyrosine (Tyr) were not significantly different between patients and controls, while aspartic acid (Asp), glutamic acid (Glu), isoleucine (Ileu), leucine (Leu) and phenylalanine (Phe) levels were lower in pituitary dwarfs than in controls. After administration of hGH, these amino acids concentrations were gradually elevated to the control level, but Asp remained decreased despite of long-term hGH therapy.

![Graph showing fasting plasma amino acids (PAA) concentrations of group I, II, III and controls. Asp, Glu, Ileu, Leu and Phe level are significantly reduced in untreated patients and are gradually normalized during hGH therapy. * P < 0.05, ** P < 0.01, *** P < 0.001 vs. controls.](image)
Changes in PAA ratio during hGH treatment. Vertical bars show mean ± sd value of individual patients, and the shaded area indicate that of overall PAA ratio in each group. Significant difference is noted between group I and II, II and III, I and control, and III and control.

2) Changes in PAA ratio during hGH therapy. – Fig. 2 depicts mean ± sd value of PAA in individual patients and that of overall PAA ratio in each group. Although individual variation was considerable, PAA ratio of group I was significantly reduced from the control value (68 ± 16% vs 82 ± 16%, P <

Changes in PAA ratio of individual amino acid. Solid circle; group I, open circle; group II, double circle; group III, and shaded area; mean ± sd of controls. Significant changes are found with the duration of hGH therapy. * P < 0.05, ** P < 0.02, *** P < 0.01 vs. group I.
Relation between plasma GH concentration and PAA ratio. No significant correlation is noted.

**Fig. 4.**

Serum GGTP activity in patients and controls. The activity is lower in untreated patients and in the early stage of hGH treatment, which is elevated to normal level after long-term hGH administration.

**Fig. 5.**
0.05), whereas this rose to the normal level (91 ± 26%\(^a\)) in the early stage of hGH therapy. In contrast, PAA ratio of group III was definitely lower than that of control (57 ± 17, \(P < 0.01\)). This indicates that the effect of hGH on PAA transport changes considerably according to duration of therapy.

As for the change in individual amino acid, almost all PAA tended to decrease after glucose administration, but a fall was especially pronounced in Val, Met, Ileu, Leu, Tyr and Phe (Fig. 3). At the early stage of hGH therapy, significant enhancement of PAA ratio was noted in Thr, Ser, Gly, Ala, Val, Met, Leu, Tyr and Phe. However, in group III patients, further reduction of all PAA ratio from pre-treatment level was observed, particularly in Ala and Ileu ratio. In short, changes in PAA ratio were prominent in glucogenic, branched-chain and aromatic amino acids.

3) Relation of PAA ratio to plasma GH level. – On the assumption that circulating GH concentration may influence PAA transport after glucose loading, the plasma GH concentration was measured. However, no significant correlation between GH level and PAA ratio was noted (Fig. 4). This indicates that time interval between the last injection of hGH and the test performed does not influence the changes in PAA ratio mentioned above.

4) Change in plasma GGTP activity. – Membrane-bound GGTP plays a significant role in cellular transport of amino acids. In preliminary experiments, GGTP activity was studied in red cell membrane, but no measurable activity was found. As an alternative, therefore, serum GGTP activity was

![Graph showing correlation between serum GGTP activity and PAA ratio. Apparent inverse relation is not statistically significant. Solid circle: group I, open circle: group II, and double circle: group III.](image-url)
The activity was lower in the patients without hGH therapy and in the early period of the treatment than that in normal controls \( P < 0.01 \), while the patients with long-term GH therapy showed a level of the activity similar to that of controls. However, there was no significant correlation between serum GGTP activity and PAA ratio (Fig. 6).

**DISCUSSION**

The concentrations of PAA have been found to be lower in untreated pituitary dwarfs than in normal children \( \text{Zachmann 1969; Stahnke et al. 1977} \). After administration of hGH for several days, certain amino acids increased and the overall amino acids pattern in the plasma became normal \( \text{Zachmann 1969} \). Our findings are consistent with these previous observations. By contrast, acute iv administration of hGH produced a decrease in all PAA concentrations after 2 h \( \text{Zachmann et al. 1972; Stahnke et al. 1977} \). Because urinary excretion of amino acids was not increased by hGH administration, reduction in plasma concentration was interpreted to be due to increased PAA transport into cells by hGH \( \text{Zachmann 1969} \). Decrease in PAA was also observed after glucose ingestion \( \text{Munro & Thomson 1953; Zinnemann et al. 1966; Swendseid et al. 1967} \), which appeared to be mediated by endogenous insulin secretion \( \text{Zinnemann et al. 1966; Felig & Wahren 1971} \). Changes in PAA balance after insulin could be accounted for by increased uptake of amino acids into muscle cells or retardation of their release. A recent study suggests that decline in PAA levels after insulin is due to inhibition of muscle release \( \text{Pozefsky et al. 1969} \). In GH deficient children, endogenous insulin secretion was diminished, but hypersensitivity to insulin actions was demonstrated \( \text{Gold et al. 1968; Costin et al. 1972} \). Exaggerated response of PAA to glucose administration in the pre-treatment group may be accounted for by increased susceptibility to insulin. In fact, the hyperresponsiveness of PAA to glucose was normalized in the early stage of hGH treatment. However, it is rather difficult to explain the response of group III on the basis of insulin hypersensitivity. Antibody to exogenous GH was not demonstrated in these patients. It seems more likely that some alterations of responsiveness to hGH occur in the cellular amino acids' transport system during long-term treatment.

Gamma-GTP is known to catalyse the membrane transport of all amino acids except proline \( \text{Montgomery et al. 1977} \). Although untreated patients with GH deficiency showed significantly decreased GGTP activity in sera and a normal activity after prolonged treatment, there was no correlation between the activity and PAA ratio. The plausible explanation for this is that (1) serum GGTP activity does not reflect that of the cell membrane, or (2) the enzyme may not account for all of the amino acids' transport into or out of
cells. So far, it seems possible that enhanced activities of amino acids' transport enzymes may play a part in hyperresponsiveness of PAA after prolonged hGH therapy.

Several investigations revealed that the stimulation of amino acids transport by GH was an acute effect which disappeared after 2–3 h; after this time the tissues were relatively refractory to further stimulation by additional GH (Kostyo 1968; Hjalmarson 1968; Nutting 1976; Nutting & Coats 1977). Refractoriness to these acute “insulin-like” actions of GH on transport required RNA and protein synthesis, suggesting that refractoriness was mediated by inhibitory protein(s) (Hjalmarson 1968), and this refractory state produced by GH appeared to be an age-dependent process (Nutting 1976). If this response pattern to GH with time is a generalized cellular phenomenon as suggested by Albertsson-Wikland & Isaksson (1978), the altered effects of GH on PAA transport during the course of hGH therapy may be explicable on this basis.

Another possible interpretation is that GH-dependent receptor regulation (down regulation) (Kahn et al. 1977) may participate in this phenomenon. Recently an evidence of this hypothesis was presented in the IM-9 lymphocytes (Lesniak & Roth 1976). The concentration of GH receptor was reported to be very sensitive to regulation by GH, and 20 ng/ml of GH concentration produced an 80% loss of receptor in vitro. Indeed, clinical observations show that serum concentration of GH administered exogenously for pituitary dwarfs is maintained at a very high level for succeeding 2–3 days. Repeated injections of hGH twice a week produce a very different profile of rhythm from that of the physiological state. Therefore, it is reasonable to deduce that the number of GH receptors may decrease under this situation, resulting in relative unresponsiveness to GH actions. The last two possibilities mentioned above may be the different expressions of the same biological process, although this awaits further investigations in future. In conclusion, amino acids transport system in peripheral tissues changes with the duration of hGH therapy for pituitary dwarfs, the actual mechanism of which remains to be elucidated.

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