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Idiopathic Leucine-Sensitive Hypoglycemia Syndrome: Insulin and Glucagon Responses and Effects of Diazoxide

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Summary

Four children, treated with diazoxide for idiopathic leucine-sensitive (ILS) hypoglycemia of infancy, had follow-up studies at 2 to 10 yr of age to assess: (1) persistence of leucine sensitivity in later childhood, (2) pancreatic glucagon responses, and (3) the hormonal and glycemic effects of diazoxide therapy. On the third day after diazoxide therapy was stopped, the mean ± S.E. baseline plasma glucose level (65.3 ± 3.4 mg/dl) was significantly ($P < 0.005$) lower than that of the controls (80.1 ± 3.1 mg/dl). Corresponding mean plasma immunoreactive insulin (IRI) and immunoreactive glucagon (IRG) values were higher than control values but the differences were not significant. After the oral administration of leucine (50 mg/kg) in the ILS children, the mean plasma IRI level rose from 15.2 ± 4.1 to 59 ± 19 μU/ml, the mean plasma glucose concentration fell to 36.0 ± 3.3 mg/dl and the mean plasma IRG level rose from 196 ± 16 to 261 ± 41 pg/ml. These responses were significantly greater ($P < 0.05$ to 0.005) than those of control children who received 150 mg/kg of leucine. Intravenous arginine administration caused similar changes in mean plasma glucose, IRI and IRG values in the ILS and control children.

During diazoxide therapy in the ILS children, the baseline mean plasma glucose level (89.5 ± 4.2 mg/dl) was significantly ($P > 0.005$) higher than without therapy. Corresponding mean plasma IRI and IRG values decreased with therapy but the differences were not significant. Diazoxide therapy blunted the changes induced by leucine administration in the ILS children but did not significantly change their response to arginine infusion.

Our results indicate that marked sensitivity to leucine persists after infancy in the ILS children. Their IRG responses are appropriate to the stimuli, indicating that their pancreatic α cells do not share the abnormality of the β cells. Diazoxide therapy increases baseline plasma glucose levels and inhibits IRI responses to leucine in ILS children, but it has little if any effect on IRG responses.

Speculation

The mechanisms for abnormal insulin response to leucine in patients with ILS hypoglycemia is not known. An abnormal regulation of insulin secretion due to lack of inhibition by somatostatin, is possible and requires investigation. Although pancreatic surgery is usually not indicated, determination of the distribution and hormone content of pancreatic α, β, and δ cells deserves careful study, should the opportunity arise.

The idiopathic leucine-sensitive (ILS) hypoglycemia syndrome has several distinctive characteristics. Hypoglycemia usually becomes manifest between 1 and 6 months of life (2) and typically follows protein-rich feedings. Restriction of protein intake and/or the administration of diazoxide, an inhibitor of insulin release, provide effective therapy for these infants (2, 3, 10, 16). Little is known of the course of this disorder after infancy. Although the abnormal insulin response to leucine administration is well known in this syndrome, glucagon responses have not been reported.

The present report describes follow-up studies of four children with ILS hypoglycemia of infancy. Plasma glucose, immunoreactive insulin (IRI) and immunoreactive glucagon (IRG) responses of the ILS children after the administration of leucine and arginine were compared with those of control children. The effects of diazoxide on the responses of ILS children were also studied.

MATERIALS AND METHODS

The ILS children, three boys and one girl, were 2, 5, 7, and 10 yr of age at the time of the study. Three had normal growth and intelligence and one was severely mentally retarded. During infancy the oral administration of 150 mg of L-leucine per kilogram of body weight (one patient received 75 mg/kg) resulted in marked hypoglycemia and hyperinsulinemia (Fig. 1). Treatment with oral diazoxide, 7.5 to 10 mg/kg/day, and modest restriction of protein intake completely prevented hypoglycemia in each patient. All of the patients developed mild hirsutism and one had transient edema during diazoxide therapy. Before the present study, the ILS children received diazoxide therapy for 6 months to 5½ years.

Five children, 2 to 4 years of age, who were evaluated for idiopathic hypoglycemia, served as controls for the oral leucine tolerance test. Controls for the arginine infusion test were five children with either familial short stature or delayed puberty who were otherwise normal.

The studies were conducted in the Clinical Research Center at Childrens Hospital of Los Angeles (CHLA). After a 2-day period of stabilization in the hospital, the ILS children were given L-leucine, 50 mg/kg orally, after an overnight fast (12 hr after the last dose of diazoxide). The test dose of L-leucine in controls was 150 mg/kg. Venous blood samples were obtained for glucose, IRI, and IRG before and 30, 45, 60, and 90 min after leucine administration. Two hours after leucine was given, three ILS children received an infusion of L-arginine hydrochloride, 500 mg/kg, over a 30-min period. The controls received the same dose of arginine. Blood samples were obtained for glucose, IRI, and IRG before and 30, 60, 90, and 120 min after the start of the arginine infusion.

After these initial studies, leucine and arginine tolerance tests were repeated in the ILS patients on the third day after diazoxide therapy was discontinued (84 hr after the last dose of diazoxide).

Plasma glucose was measured by a glucose oxidase method (12), and plasma IRI by radioimmunoassay (17). Plasma IRG was measured by radioimmunoassay of a modification of the method of Unger (5, 27) using Unger's pancreatic glucagon antiserum 30K. Data were analyzed for statistical significance by paired and unpaired Student's *t* test (25).

This investigation was reviewed and approved by the Committee on Clinical Investigations and Publications of CHLA. Informed parental and/or patient consent was obtained before the patients entered the study.

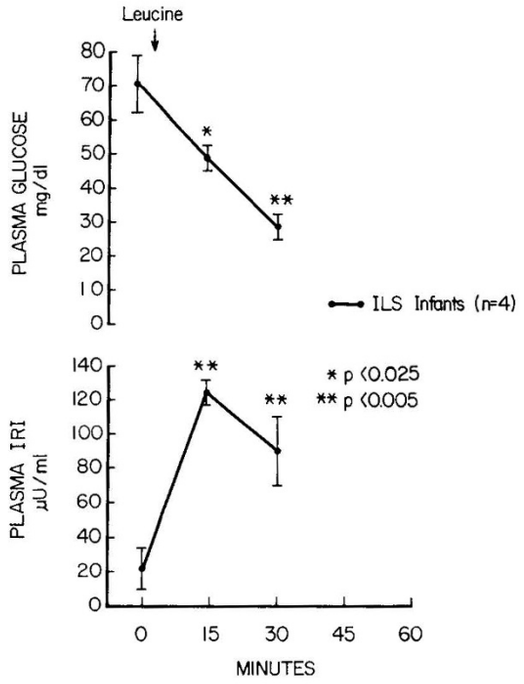

Fig. 1. Mean ± S.E. plasma glucose and IRI responses after the oral administration of L-leucine, 150 mg/kg, in the four ILS infants at the time of initial diagnosis. The *asterisks* indicate the significance of the change from baseline values.

RESULTS

The responses of the ILS children without diazoxide therapy are presented first for comparison with those of the controls and with the effects of diazoxide administration.

ILS CHILDREN WITHOUT DIAZOXIDE THERAPY

Baseline Values. The mean ± S.E. baseline plasma glucose level in ILS children without diazoxide therapy was 65.3 ± 3.4 mg/dl and lower than the mean value of 80.1 ± 3.1 mg/dl in controls ($P < 0.005$). The mean baseline plasma IRI and IRG values of ILS children were 15.2 ± 4.1 μU/ml and 196 ± 16 pg/ml, respectively. Both of these levels were higher than those of controls (IRI, 9.3 ± 1.2 μU/ml and IRG, 177 ± 12 pg/ml), but these differences were not significant.

Response to Leucine. Figure 2 shows the effects of leucine administration in control and ILS children. In the ILS children, mean plasma glucose values fell and mean plasma IRI concentrations rose immediately after the administration of leucine. In the controls, there was minimal change in these values. Mean plasma glucose values were significantly lower than control values at 15, 30, 45, 60, and 90 min and mean IRI values were significantly greater than control values at 15 and 30 min. The mean plasma IRG concentration was unchanged at 15 min in the ILS children, then it rose to a peak at 45 min. The percent increase in IRG levels above the baseline was significant at 30, 45, and 60 min. In the controls, the mean plasma IRG level remained unchanged after leucine administration. By 90 min the plasma glucose, IRI and IRG values in the ILS children were all approaching baseline levels.

Response to Arginine. The effects of arginine infusion in ILS patients and in controls are shown in Figure 3. The mean plasma glucose IRI, and IRG concentrations rose at the end of the arginine infusion in both ILS patients and in controls, and there were no significant differences between the responses of patients and controls.

ILS CHILDREN RECEIVING DIAZOXIDE THERAPY

Baseline Values. With diazoxide therapy the baseline mean plasma glucose level was 89.5 ± 4.2 mg/dl and higher than the mean plasma glucose level of 65.3 ± 3.4 mg/dl without therapy ($P < 0.005$). The baseline mean plasma IRI and IRG levels with diazoxide therapy were 13.2 ± 1.4 μU/ml and 182 ± 18 pg/ml, respectively. These values were slightly, but not significantly lower than the IRI and IRG values of 15.2 ± 4.1 μU/ml and 196 ± 16 pg/ml without treatment.

Response to Leucine. After the administration of leucine to the ILS children during diazoxide therapy, the mean percent fall in plasma glucose concentration was significantly less than the fall in glucose without diazoxide (Fig. 4). With diazoxide therapy mean plasma IRI values increased significantly less than without diazoxide treatment. The mean plasma IRG concentration increased less with diazoxide therapy than without, however, this difference was not significant.

Response to Arginine. There were no significant differences in the percentage increase in mean plasma glucose, IRI, or IRG concentrations after arginine administration in the patients receiving diazoxide compared to the responses without diazoxide.

DISCUSSION

Marked sensitivity to leucine is characteristic of ILS hypoglycemia syndrome. However, sensitivity to leucine is not unique to

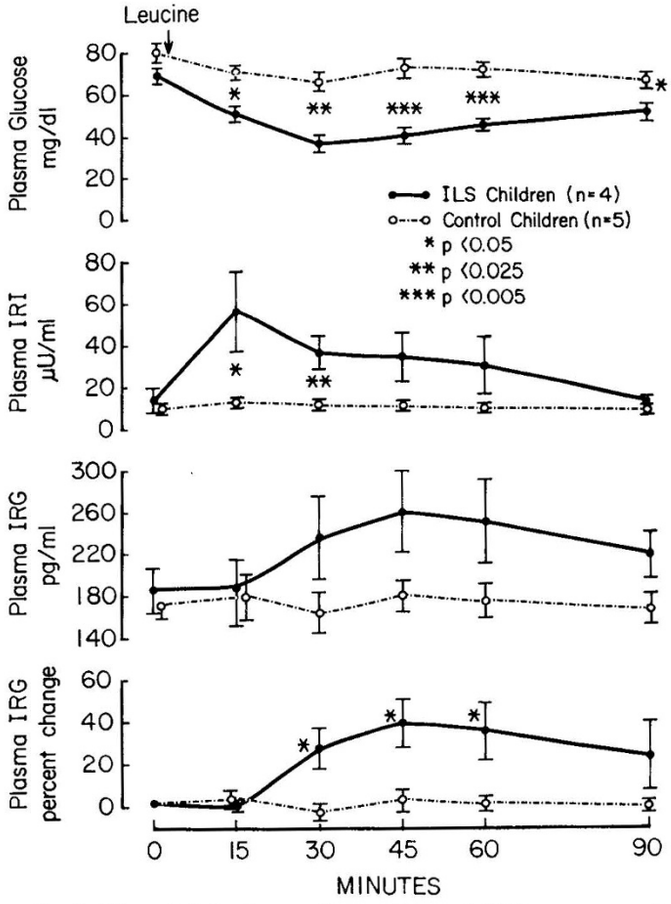

Fig. 2. Mean ± S.E. plasma glucose, IRI, and IRG responses and percent change in IRG after the oral administration of L-leucine in five control children (*dashed lines*) and four ILS children (*solid lines*). The test dose of L-leucine was 150 mg/kg for the controls and 50 mg/kg for the ILS children. The *asterisks* indicate the significance of the differences between ILS children and controls, except for percent change in IRG, where peak values are compared to baseline levels.

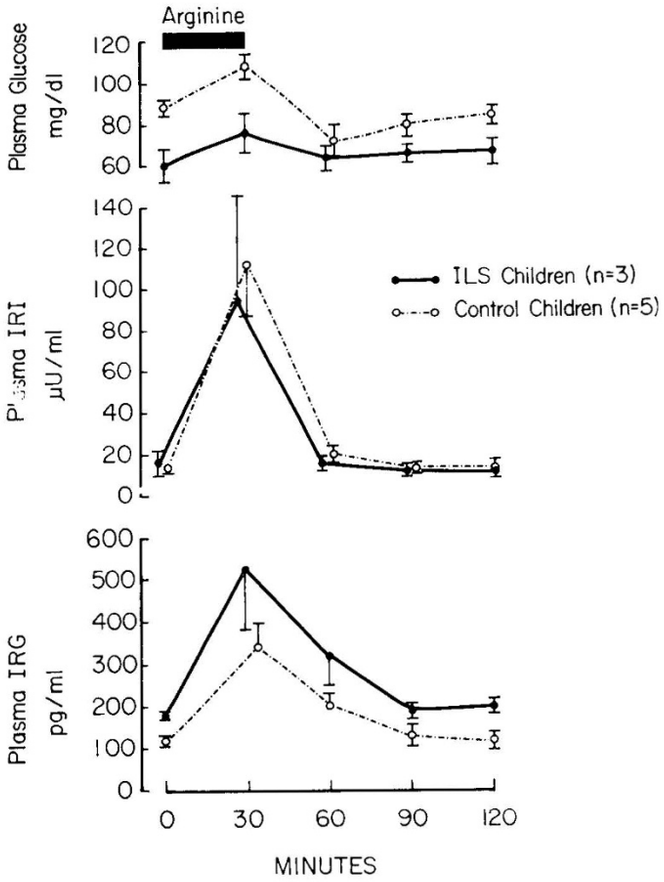

Fig. 3. Mean ± S.E. plasma glucose, IRI, and IRG responses after intravenous arginine infusion in five controls (*dashed lines*) and three ILS children (*solid lines*). There were no statistically significant differences between corresponding values for these two groups.

this syndrome since leucine administration can provoke hypoglycemia in several hyperinsulinemic states in adults and children (6, 9). This has led some investigators to question the specificity of this syndrome (26). However, the clinical similarities among affected infants and our patients, suggest that ILS hypoglycemia is a distinct form of hyperinsulinism.

Because pancreatic surgery was not required for treatment in our patients, the histologic appearance of their pancreases is not known. Although our patients may have a histologic abnormality of the islets, nesidioblastosis or islet cell adenoma are unlikely since hypoglycemia due to these defects is usually refractory to diazoxide therapy (11, 22–24).

As our patients grew older, they became less prone to hypogylcemia. However, our present studies show persistence of marked leucine sensitivity as late as 10 yr of age. These findings and the observations of others, indicate that an abnormality of insulin secretion persists in patients with ILS hypoglycemia longer than previously suspected, perhaps into adult life (4, 18).

The mechanism for leucine-induced hyperinsulinism in this syndrome is unknown, but it may be an exaggeration of the normal response to leucine (8). In our controls and in normal children reported previously (15), there was a slight rise in plasma IRI levels and a slight fall in plasma glucose values after ingestion of 150 mg/kg of L-leucine. In contrast, our ILS patients were markedly sensitive to as little as 50 mg/kg of L-leucine. Leucine appears to act directly on islet cells since it stimulates insulin release in isolated islet preparations (14).

We previously observed that hyperinsulinism and hypoglycemia also occur in ILS children after intravenous tolbutamide administration (13). The normal IRI response of our patients after arginine infusion suggests that beta cell function, with respect to arginine, is normal in ILS patients and that arginine causes insulin release by a mechanism different from that of leucine and tolbutamide, as suggested by others (7).

The role of glucagon in the regulation of blood glucose in ILS children has not been reported previously. In the fasting state, IRG concentrations were similar in our ILS patients and in controls. Although perfusion of the rat pancreas with supraphysiologic concentrations of leucine stimulates immediate release of IRG (20), neither the oral administration of leucine in our controls nor the intravenous administration of leucine in humans or intact dogs (19, 21) elicited a rise in plasma IRG levels.

In our ILS children, plasma IRG levels increased after leucine administration but not until more than 15 min after the dose was given (Fig. 2). The delay in IRG response suggests that it was secondary to hypoglycemia and not a manifestation of pancreatic alpha cell hypersensitivity to leucine. This observation and the normal IRG response to arginine infusion in our patients indicate that their α cell function was normal.

Diazoxide therapy was effective in maintaining normoglycemia in our patients. The most likely mechanism for the beneficial effect of diazoxide is inhibition of insulin secretion, although other factors may be involved (1).

Diazoxide may have had some effect on glucagon secretion in our patients since there was a tendency for baseline IRG levels to be lower during therapy. In intact dogs, diazoxide slightly lowered resting plasma IRG levels (1), and in the perfused rat pancreases, diazoxide inhibited both leucine- and arginine-induced release of IRG (28). There was a negligible inhibition of the IRG response after arginine by diazoxide therapy in our patients. Although the attenuated IRG response to leucine during therapy could reflect

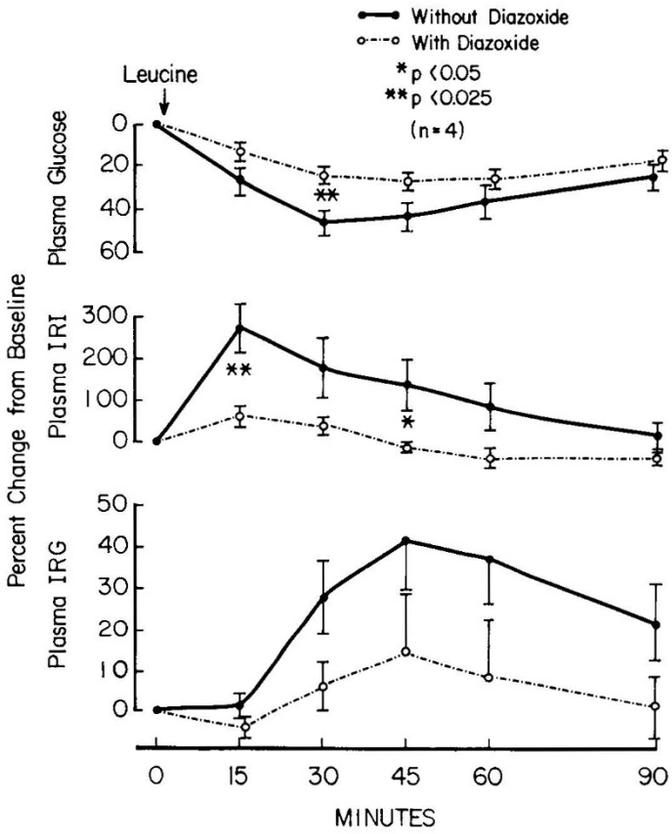

Fig. 4. Mean ± S.E. percent change from baseline levels of plasma glucose, IRI, and IRG after L-leucine administration with diazoxide (*dashed lines*), as compared to without diazoxide (*solid lines*) in four ILS patients. The *asterisks* indicate the significance of the differences between the responses with and without diazoxide.

α cell inhibition by diazoxide, the blunted fall in plasma glucose is a more likely explanation.

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