Decreased Cyclic Guanosine 3',5' Monophosphate and Guanylate Cyclase Activity in Leprechaunism: Evidence for a Postreceptor Defect

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ABSTRACT. Patients with leprechaunism have hyperinsulinemia and extreme insulin resistance. The mechanism of the insulin resistance has not been delineated. To examine postreceptor events in this unusual syndrome we have assayed the enzyme guanylate cyclase [E.C.4.6.12], which is modulated by insulin, and the concentration of the intracellular messenger cyclic GMP in liver from two children with leprechaunism and extreme insulin resistance. Both patients exhibited down regulation of the red blood cell insulin receptors, but normal insulin receptor binding to Ebstein-Barr transformed IM-9 lymphocytes and monocytes. There was no evidence of antireceptor or antiinsulin antibodies. Activity of liver guanylate cyclase expressed as pmol/mg protein/10 min incubation in the soluble and particulate fractions were, respectively, Ark-1 133 ± 18, 25 ± 6; Ark-2 129 ± 17, 23 ± 8; control children (six average) 287 ± 16, 55 ± 9. The concentration of cyclic GMP was also 50% lower (0.08 ± 0.03 in Ark-1 and 0.07 ± 0.04 in Ark-2), compared to 0.19 ± 0.07 pmol/mg protein/min in the control livers. There was no change in adenylate cyclase activity in children with leprechaunism *versus* the control children. These data suggest an abnormality of a postreceptor event in this rare genetic disease. These data, however, do not rule out that in some cases of leprechaunism a receptor binding abnormality may be the primary defect. We speculate that a defect in insulin action distal to plasma membrane receptor binding may be etiological in this unusual syndrome. (*Pediatr Res* 20: 329–331, 1986)

Abbreviation

Cyclic GMP, cyclic guanosine 3',5' monophosphate

Leprechaunism is a rare inherited disease characterized by pixie-like facies, hirsutism, clitoromegaly, and sparse fat stores (1, 2). In addition, patients with this disorder exhibit extreme insulin resistance, intrauterine growth retardation, and disordered carbohydrate metabolism (1–3). We report two patients with leprechaunism who had marked hyperinsulinemia (2000–

10,000 uU/ml), severe insulin resistance, disordered carbohydrate metabolism, decreased insulin receptor binding in liver and red blood cells, and a normal number of receptors for insulin in Ebstein-Barr transformed lymphocytes and fibroblasts (3–5). The circulating plasma insulin was normal on the basis of chromatographic, electrophoretic, and immunologic criteria in one of these patients (Ark-1) (3). This patient's purified insulin binds normally to insulin receptors on cultured IM-9 lymphocytes (5) and rat fat cells (3). Antiinsulin and antiinsulin-receptor antibodies were not detected in Ark-1's plasma, and plasma levels of glucagon, growth hormone, and cortisol were normal (3). The above studies and studies on another patient with leprechaunism have suggested that there may be a postinsulin-receptor defect in at least some patients with this disorder (6, 7).

Guanylate cyclase is a postreceptor enzyme that may be modulated by interaction with insulin (8–10). Diabetic animals have decreased activity of this enzyme in peripheral tissues, and this activity can be restored with either exogenous insulin (8, 9) or endogenous insulin via pancreatic transplantation (10). The present study examines the possibility that some patients with leprechaunism, known insulin resistance, and normal insulin receptor binding may have altered activity of guanylate cyclase, indicating a postreceptor abnormality of insulin action.

MATERIALS AND METHODS

Cases studied. Ark-1 has been previously described (4). She weighed 2600 g at birth and was noted to have a prominent clitoris (11 mm) and labia majora, hypertrichosis, coarse facial features, and decreased subcutaneous tissue. At 11 months of age marked pachyderma was noted over the ears, elbows, knees, and dorsum of the feet. Hypertrichosis and velvet-like hypermelanotic skin were also present. The patient's lips, labia majora, and anus were hyperkeratotic and rugated. The breasts had prominent nipples but no mammary tissue. Laboratory investigation revealed only an elevated 24-h urinary glucose. A skin biopsy was compatible with acanthosis nigricans. At age 2½ yr she underwent tonsillectomy, adenoidectomy, and placement of pneumatic ear tubes because of severe recurrent otitis media and sinusitis attributed to excessive tissue in the sinuses and middle ears. At age 3 yr she had early breast development with palpable mammary tissue. Hypertrichosis increased, resulting in confluent eyebrows, and her acanthosis nigricans worsened. By 5 yr of age her dental age was 14 yr and she had required the removal of five teeth. At 6 yr colonoscopy and upper endoscopy, performed for recurring intermittent abdominal distention and cramps, revealed multiple nodular areas of lymphoid hyperplasia from anus to cecum. Although her esophagus and upper gastrointes-

tinal tract were histologically normal, the gastric rugae were hypertrophied and the duodenal folds thickened. Abdominal and pelvic ultrasound revealed large kidneys. Her neurologic development has been normal and she is doing well in school.

Laboratory studies have consistently shown fasting hypoglycemia (<20 mg/dl), postprandial hyperglycemia (>250 mg/dl), marked hyperinsulinemia (>1,000 uU/ml), severe insulin resistance (<20% decrease in blood sugar with 0.3 to 1.0 U/kg of regular intravenous insulin) and elevated glycosylated hemoglobin (>13%). Adrenal, gonadal, and pituitary hormone levels were normal with normal response to gonadotropin-releasing hormone and thyrotropin releasing hormone.

Ark-2 was initially seen at the age of 10 days. She was born weighing 1400 g after a 37-wk gestation to a 23-yr-old mother. Neonatal course was characterized by fasting hypoglycemia (blood glucose 20–30 mg/dl) and postprandial hyperglycemia (blood glucose 300–500 mg/dl). Physical examination revealed a small, hirsute infant with thick, wrinkled skin and severe acanthosis nigricans in the neck and axillary areas. She had dysplastic nails, bilateral inguinal hernias, hypertrophic external genitalia with marked clitoral enlargement, rectal prolapse, and absent subcutaneous fat. Laboratory studies were normal except for fluctuating serum glucose concentrations, consistent hyperinsulinemia, and absent ketonemia. Family history is significant in that she had a brother with the same syndrome. The patient died at 6 months of age weighing less than 2500 g. Detailed findings of her clinical course and laboratory findings have been previously published (4).

Guanylate cyclase assay. One gram of liver from Ark-1 was obtained at open surgical biopsy at 3½ yr of age. Liver from Ark-2 was obtained within 2 h after death from an acute hypoglycemic episode. The control livers used for this study were obtained from patients aged 6 months to 4 yr who died in a variety of accidental deaths and were all obtained within 2 h of death. There was no evidence of trauma to either the liver samples of the patients with leprechaunism or the control patients' liver samples. The livers were stored at −70° C until time for study. All studies were approved by the Human Research Advisory Committee at the University of Arkansas and permission was obtained from the parents for these studies. Guanylate cyclase was assayed as previously described (11, 12). Livers were homogenized in cold 0.3 M Tris Hcl (pH 7.6) and centrifuged at 37,000 × g in a refrigerated centrifuge at 4° C for 15 min. The reaction mixture consisted of 20 mM Tris-HCl (pH 7.6); 4 mM MnCl$_2$; 2.67 mM cyclic GMP (used to minimize destruction of ^{32}P-labeled cyclic GMP); a guanosine triphosphate regenerating system consisting of 5 mM creatine phosphate, 11.25 U of creatine phosphokinase (E.C.2.7.3.2); 100 µg of bovine serum albumin; 20 mM caffeine; and 1.2 mM [α-^{32}P] guanosine triphosphate (approximately 5 × 10^5 counts/min). The final pH of the reaction mixture was 7.6. The volume of the supernatant or particulate fractions was 25 µl and final volume of the cyclase assay, which includes the above supernatant or particulate fractions, the reaction mix, and the radioactive isotopes, was 75 µl. The enzyme preparations of guanylate cyclase had 0.1 to 0.2 mg of protein (11, 12). The reaction was allowed to proceed for 10 min at 37° C, following which it was terminated by adding 10 µl of 0.1 EDTA (pH 7.6) containing about 30,000 cpm of [^{3}H]-cyclic GMP (to estimate recovery in subsequent steps) and boiling for 3 min. After cooling in an ice bath, the [^{3}H]-cyclic GMP formed was isolated by sequential chromatography on Dowex 50 W × 4 H$^+$ (200–400 mesh) and alumina using the modification described in detail previously (12). In this assay system, cyclic GMP production was linear with time for 20 min and with increased concentration of protein in the liver fractions from 50 to 400 µg. All of the ^{32}P-containing material was identifiable as cyclic GMP as determined by thin-layer chromatography on PEI-cellulose (Brinkman, Westbury, NY) using 1 M LiCl as solvent and on Chromar sheets (Mallinckrodt Chemical Works, St. Louis, MO) developed with absolute alcohol and concentrated NH$_4$OH (5:2 v/v). All reagents were of analytical grade and from the same

sources as described previously (11, 12). Each assay was conducted in triplicate and repeated in three separate experiments. Cyclic GMP tissue concentrations were measured by radioimmunoassy (8). Adenylate cyclase was measured as previously described (13, 14). The particulate liver fractions containing 0.09 to 0.12 mg of protein were incubated at 37° C for 15 min with 2.5 mM [α-^{32}P] ATP, 3.5 × 10^6 cpm; 8 mM theophylline; 3 mM Mg Cl$_2$; 21 mM Tris HCl, pH 7.7; 0.9 mM phosphenolpyruvate; 0.2 mg/ml of pyruvate kinase; 26 mM potassium chloride; and 0.8 mg/ml of bovine serum albumin. The total volume of the incubation mixture was 65 µl.

RESULTS

Guanylate cyclase activity was decreased in soluble and particulate fractions of livers from patients with leprechaunism as compared to age-matched controls (Table 1). During a 10-min incubation, concentrations of cyclic GMP accumulated secondary to activation of the enzyme guanylate cyclase in control livers were 55 ± 9 and 287 ± 16 pmol/mg protein in the particulate and supernatant fractions, respectively (Table 1). These concentrations are very similar to those found by our laboratory in adult human livers (15). By contrast, the activity of guanylate cyclase from the livers of patients with leprechaunism was approximately one-half of this value, *i.e.* the amount of cyclic GMP accumulated in pmol/mg protein in 10-min incubations was Ark-1 25 ± 6, Ark-2 23 ± 8 in the particulate fractions and Ark-1 133 ± 18, Ark-2 129 ± 17 in the supernatant fractions.

Measurement of hepatic cyclic GMP concentrations by radioimmunoassay from the control patients was similar to that

Table 1. *Guanylate cyclase activity in leprechaunism*

	Guanylate cyclase (pmol cyclic GMP/mg protein/10 min)*		
	Particulate	Supernatant	*p*†
Control	55 ± 9	287 ± 16	
Ark-1	25 ± 6	133 ± 18	<0.001
Ark-2	23 ± 8	129 ± 17	<0.001

* Hepatic supernatant and particulate fractions of 37,000 × g centrifugation were assayed as described in the text. Each value is the mean ± SEM of triplicate samples determined in three separate experiments. The value for the control is the average of six different normal livers.

† Significance compared to control determined by Student's *t* test for unpaired values.

Table 2. *Hepatic cyclic GMP concentrations in leprechaunism*

	pmol cyclic GMP/mg protein/min*	*p*†
Control	0.19 ± 0.07	
Ark-1	0.08 ± 0.03	<0.001
Ark-2	0.07 ± 0.04	<0.001

* Each value represents the mean ± SE of duplicate determinations repeated in three separate experiments.

† Significance compared to control determined by Student's *t* test for unpaired values.

Table 3. *Adenylate cyclase activity in leprechaunism*

	Adenylate cyclase (pmol cyclic AMP/mg protein/10 min)*	*p*†
Control	68 ± 9	
Ark-1	76 ± 12	NS
Ark-2	62 ± 11	NS

* Each value represents the mean ± SE of triplicate determinations. The control value is the average of six different normal livers.

† Significance of comparison with control was determined by the Student's *t* test.

previously observed in normal adult human tissue (15). The cyclic GMP concentration from the patients with leprechaunism, however, showed a lower concentration of this nucleotide, 0.08 ± 0.03 pmol/mg protein/min in Ark-1 and 0.07 ± 0.04 in Ark-2, as compared to 0.19 ± 0.07 pmol/mg protein/min in age-matched control subjects. These data demonstrate significantly lower concentrations of hepatic guanylate cyclase and cyclic GMP concentrations in hepatic slices from both patients with leprechaunism (Table 2) using the Student's t test, $p < 0.001$. The data were also tested using the Tukey nonparametric test and found to be significant at the 0.05 level (16). Adenylate cyclase was unchanged in the livers of the patients with leprechaunism *versus* the six control patients (Table 3). The observations of normal insulin receptor binding in some tissues, decreased activity of the enzyme guanylate cyclase, and decreased concentration of the cyclic GMP nucleotide would be consistent with a postreceptor defect in insulin action; however, an alternate interpretation that cannot be ruled out at present is that they are a result of a primary defect in the insulin receptor interaction with decreased cellular signaling.

DISCUSSION

The etiology of the insulin resistance in leprechaunism appears to be heterogenous. Some patients with this disorder have a decrease in insulin receptor numbers (17), others have structure alterations of the insulin receptor (18–20), while others would appear to have a postreceptor defect (3, 6, 7) or a combination of abnormalities.

Despite multiple studies, the exact nature of the defect in this interesting group of patients has not been identified. The present investigation indicates that activity of one enzyme that has been shown to be modulated by insulin (8–10) *in vivo* and directly stimulated by insulin *in vitro* (8, 21) was decreased in the two patients with leprechaunism that were studied. The regulation of receptor binding affinity is intimately associated with the action of those hormones which activate adenylate cyclase (22, 23), however, the exact interrelationships of receptor binding and guanylate cyclase activity secondary to hormones such as insulin has not been determined. Whether the abnormal binding activity of insulin in our two patients is directly related to the decreased guanylate cyclase activation remains to be determined.

Decreased guanylate cyclase activities have previously been observed in the livers of diabetic rats and hamsters (8–10). The cyclic GMP levels usually parallel guanylate cyclase changes as seen in our patients. Insulin fully corrects the decreased guanylate cyclase within minutes after injection in diabetic animals suggesting a direct interaction between insulin and guanylate cyclase rather than an effect of insulin on protein synthesis of this enzyme which would require more time to be accomplished.

In patients with leprechaunism who have normal insulin receptor binding as reported in some patients with this disorder (3, 6, 7) the decreased activity of the enzyme guanylate cyclase and the decreased concentration of cyclic GMP would be consistent with a postreceptor defect in insulin action. If, on the other hand, there is a defect in the interaction of insulin with its receptor in patients with leprechaunism (17–19), the findings of the present investigation would be consistent with either 1) there being only one defect with the primary defect being in the interaction of insulin with its receptor and the present findings on the guanylate cyclase–cyclic GMP system being secondary to this primary defect, or 2) that there are multiple defects in the syndrome with the insulin interaction with its receptor and insulin's interactions with the enzyme guanylate cyclase being at least two of an unknown number of defects in this disease.

Further, some patients with leprechaunism have been reported to have decreased insulin, epidermal growth factor, multiple stimulating activity, and somatomedin C receptors (17, 18, 24). Others have been reported to have impaired postbinding actions of insulin, somatomedin, and epidermal growth factor (6, 7, 24),

suggesting a primary defect in a final common pathway of growth factor-receptor mediating signaling. Since somatomedin C (25) and epidermal growth factor (26), as well as insulin, enhance guanylate cyclase activity, one might expect that patients with abnormalities of the receptor for these growth factors may also have decreased guanylate cyclase activity and decreased cyclic GMP levels. Whether or not all patients with leprechaunism are characterized by decreased guanylate cyclase activity and decreased intracellular messenger cyclic GMP awaits further investigation.

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