Glucose intolerance in chronic renal failure —A study from the viewpoint of insulin receptor—

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〈Abstract〉

In order to clarify the pathogenesis of glucose intolerance in chronic renal failure, we investigated it from the viewpoint of insulin receptor. IV-GTT (0.5 g/kg) was performed in 10 undialyzed and 12 dialyzed patients with chronic renal failure and 6 normal subjects, and their insulin receptors were examined. Σ IRI (the sum of IRI during IV-GTT) in the undialyzed group was $445 \pm 78 \mu\text{U}/\text{m}l$, which was significantly higher than that ($260 \pm 38 \mu\text{U}/\text{m}l$) in the normal group. The K value (glucose disappearance rate) in the undialyzed group was 1.32 ± 0.30 , which was significantly lower than that (1.92 ± 0.25) in the normal group and that (1.82 ± 0.34) in the dialyzed group. No significant difference in K value was recognized between the normal and dialyzed groups.

The specific binding of ^{125}I -insulin to erythrocytes (2.4×10^9 cells/ $\text{m}l$) in the undialyzed group was $3.99 \pm 0.76\%$ which was significantly lower than that ($5.50 \pm 0.69\%$) in the normal group. However, no significant difference was observed between the normal and dialyzed groups. When competition data for the three groups were subjected to Scatchard analysis, the difference in binding to erythrocytes appeared to be due to the difference in receptor concentration rather than receptor affinity (receptor concentration : 42, 58, and 64 sites/erythrocyte in the undialyzed, dialyzed, and normal groups, respectively). In the dialyzed group, the correlation coefficients between the specific binding of ^{125}I -insulin and K value ($r = 0.542$) were higher than those between the binding and fasting IRI, Σ IRI ($r = -0.381, -0.272$).

From these results, it is suggested that glucose intolerance in chronic renal failure is founded on a decrease in insulin receptor concentration, and that hemodialysis improves this glucose intolerance through increased sensitivity to insulin action in the peripheral tissue with accompanying normalization of the insulin receptors.

腎不全における耐糖能異常の成因について—インスリン受容体からの検討—

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慢性腎不全に耐糖能異常が存在することはよく知られているが、その成因については不明な点が少なくない。インスリン抵抗性もその一因と考えられているが、この点をさらに明らかにするためにインスリン受容体の面から検討した。未透析腎不全患者 10 名、透析患者 12 名および正常者 6 名を対象に赤血球のインスリン受容体を検討し、0.5 g/kg の IVGTT を行い、K 値 (血糖消失率) および Σ IRI (IRI の総和) を求めた。K 値は未透析群 (1.32 ± 0.30) では正常群 (1.92 ± 0.25)、透析群 (1.82 ± 0.34) と比べて低値を示したが、正常群と透析群との間には差がなかった。 Σ IRI は未透析群 ($445 \pm 78 \mu\text{U}/\text{m}l$)、透析群 ($420 \pm 80 \mu\text{U}/\text{m}l$) は正常群 ($260 \pm 38 \mu\text{U}/\text{m}l$) と比べて高値を示したが、未透析群と透析群との間には差がなかった。インスリン特異結合率 (赤血球 2.4×10^9 個/ $\text{m}l$ への $0.2 \text{ ng}/\text{m}l$ の ^{125}I -イ

ンスリン)は未透析群 (3.99±0.76%)は低値を示したが、正常群 (5.50±0.69%)と透析群 (5.11±0.80%)との間には有意差がなかった。Scatchard 解析より、未透析群におけるインスリン特異結合率の低下はインスリン受容体数の減少によるものと思われた (正常群 64 個/cell ; 透析群 58 個/cell ; 未透析群 45 個/cell)。

さらに、耐糖能異常とインスリン受容体との関係を明らかにするために、インスリン特異結合率とK値および空腹時 IRI, ΣIRI との関係をみたが、透析群において特異結合率とK値との間に正の相関を認めた。このことより、未透析腎不全患者の耐糖能異常はインスリン受容体数の低下によるものと思われ、透析療法はインスリン受容体レベルでの改善により耐糖能障害を正常化させるものと思われた。

Introduction

The presence of impaired glucose metabolism in patients with chronic renal failure has been widely recognized^{1,2,3}. However, the exact etiology of such glucose intolerance remains unclear. It is suggested that insulin resistance may represent one of its etiologies based on the finding of a delayed, diminished fall in blood glucose concentration in spite of a high serum insulin level during intravenous glucose injection¹. In this connection, Gambhir et al.⁴ reported a decreased insulin receptor concentration in the erythrocytes of uremic patients. However, when the insulin receptors of uremic patients are investigated, the relationship between glucose intolerance and the insulin receptors should also be considered in the same patients.

In the present study, we examined the glucose metabolism of undialyzed patients with chronic renal failure and their insulin receptors, and furthermore investigated the effect of hemodialysis on their glucose intolerance.

Subjects and Methods

10 undialyzed uremic patients aged from 25-48 years, and 12 patients on chronic hemodialysis for more than two years with ages ranging from 29-47 years, were studied. The clinical profile of these patients is summarized in Table 1. 6 age-matched, healthy men and women were selected as controls. All of them were non-obese, without past and family histories of diabetes. After an over night fast, about 12 ml of blood was collected in a tube containing sodium heparin for insulin receptor assay. An intravenous glucose tolerance test (IV-GTT) was then carried out. Glucose was administered intravenously at a dose of 0.5 g/kg body weight and blood was obtained before and at 10, 20, 30, 60, 90, and 120 min after termination of the glucose injection for measurement of the blood glucose and serum insulin concentrations.

Blood glucose was measured by the glucose-oxidase method and serum insulin by radioimmunoassay using a double-antibody method⁵. The glucose disappearance rate (K value), which was expressed as the degree of glucose intolerance, was calculated according to the method described by Lundbaek⁶. ΣIRI was the sum of the IRI at 0, 10, 20, 30, 60, 90, and 120 min.

Insulin receptor assay in erythrocytes was performed by a modification of the method of Gambhir et al.⁷. The cell pellet was suspended in 2 vols of physiological saline, layered over a mixture of Ficoll-hypaque and centrifuged at 400G for 15 min at room temperature. This process was repeated twice to minimize contamination

	Number	Age yr	% IBW	BUN mg/dl	Cr mg/dl	Ca ⁺⁺ mEq/l	Ht %	FBG mg/dl	IRI μU/ml
renal failure (Undialyzed)	10	39 25~48	93 81~108	86 51~114	8.9 3.9~10.6	4.2 3.8~4.9	29 24~39	91 76~111	21 14~36
renal failure (Dialyzed)	12	43 29~47	104 92~106	72 64~88	8.7 7.0~12.4	4.9 4.2~5.3	24 16~37	84 72~108	24 10~32
normal	6	35 30~47	97 88~118	18 16~24	1.3 0.6~1.7	5.1 4.8~5.3	43 38~49	87 67~98	13 9~19

IBW=Ideal body weight mean
range

Table 1 Clinical profile of subjects

Figure 1 ΣIRI and K value in undialyzed, dialyzed and normal groups

from leucocytes, especially mononuclear cells. The resultant erythrocyte pellet was washed three times with Tris-HEPES buffer (50 mM Tris, 50 mM HEPES, 50 mM NaCl, 10 mM MgSO₄, 5 mM KCl, 10 mM CaCl₂, 2 mM EDTA, 10 mM glucose, pH 8.0) containing 1% bovine serum albumin. The erythrocytes so obtained were resuspended in Tris-HEPES buffer to give about 3.0×10^9 cells/ml. ¹²⁵I-labeled insulin was prepared with chloramine T according to the method of Hunter et al.⁸⁾. 400 μl of the erythrocyte suspension and 50 μl of porcine monocomponent insulin (final concentration, 0.5–200 ng/ml) and 50 μl of ¹²⁵I-insulin (final concentration, 0.2 ng/ml) were incubated in a 15°C water-bath for 210 min. After the incubation, duplicate 100 μl aliquots of the incubation mixture were transferred to prechilled plastic tubes containing 100 μl of dibutylphthalate and 200 μl of assay buffer. The microfuge tubes were then centrifuged for 150 sec in a Beckman Microfuge and the radioactivity in the cell pellets was determined. The insulin binding was expressed as the cell-associated ¹²⁵I-insulin subtraction of nonspecific binding, determined in the presence of excess (10⁵ ng) unlabeled insulin. The degradation of the ¹²⁵I-insulin during the binding assay, as determined by the TCA precipitation method, amounted to less than 5%.

Results

The fasting blood glucose level in undialyzed uremic patients was 91 ± 10 mg/dl, that in dialyzed patients was 84 ± 8 mg/dl, and that in normal persons was 87 ± 9 mg/dl. There was no significant difference among these three groups. The ΣIRI value in the undialyzed group was 445 ± 78 μU/ml, which was significantly higher than that (260 ± 38 μU/ml) in the normal group ($P < 0.01$). However, no significant difference was recognized between the undialyzed group and dialyzed group (420 ± 80 μU/ml). In spite of the high ΣIRI level, the K value in the undialyzed group was 1.32 ± 0.30 which was significantly lower than that (1.92 ± 0.25) in the normal group and that (1.82 ± 0.34) in the dialyzed group ($P < 0.05$) (Figure 1).

The insulin fraction specifically bound to erythrocytes (bound/free insulin) in normal subjects was $5.50 \pm 0.69\%$ at a tracer concentration of insulin (0.2 ng/ml). In the undialyzed and dialyzed groups, the values ranged widely from 2.21 to 5.28% ($3.99 \pm 0.76\%$) and from 3.62 to 6.51% ($5.11 \pm 0.80\%$), respectively. The value in the undialyzed group was significantly lower than that in the normal group ($P < 0.01$). However, no significant

change in specific ^{125}I -insulin binding was recognized between the dialyzed and normal groups (Figure 2). When competition data for the three groups were subjected to Scatchard analysis⁹⁾, the plots were curvilinear and parallel to each other. It seems likely therefore that the difference in binding to each of the erythrocytes from the three groups was due to a difference in receptor concentration rather than receptor affinity. The mean concentrations of insulin receptor, obtained by extrapolating the curves to their points of intersection on the abscissa, were 45, 58, and 64 sites/erythrocyte for the undialyzed, dialyzed and normal groups, respectively (Figure 3).

Correlation coefficients were also calculated between the binding and fasting IRI or ΣIRI as well as between the binding and K value. In the undialyzed group, there was no significant correlation between the binding and other parameters. On the other hand, in the dialyzed group, a significant correlation was found between the binding and K value ($r=0.542$, $P<0.05$). However, the values for the coefficients of fasting IRI and ΣIRI were lower than that between the binding and K value ($r=-0.381$, -0.272 , respectively) (Table 2).

Discussion

Our data indicate a high incidence of abnormal glucose tolerance in undialyzed patients with chronic renal failure and that hemodialysis can improve such glucose intolerance. These results are in agreement with the reports of previous investigators^{1,10,11)}. The oral glucose tolerance test (OGTT) is usually applied in demonstrating abnormalities of glucose metabolism. However, OGTT may be affected by abnormalities of glucose absorption. Uremic patients frequently have gastrointestinal diseases. Therefore, it is not adequate to apply OGTT for identifying glucose intolerance in uremic patients. For this reason, they were subjected to IV-GTT this time.

Concerning the finding that in spite of a high insulin level during IV-GTT, glucose intolerance is frequently present in undialyzed uremic patients, insensitivity to the action of insulin in the peripheral tissue is suggested. Hyperglucagonemia, elevated growth hormone levels¹²⁾ and humoral factors¹⁾ are considered to be involved in the pathogenesis of insulin resistance in chronic renal failure. Since it is generally accepted that binding of insulin to specific receptors located on the plasma membranes of target tissue cells represents the initial step in its biological effect, it is necessary to consider such insulin resistance from the viewpoint of the insulin receptors. Although the main target organs of insulin action are the liver, muscle and adipose tissue, it is difficult to apply them clinically for insulin receptor assay. Despite the possibility of anemia, since a little blood is sufficient to isolate adequate erythrocytes for insulin receptor studies, we employed erythrocytes from uremic patients.

When discussing insulin receptors, the ^{125}I -insulin binding is generally regarded as reflecting the insulin receptor since a second messenger of insulin is unknown¹³⁾. As shown in Figure 2, the ^{125}I -insulin specific binding in the undialyzed group was significantly lower than that in the normal group. Scatchard analysis of the binding curves revealed that the change in insulin binding to erythrocytes was associated with a decrease in the insulin receptor concentration in undialyzed patients. Regarding the mechanism of this decrease in insulin receptor, there have been many reports supporting "down regulation"; that is, insulin receptors are regulated by the insulin concentration itself^{14,15)}. However, it seems unlikely that the decrease in insulin binding in the undialyzed patients was simply a result of their hyperinsulinemia. Because, the insulin binding of dialyzed patients increased to the level of that of normals without a remarkable change in serum insulin level. The possibility exists therefore of the presence of other factors affecting the insulin receptor. Abnormality of humoral factors is well recognized in chronic renal failure. Furthermore, since it is reported to take more than several months for the improvement of glucose intolerance by hemodialysis¹¹⁾, we assume that this factor may be a "middle molecular weight substance" which is not easily dialyzed with usual hemodialysis.

Examination of the relationship between the binding and other parameters such as fasting IRI and ΣIRI , revealed a correlation with the K value rather than fasting IRI or ΣIRI . It seems possible therefore that hemodialysis may cause the insulin receptor concentration to normalize and improve the glucose intolerance through an increased sensitivity to insulin action in the peripheral tissue.

Figure 2 Specific ^{125}I -insulin binding (%) to erythrocytes in undialyzed, dialyzed and normal groups

Figure 3 Scatchard plots of ^{125}I -insulin specific binding to erythrocytes

	Undialyzed	Dialyzed
Fasting IRI	$r = -0.342$	$r = -0.381$
Σ IRI	$r = -0.116$	$r = -0.272$
K-Value	$r = 0.277$	$r = 0.542^*$

* $p < 0.05$

Table 2 Correlation coefficients between ^{125}I -insulin specific binding and each parameters

It is considered that although erythrocytes are not target cells for insulin action and differ from other human cells in that they lack nuclei and do not internalize hormones¹⁶⁾, their insulin receptors parallel those in monocytes or adipocytes^{17,18)}. On the other hand, it has been reported that the number of insulin receptors in erythrocytes decreases with their age and that reticulocytes are rich in insulin receptor numbers^{19,20)}. The erythrocytes of uremic patients have a shorter life span than those of normal persons and are rich in reticulocytes²¹⁾. Therefore, when we employ erythrocytes for insulin receptor assay, it is necessary to take into account the unique features of the erythrocyte insulin receptors.

Conclusion

10 undialyzed patients, 12 dialyzed patients with chronic renal failure and 6 normal persons were subjected to IV-GTT and their insulin receptors were examined.

- 1) In spite of a high ΣIRI level, the K value in the undialyzed group was significantly lower than that in the normal group.
- 2) The insulin receptor concentration in the undialyzed group was lower than that in the normal group.
- 3) No significant difference in K value or insulin receptor concentration was observed between the dialyzed and normal groups.

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