Increased ^{125}I -Insulin Receptor Binding to Erythrocytes of Hypoglycemic Infants and Children

GEORGE P. CHROUSOS,⁽¹⁴⁾ HELENA WACHLICHT-RODBARD, ANTHONY J. ADAMS, JESSE ROTH, AND MARVIN CORNBLATH

Neonatal and Pediatric Medicine Branch, National Institute of Child Health and Human Development, and The Diabetes Branch, National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health, Bethesda, Maryland, USA

Summary

The measurement of ^{125}I -insulin specific binding to erythrocytes obtained from seven infants and children with various hypoglycemic syndromes showed a significant increase in six patients with recurrent, documented, symptomatic hypoglycemia (percent specific ^{125}I -insulin binding 9 to 14 versus $6.1 \pm 1.4\%$; mean ± 2 S.D. for 13 controls). The increase was due to an increased number of receptors sites per cell rather than to increased affinity for insulin. The patients included three children with nesidioblastosis, all after 90% pancreatectomy, two with leucine sensitivity, and two with glycogen storage disease type I. One of the patients with leucine sensitivity, who for 2 years before the study had no hypoglycemia, had normal insulin binding (4.8%). All seven patients had normal fasting basal plasma insulin values ($<10 \mu\text{U/ml}$). Thus, symptomatic hypoglycemia correlated better with increased ^{125}I -insulin binding than with plasma insulin values.

Furthermore, Diazoxide therapy in two patients caused a mild but consistent decrease in the number of insulin receptor sites, and the institution of continuous nocturnal nasogastric feedings in a patient with glycogen storage disease type I was followed by amelioration of the hypoglycemia and a marked increase in ^{125}I -insulin specific binding from 5.2 to 9.5%.

Speculation

^{125}I -Insulin binding to receptors of various cells or tissues may be altered in a variety of clinical situations. Thus, erythrocyte insulin binding sites are significantly decreased in adult-onset, nonobese diabetics that are non-insulin dependent. This patient population has chronic hyperglycemia, the opposite state from our patients with chronic hypoglycemia. Inasmuch as our patients had increased insulin binding sites and normal basal plasma insulin values, we may speculate that prolonged hypoglycemia and therefore tissue glucose depletion may be associated with an increase in insulin receptors and therefore an increase in the insulin bioeffect. This may explain why these patients become hypoglycemic in the presence of normal plasma insulin values. Increased erythrocyte ^{125}I -insulin binding may prove to be another index of long-term hypoglycemia and may perhaps prove more reliable than isolated plasma insulin or glucose values.

Hypoglycemia as a chronic recurrent state has been associated with an absolute deficiency of available substrates or with excess insulin action resulting in either a block in hepatic glucose production and/or an increase in peripheral utilization. These effects of insulin are not always associated with high plasma insulin values, especially in infants or children with symptomatic hypoglycemia. Inasmuch as the insulin bioeffect depends upon the activity of the hormone-receptor complex and its subsequent

intracellular events and not only on the level of circulating hormone, insulin receptors were measured on erythrocytes of children with chronic hypoglycemia. The binding of insulin to erythrocytes has been investigated extensively in both adults and children (5, 6, 8, 11). The circulating erythrocyte provides an easily obtainable cell system that may reflect insulin binding activity in other tissues such as monocytes and adipocytes, not ordinarily available for study in infants or small children.

MATERIALS AND METHODS

SUBJECTS

The profile of the studied patients is shown in Table 1. Our control values were obtained from 13 age-matched control children (ages 6 to 48 months) and 17 normal adults. All bloods were obtained in the morning after at least 3 hr fasting for the patients, because a longer period of fasting resulted in hypoglycemia, and after 8 hr for the controls (13).

ASSAYS

^{125}I -Insulin binding to erythrocytes was measured according to the method of Gambhir *et al.* (13) as modified previously and described in detail (11). The binding assays were done concurrently in both patients and controls. Plasma insulin was determined by radioimmunoassay (12). Plasma glucose was measured by the glucose oxidase method (Beckman glucose analyzer model ERA-2001; Beckman Instruments, Fullerton, CA) (7).

^{125}I -INSULIN BINDING DATA ANALYSIS

The ^{125}I -insulin binding data are reported in two ways: (1) the percentage of total radioactivity bound specifically to receptors is plotted as a function of the logarithm of total insulin concentration (competition curve), and (2) the bound-to-free ratio (B/F) of ^{125}I -insulin is plotted as a function of bound hormone (B, Scatchard plot) (9). For binding of insulin to its receptors, this plot is curvilinear. To evaluate the affinity of the binding sites for insulin, we used the slopes of the Scatchard analysis curves and the concentration of unlabeled insulin causing 50% inhibition of ^{125}I -insulin specific binding (ID_{50}).

RESULTS

The percent ^{125}I -insulin that was specifically bound to erythrocytes of patients with chronic hypoglycemic states is shown in the lower panel of Figure 1, and the corresponding basal plasma insulin values are shown in the top panel. Simultaneously performed plasma glucose values ranged between 22 and 95 mg/dl. All patients except patient 5 had previously documented episodes

Table 1. Hypoglycemic patient profile

Patients	Age (mos.)	Sex	Diagnosis	Symptomatic hypoglycemia	% ideal wt.	Treatment
1. J. L.	32	F	Nesidioblastosis after 90% pan- createctomy	+	106	Diazoxide
2. G. E.	48	F	Nesidioblastosis after 90% pan- createctomy	+	170	Diazoxide
3. E. L.	6	F	Nesidioblastosis after 90% pan- createctomy	+	95	Diazoxide
4. H. S.	28	F	Leucine sensitivity	+	90	Diazoxide
5. M. R.	168	M	Leucine sensitivity	None for 2 years	100	Diazoxide
6. T. M.	26	F	Glycogen storage disease type I	+	120	Continuous nocturnal na- sogastric feedings
7. M. T.	22	M	Glycogen storage disease type I	+	82	Continuous nocturnal na- sogastric feedings

Fig. 1. The values of percent ^{125}I -insulin specific binding to erythrocytes of patients with hypoglycemic syndromes are shown in the *bottom panel*, and the corresponding basal plasma insulin values are shown in the *top panel*. Each patient had one or more determinations as shown by the number of points. (The same symbol when used in both panels indicates ^{125}I -insulin specific binding and plasma insulin value obtained from the same blood sample. These same symbols are subsequently used in Figs. 2 to 4.) The shaded zone indicates the control values (mean $\pm$ 2 S.D., $N = 13$) for percent ^{125}I -insulin specific binding. The broken line in the top panel indicates the upper limit of normal for basal plasma insulin values. Lower specific binding values of patient 1 (● and ○) and patient 4 (●) were obtained while the patients were receiving Diazoxide.

For the ^{125}I -insulin binding assays, erythrocytes (4.5×10^9 cells/ml) were incubated for 90 min at 15°C in the presence of radiolabeled insulin (100 to $150 \mu\text{Ci}/\mu\text{g}$) and increasing concentrations of cold insulin (0.1 to 10^5 ng/ml) in a total volume of 0.5 ml. Nonspecific binding was defined as the radioactivity of the cell pellet in the presence of 10^5 ng/ml of unlabeled insulin.

of prolonged symptomatic hypoglycemia. Patient 5, a 14-year-old boy with leucine sensitivity, had not had hypoglycemia for at least 2 years before the study.

The shaded area in the lower panel represents the mean $\pm$ 2 S.D. from 13 control subjects ages 6 to 58 months. Inasmuch as

Fig. 2. Representative competition curves and Scatchard plots (*inset*) of the three patients with nesidioblastosis and 90% pancreatectomy (symbols are the same as in Fig. 1). The shaded area represents the control values ($\pm$ 2 S.D.; $N = 13$) and the lower line in the inset is the mean of the Scatchard plot curves from control subjects. The same control values for the competition curves and Scatchard plots are shown in Figures 3 and 4.

these normal data did not differ from those obtained in 17 normal adult subjects, it was possible to compare the data of the 14-year-old patient (patient 5).

All patients with symptomatic hypoglycemia had increased ^{125}I -insulin specific binding. Patient 5 had normal binding (Fig. 1). From both the competition curves (ID_{50} of patients with increased ^{125}I -insulin specific binding ranged within 8 to 60 ng of unlabeled insulin per ml, *i.e.*, within the $\pm$ S.D. limits for ID_{50} obtained from 13 controls) and from the parallelism of Scatchard plot curves (Figs. 2 to 4), it is apparent that the increased insulin specific binding of our patients was due to an increased number of binding sites rather than to increased affinity of the receptors for insulin.

Patients 1 and 4 were studied before and at least 3 months after institution of Diazoxide therapy (Figs. 1 to 3). Insulin specific binding was increased in both under either condition. While undergoing treatment, however, the specific binding of both was consistently lower than before therapy.

Patients 6 and 7 with glycogen storage disease type I had high insulin binding while receiving treatment with continuous nocturnal nasogastric tube feedings (Figs. 1 and 4). Before starting this therapy, patient 7 had normal insulin specific binding.

Fig. 3. Results presented as in Figure 2. ^{125}I -Insulin binding to erythrocytes from two patients with leucine sensitivity. The nonsymptomatic patient 5 [M. R. (■)] had normal binding. The symptomatic patient 4 (H. S.) had lower binding while receiving Diazoxide therapy (●).

DISCUSSION

In six patients with chronic symptomatic hypoglycemia and normal basal plasma insulin values, the percent ^{125}I -insulin specific binding to erythrocytes was increased. This increase was due to an increase in number of binding sites rather than to an increase in affinity of receptors for insulin. The physiologic significance of this increase is not known. It appears, however, that this phenomenon may be the reverse of what happens in adult onset, nonobese, non-insulin-dependent diabetics in whom there is chronic hyperglycemia and decreased number of insulin binding sites on erythrocytes without any change in their affinity for insulin (8).

In patients with chronic hypoglycemia and therefore chronic glucose depletion of cells, an increase in insulin receptors may represent an adaptive mechanism to increase the effective insulin action on the cell. Inasmuch as the mature erythrocyte can neither synthesize nor move new integral proteins such as the insulin receptor from the inside of the cell into the plasma membrane, it would appear that these changes in receptor number occurred while the erythrocyte was still immature. Thus, the change in the receptor number may indicate hypoglycemia that has been present long before the measurement of ^{125}I -insulin binding. This then may be an index of prolonged hypoglycemic states of greater value than isolated basal plasma insulin or blood glucose determinations. Increased ^{125}I -binding also occurs in young erythrocytes, i.e., reticulocytes (1, 10); however, none of the patients studied nor any of the controls had anemia or any other conditions associated with reticulocytosis. Certainly, the increased binding in our patients may help explain their symptomatic hypoglycemia in the presence of relatively low plasma insulin values, especially if these changes reflect those in liver and other tissues. An increase in bioeffect of insulin would result in the shut down of glucose secretion from the liver and an increase in glucose removal and consumption in other target tissues.

The previously noted increased sensitivity to symptomatic hypoglycemia in children with glycogen storage disease type I after therapy with continuous nocturnal nasogastric tube feedings (4) may be in part explained by an increase in the insulin bioeffect secondary to increased number of insulin receptors.

Fig. 4. Results presented as in Figures 2 and 3. Both patients with glycogen storage disease type I and high ^{125}I -insulin specific binding with normal affinity (inset). They were both receiving continuous nocturnal nasogastric feedings. One of the patients tested before the initiation of tube feedings (□) had normal binding.

Further studies of the effects of Diazoxide therapy are warranted to determine whether, in addition to its known action on inhibiting the secretion of insulin from the beta cell (2), Diazoxide may also have some peripheral effects on insulin receptors.

REFERENCES AND NOTES

- Eng, J., Lee, L., and Yalow, R. S.: Influence of the age of erythrocytes on their insulin receptors. *Diabetes*, 29: 164 (1980).
- Fajans, S., Floyd, J., Knopf, R., Rull, J., Guntsche, E., and Conn, J.: Benzothiadiazine suppression of insulin release from normal and abnormal islet tissue in man. *J. Clin. Invest.*, 45: 481 (1966).
- Gambhir, K. K., Archer, J. A., and Bradley, C. J.: Characteristics of human erythrocyte insulin receptors. *Diabetes*, 27: 701 (1978).
- Greene, H. L., Slonim, A. E., Burr, I. M., and Moran, J. R.: Type I glycogen storage disease: five years of management with nocturnal nasogastric feeding. *J. Pediatr.*, 96: 590 (1980).
- Herzberg, V. L., Boughter, M. J., Carlisle, S. K., Ahmad, F., and Hill, D. E.: ^{125}I -Insulin binding to cord blood erythrocytes of varying gestational age and comparison with adult values. *Pediatr. Res.*, 14: 4 (1980).
- Kappy, M. S., and Plotnick, L.: Studies of insulin binding in children using human erythrocytes in small amounts of blood. *Diabetes*, 28: 1001 (1979).
- Marks, U.: An improved glucose-oxidase method for determining blood, CSF and urine glucose levels. *Clin. Chim. Acta*, 4: 395 (1959).
- Robinson, T. J., Archer, J. A., Gambhir, K. K., Hollis, V. W., Jr., Carter, L., and Bradley, C.: Erythrocytes: a new cell type for the evaluation of insulin receptor defects in diabetic humans. *Science (Wash. D. C.)*, 205: 200 (1979).
- Scatchard, J., Coleman, J., and Shon, A.: Physical chemistry of protein solutions. VII. The binding of some small anions to serum albumin. *J. Am. Chem. Soc.*, 79: 12 (1957).
- Thomopoulos, P., Testa, U., Flamier, A., and Bethelie, M.: Insulin receptors and protein synthesis in bone marrow cells and reticulocytes. *Diabetes*, 29: 820 (1980).
- Wachslight-Rodbard, H., Gross, H. A., Rodbard, D., Ebert, M. H., and Roth, J.: Increased insulin binding to erythrocytes in anorexia nervosa. *N. Engl. J. Med.*, 300: 882 (1979).
- Yalow, R., and Berson, S.: Immunoassay of endogenous plasma insulin in man. *J. Clin. Invest.*, 39: 1157 (1960).
- Appropriate informed consent was obtained for this study.
- Requests for reprints should be addressed to: Dr. George P. Chrousos, Department of Health and Human Services, NIH, Building 10, Room 13N-260, Bethesda, MD 20205 (USA).
- Received for publication November 18, 1980.
- Accepted for publication February 10, 1981.