Increased Affinity of Insulin Receptor on Hepatocytes from Streptozotocin-Induced Diabetic Rats

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Abstract

When we use Scatchard analysis for insulin receptor on intact cells, internalization of insulin may affect the Scatchard analysis. In this study, results from Scatchard analysis for insulin receptor on hepatocytes of streptozotocin (STZ)-induced diabetic rats were compared with those from association and dissociation studies and those from Scatchard analysis using hepatocyte membrane which does not internalize insulin.

Insulin binding was increased in both hepatocytes and the membranes from STZ-treated rats. Scatchard analysis revealed that both the receptor number and affinity constant were increased. In the dissociation study, the increase in the affinity constant was revealed to be due to a decrease in the dissociation rate constant. The association rate constant was comparable in STZ and control rats. The Scatchard plot was more concave in STZ rats than in controls, suggesting that the magnitude of the negative cooperative effect was greater in STZ rats. This was confirmed by measuring the dissociation rate constant in the presence or absence of unlabeled insulin. Increases in the receptor number, affinity constant, and negative cooperative effect in STZ rats were observed both in hepatocytes and in hepatocyte membranes.

Previous studies have shown that the affinity of insulin receptor is altered in a number of situations, such as acute glucose ingestion (Muggeo *et al.*, 1977), hypoglycemia (Bar *et al.*, 1977), acidosis (De Meyts *et al.*, 1976), fasting (Bar *et al.*, 1976; DeFronzo *et al.*, 1977; Olefsky *et al.*, 1978) and streptozotocin (STZ)-induced insulin deficiency (Kobayashi *et al.*, 1979). We have also reported that not only the num-

ber but also the affinity constant of insulin receptors is increased in hepatocytes from prolonged insulin deficiency in rats induced by streptozotocin (50mg/kg) (Okamoto *et al.*, 1982). But all these results were obtained from Scatchard analyses. On the other hand, Carpentier *et al.*, reported using the electron microscopic method which showed that ^{125}I -insulin bound to hepatocytes was internalized even at 20°C (Carpentier *et al.*, 1979). Furthermore, it has been reported that the internalization of in-

sulin during binding studies affects Scatchard analysis (Olefsky *et al.*, 1978). These facts suggest that Scatchard analysis cannot be used in some conditions, and that it is important to re-evaluate the results obtained by Scatchard analysis in intact cells with method which are not affected by internalization.

To ensure and to elucidate the mechanisms responsible for increased affinity of insulin receptors in STZ rat, the following two methods were used. First, association and dissociation rate constants of insulin receptors were measured in hepatocytes. The ratio of these constants represents the affinity constant. Second, results of Scatchard analyses of binding curves from intact hepatocytes were compared with those from hepatocyte membranes, which do not internalize insulin.

Material and Methods

Animals

Male Wistar rats weighing 160–200 g were used in all experiments. Hypoinsulinemic diabetic rats were produced by injecting 50 mg/kg of streptozotocin dissolved in citrate buffer (pH 4.5) into a tail vein of rats fasted overnight. They were used for experiments 8 weeks after the injection. All animals were fed *ad libitum* until the experiments. Preparation of isolated hepatocytes and cell membranes: Hepatocytes were isolated according to a modification of the method of Berry and Friend (1969) as described previously (Okamoto *et al.*, 1982). More than 90% of the hepatocytes were found intact judged from their ability to exclude trypan blue after incubation. Plasma membranes were prepared from the isolated hepatocytes by the method of Neville (1968) except that the minimum essential medium was used as diluent. Protein determinations were performed according to the method of Lowry *et al.* (1951).

Iodination of insulin

^{125}I -insulin was prepared by the chloramin T-method with specific activities of 100–150 $\mu\text{Ci}/\mu\text{g}$ and purified on a Sephadex G 75 column

(1×60 cm). Degradation of ^{125}I -insulin was checked by the TCA precipitation method before each experiment. The precipitability was $96\pm0.8\%$

Binding study

The binding study on hepatocytes was performed as previously described (Okamoto *et al.*, 1982). The hepatocytes, suspended to give a final concentration of $0.5\times10^6/\text{ml}$ in the minimal essential medium, were incubated with ^{125}I -insulin and unlabeled porcine insulin over a range of concentrations from 0.1 to 1000 ng/ml for 2 hours at 15°C. Nonspecific binding, defined as the radioactivity in cells in the presence of 2×10^5 ng/ml of unlabeled insulin, was approximately 0.4% of total radioactivity. Degradation of ^{125}I -insulin during the binding assay determined by the TCA precipitation method was less than 13% of the total radioactivity. The binding study with hepatocyte plasma membranes was performed by the method of Cuatrecasas *et al.* (1971). The membrane (150 $\mu\text{g}/\text{ml}$) was incubated with ^{125}I -insulin, using the same buffer for the same incubation period as those in the study with hepatocytes. Degradation of ^{125}I -insulin during the binding assay was less than 6.2%. The receptor number and “empty site” affinity constant (K_A) were calculated from Scatchard plots (Scatchard, 1949) by the method of De Meyts *et al.* (1975).

Association study

Isolated hepatocytes were incubated in the buffer (15°C) containing 0.6 ng/ml of ^{125}I -insulin. At the indicated time, 2 ml aliquot was layered on 0.5 ml of a mixture of dibutylphthalate and olive oil in a small test tube and centrifuged for 150 seconds in a Bechman microcentrifuge. Radioactivities in cell pellets were then counted. Non specific binding was determined as the binding in the presence of 2×10^5 ng/ml of insulin.

Dissociation study

All dissociation studies were performed at 15°C with a modification of the method of Olefsky *et al.* (1978). Hepatocytes were preincubated with ^{125}I -insulin (1 ng/ml) in a total volume of 3 ml. After steady state binding was achieved by incubating for 120 minutes, the cells were centrifuged for 2 minutes at 500 rpm. The buffer was removed and replaced with an equal amount of insulin-free media at 15°C. Aliquots

of the cell suspension (100 μ l) were distributed into tubes containing 1.9 ml of the buffer at 15°C with or without unlabeled insulin (1000 ng/ml). The cells in these tubes were then centrifuged in a Beckman microcentrifuge on 0.5 ml of mixture of dibutylphthalate and olive oil at the time points indicated, and radioactivity remaining in the cell layer was determined. Nonspecific binding was subtracted from total binding. Internalized insulin during the preincubation for 2 hours at 15°C was determined with a slight modification of the method of Haigler *et al.* (1980), using a buffer at pH 3.5. After 2 hours preincubation of the cells with 125 I-insulin at 4°C, 95% of cell associated insulin was dissociable by this method, suggesting that the method was available to estimate the surface-bound insulin for hepatocytes. The cell surface-bound insulin was used for calculating the dissociation of insulin.

Blood glucose was measured with a Dextrometer®/Dextrostix (Ames Division, Miles Laboratory Inc.). Plasma immunoreactive insulin concentrations were determined by the polyethylene glycol method (Desbuquois and Aurbach, 1971), using rat insulin as a standard. Results were expressed as mean $\pm$ SEM. Statistical analyses were performed by Student's *t*-test.

Results

Animals

Body weight, blood glucose and plasma insulin levels in control and STZ-treated rats are summarized in Table 1. Blood glucose levels were markedly increased, whereas insulin concentrations were decreased in STZ rats. The weight gain was attenuated in diabetic rats.

Binding study

Insulin binding to hepatocytes was shown in Fig. 1-a. Specific insulin binding at a tracer concentration of insulin (0.1 ng/ml) was $15.9 \pm 1.9\%$ in the STZ rat and $6.6 \pm 1.1\%$ in the control. The receptor concentration and affinity constant obtained from Scatchard plot were $60 \times 10^4/\text{cell}$ and $3.8 \times 10^8 \text{M}^{-1}$, respectively, in the STZ rat, while $40 \times 10^4/\text{cell}$ and $2.1 \times 10^8 \text{M}^{-1}$ in the

Table 1. Characteristics of Experimental Animals

	Control Rats	STZ Rats
Body Weight	400 ± 30	$203 \pm 18^{**}$
Blood Glucose (mg/dl)	131 ± 6	$330 \pm 9^{**}$
Plasma Insulin (ng/ml)		
Portal Vein	7.7 ± 1.1	$0.6 \pm 0.1^{**}$
IVC***	3.4 ± 0.4	$0.3 \pm 0.1^{**}$

Values given are Mean $\pm$ S.E.M.

** $P < 0.01$

*** IVC stands for inferior vena cava.

Fig. 1-a. Specific 125 I-insulin binding to hepatocytes from STZ (○) and control (●) rats. (** $P < 0.01$, * $P < 0.05$)

Fig. 1-b. Scatchard plots of the binding data (Fig. 1-a) in hepatocytes from STZ (○) and control (●) rats. Receptor numbers and affinity constants are shown in the text.

control (Fig. 1-b). The increase in insulin binding found in STZ rats was thus accounted for by both increased receptor concentrations and affinity constant. In addition, the Scatchard curve was more concave in STZ rats than in control rats. This finding indicates that the magnitude of the negative cooperative effect is apparently greater in STZ rats than control rats. On the other hand, internalized insulin during the incubation for 2 hours measured by a modified method of Haigler *et al.* (1980) was $1.93 \pm 0.18\%$ of total radioactivity for controls and $2.12 \pm 0.25\%$ for STZ rats. The ratio of the internalized insulin to specifically bound (cell associated to be exact) insulin was $28.2 \pm 1.99\%$ for controls and $15.5 \pm 2.35\%$ for STZ rats respectively. These ratios were remarkable and the difference between them was significant ($P < 0.01$). Therefore the internalization might affect the results obtained with Scatchard analysis. To confirm the increase in the affinity constant and the cooperative effect in STZ rats found in the binding study, the association and dissociation rate constants were measured.

Association study

Eq 1 shows a reaction for the insulin-receptor binding, where [I] represents the free insulin concentration, [R] free receptor concentration, and [IR] insulin-receptor complex concentration. Eq 2 shows the relationship of K_A (affinity constant), K_a (association rate constant) and K_d (dissociation rate constant). In the initial part of the association study, the association rate constant can be estimated by Eq 3. V_a represents the rate of association of insulin to its receptors, and [I] and [R] are almost equal to $[I_0]$ (tracer concentration of insulin) and $[R_0]$ (free receptor concentration at the start of the study), respectively.

$$K_A = \frac{K_a}{K_d} = \frac{[IR]}{[I] \times [R]} \quad \text{Eq (2)}$$

$$V_a = K_a \times [I] \times [R] \quad \text{Eq (3)}$$

Fig. 2-a shows the initial part of the time course of insulin binding. The actual amount bound to hepatocytes from STZ-treated rats was higher than control cells, although the shapes of the curves were similar. To compare the rate of association on a uniform basis, the ratio of bound insulin to the receptor number was calculated as shown in Fig. 2-b. With this an-

Fig. 2-a. Initial part of time course of insulin binding to hepatocytes ($0.5 \times 10^6/\text{ml}$) from STZ (○) and control (●) rats. Data are expressed as the percentage of ^{125}I -insulin specifically bound at each time point. (* $P < 0.05$)

alysis, the curves were essentially superimposable, indicating that K_a is similar for the both groups. The association rate constants calculated from the initial 5 minutes were $1.0 \times 10^5 \text{ M}^{-1} \text{ S}^{-1}$ for both groups.

Dissociation study

Hepatocytes were allowed to associate with a tracer concentration of ^{125}I -insulin (1 ng/ml) for 2 hrs at 15°C . Then, the dissociation studies were performed by dilution with (dilution plus insulin) or without (dilution only) addition of 1000 ng/ml unlabelled insulin. Internalized (or non dissociable)

insulin during the incubation for 2 hours before the dissociation study was subtracted from cell associated insulin at the time points indicated during the dissociation study. The dissociation rate constant (K_d) was derived from the initial 5 min period of the dissociation study (Fig. 3) using, Eq 4, where V_d equals the rate of dissociation of insulin from the receptors.

$$V_d = K_d \times [\text{IR}] \quad \text{Eq (4)}$$

The dissociation rate constant estimated from the "dilution only" study was decreased in the STZ rats ($1.7 \times 10^{-4} \text{ S}^{-1}$ v.s.

Fig. 2-b. Time course of insulin binding corrected by receptor number [(insulin bound)/(receptor number)]. Receptor number and bound insulin were derived from Fig. 1-b and Fig. 2-a respectively. Association rate constants were calculated from the results for the initial 5 minutes of the study. (STZ ○, control ●)

Fig. 3. Dissociation of ^{125}I -insulin from hepatocyte insulin receptor in the absence of insulin (dilution: STZ ○, control ●) in the presence of insulin (1000 ng/ml; dil.+insulin: STZ △, control ▲). Dissociation rate constants were calculated from the results for the initial 5 minutes of the study. (* $P < 0.05$)

$3.1 \times 10^{-4} \text{S}^{-1}$). ^{125}I -insulin dissociated from hepatocytes of both STZ and control rats was faster in the "dilution plus insulin" group than in the "dilution only" group. Thus higher fractional receptor occupancy produced by the unlabeled insulin accelerates the dissociation (negative cooperativity). The magnitude of this effect was greater in STZ rats. The dissociation rate was

comparable for control and STZ rat hepatocytes in the presence of 1000 ng/ml insulin, while slower in STZ rats in the absence of insulin. The results agree with the observation that the Scatchard plot is more concave in STZ rats than in control rats.

Insulin binding to hepatocyte membranes

Insulin binding to hepatocyte membranes is shown in Fig. 4-a. Specific insulin binding at the tracer concentration of insulin (0.1 ng/ml) was $10.6 \pm 1.8\%$ in STZ rats and $6.7 \pm 0.7\%$ in control rats. The receptor concentration and affinity constant obtained from the Scatchard plot (Fig. 4-b) were $2.5 \times 10^{11}/150 \mu\text{g}$ protein and $3.1 \times 10^8 \text{M}^{-1}$ in STZ rat hepatocyte membranes, and $2.0 \times 10^{11}/150 \mu\text{g}$ protein and $1.8 \times 10^8 \text{M}^{-1}$ in controls, respectively. Thus, the binding of insulin to the membranes was accounted for by increases in both the receptor concentration and affinity, as in the case of intact hepatocytes.

Fig. 4-a. Specific ^{125}I -insulin binding to hepatocyte membranes from STZ (○) and control (●) rats. (* $P < 0.05$)

Fig. 4-b. Scatchard plots of the binding data (Fig. 4-a) in hepatocyte membranes from STZ (○) and control (●) rats. Receptor number and affinity constant are shown in the text respectively.

Discussion

Many studies have previously reported about insulin receptor on intact cells. However, the internalization of insulin (Carpentier *et al.*, 1979) or insulin receptor (Gorden *et al.*, 1982) could occur in intact cells even at a temperature as low as 20°C , which might affect Scatchard analysis. Using isolated rat hepatocyte, Gorden *et al.* showed that 16% of cell associated grains (photo-reactive insulin) were internalized into the cells at 20°C during 30 minutes of preincubation and the internalized grain increased to 51% at 37°C and to 29% at 20°C in 30 minutes incubation after photolysis, suggesting significant internalization of the insulin-receptor complex. Olefsky *et al.* (1978) compared the insulin binding curve obtained from rat intact hepatocytes with that from hepatocyte membranes at 37°C , and found that the curves were similar as long as the

insulin concentration was less than 100 ng/ml. At higher insulin concentrations, however, the curves showed different shapes. They ascribed such differences between intact cells and cell membranes to the internalization of insulin in the former. Thus these studies indicated that it is important to re-evaluate or to ensure the results obtained by Scatchard analysis in intact cells with methods which are not affected by the internalization. For this purpose we used two methods described in the study with hepatocytes from STZ treated rat because insulin receptor on the cells was previously reported to be changed (Okamoto *et al.*, 1982; Kobayashi *et al.*, 1979).

To compare the results from hepatocytes with those from hepatocyte membranes or those obtained from the association and dissociation study, internalization of insulin and its receptor must be minimized. Since the internalization is known to occur in a temperature dependent manner (Carpentier *et al.*, 1979; Gorden *et al.*, 1982), we chose 15°C for the binding assay in the present study.

This study demonstrated that the association rate constant in STZ rat hepatocytes was comparable to that in control cells, whereas the dissociation rate constant was decreased in STZ rats. Since the affinity constant is equal to the ratio of association to dissociation rate constant, the decreased dissociation rate constant in STZ rats seems responsible for the increased affinity constant observed in the Scatchard plot. Furthermore, the increased binding affinity was confirmed in the present experiments using hepatocyte membranes which do not internalize insulin. As shown in Fig. 4-a, a significant difference in insulin binding to the membranes was seen at the low insulin concentration (≤ 10 ng/ml), with a less remarkable difference at the high concentration (≥ 25 ng/ml), suggesting an increase in the affinity constant and a less obvious increase in the receptor number. The un-

equivocal increase in the affinity constant and tendency toward the increase in the receptor number were also observed by Scatchard analysis with hepatocyte membrane (Fig. 4-b). This is similar to the findings in normal rats by Olefsky *et al.* (1978).

In our present studies, the Scatchard analysis gave a more concave curve in STZ rats than in normal rats. The ratio of K_e/K_f , which was defined as representing the potency of the cooperative effect (DeMeyts *et al.*, 1973), was increased in both intact hepatocytes and hepatocyte membranes from STZ rats. This suggests an increase in negative cooperativity in STZ rats. In fact, our dissociation study demonstrated that the dissociation of insulin in STZ rats was slower than in controls in the absence of insulin, but almost comparable to controls in the presence of insulin. These results are in accordance with the results of Scatchard analysis. It is interesting to note that the dissociation rate constant of insulin receptors from STZ rats was almost the same as that of control rats at the high concentration of insulin. This indicates that the decreased dissociation rate constant in STZ rats depends on the concentration of insulin.

As described above, we could ensure the increase in the number and affinity of insulin receptor on hepatocytes from STZ rat with two different methods. The increase in affinity was revealed to be due to a decrease in the dissociation rate constant by the dissociation study. An increase in negative cooperativity in the receptor from STZ rat found by Scatchard analysis was also shown directly by the dissociation study. These results suggest that we can use intact cells for the study of the receptor as long as we chose proper experimental conditions. But some factors, such as internalization of insulin and its receptor or degradation of insulin during binding studies, may affect the results obtained from Scatchard analysis in intact cells. In fact, the ratios of in-

ternalized insulin to cell associated insulin in both control and STZ rats were quite large and the difference between the ratios was significant. Using isolated rat hepatocyte, Gorden *et al.*, showed that 29% of the cell associated insulin (or insulin-receptor complex) was internalized at 20°C in 60 minute incubation (Gorden *et al.*, 1982). Their values as well as ours for the internalization are rather greater than those reported by Olefsky *et al.* (1982) in adipocytes and IM-9 lymphocytes. As suggested by Olefsky *et al.*, the degree of internalization may differ among the cells. They also said that only the intact surface bound (acid-extractable) fraction should be considered when analyzing insulin-binding data according to the method of Scatchard.

In addition to the internalization of insulin or its receptor, degradation of insulin is another important factor that affects the Scatchard analysis and it must be minimized. We used a low temperature (15°C) for this purpose. At this temperature, insulin degradation was less than 13% (8.4% for the mean) during the 2 hours incubation. Thus, the decrease in insulin binding to cells due to the decrease in intact insulin in the media was estimated to be less than 10% of the total binding. Bacitracin is usually used to inhibit the degradation of insulin by proteolytic enzymes. However, it was reported that bacitracin caused a rapid and marked increase in insulin binding to the plasma membrane of rat adipocyte (Hammons *et al.*, 1982). Hammons *et al.*, showed that the increase was due to the increase in the half-time of dissociation, suggesting the slowing of insulin dissociation. It means a decrease in the dissociation rate constant and an increase in the affinity constant. For these reasons we did not use bacitracin in our study.

An increase in insulin binding is a desirable change for insulin deficit animals such as STZ rat to use little insulin in their blood. But this change may not be due to

the regulation or adaptation to hypoinulinemia. Ronnett *et al.*, reported using the heavy isotope density shift method in which insulin does not affect the rate of appearance of the newly synthesized active receptor at the plasma membrane; rather the ligand increase the net rate of loss of receptor from the cell surface (Ronnett *et al.*, 1983). The increase in the number of receptors in STZ rat may therefore be only a result of a decrease in the degradation of the receptor. The increase in the affinity constant in STZ rat was revealed to be due to a decrease in the dissociation rate constant. But other mechanisms remain obscure. They may be explained by a decrease in receptor degradation. If a high affinity state (or site) receptor is bound to insulin and degraded faster than low affinity state (or site) receptor, the mean affinity will be decrease at high insulin concentrations due to a decrease in the affinity of the receptor. And mean affinity will be increased at low insulin concentrations. But a change in the affinity constant could be observed even at a membrane which do not internalize the receptor, suggesting that the affinity change in STZ rat is due to other mechanisms which mainly change the dissociation rate constant.

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References

- Bar, R., S. Gorden, J. Roth, C. R. Kahn and P. DeMeyts (1976). Fluctuation in the affinity and concentration of insulin receptors on circulating monocytes of obese patients.

- Effects of starvation, refeeding and dieting. *J. Clin. Invest.* **59**, 1123-1135.
- Bar, R., S. Gordon, J. Roth and C. W. Seibert (1977). Insulin receptors in patients with insulinomas: changes in receptor affinity and concentration. *J. Clin. Endocrinol. Metab.* **44**, 1210-1213.
- Berry, M. N. and D. S. Friend (1969). High-preparation of isolated rat liver parenchymal cells. A biochemical and fine structural study. *J. Cell Biol.* **43**, 506-520.
- Carpentier, J. L., P. Freychet, A. Le Cam and L. Orch (1979). Lysosomal association of internalized ^{125}I -insulin in isolated rat hepatocytes. *J. Clin. Invest.* **63**, 1249-1261.
- Cuatrecasas, P., B. Desbuquois and F. Krug (1971). Insulin-receptor interactions in liver cell membranes. *Biochem. Biophys. Res. Commun.* **44**, 333-339.
- DeFronzo, R., R. Sherwin, V. Soman, R. Hendler and P. Felig (1977). Dissociation of insulin binding to and insulin action in intact man during starvation and refeeding. *Clin. Res.* **25**, 494a.
- DeMeyts, P., J. Roth, D. Neviller Jr., J. R. Gavin and M. A. Lesniak (1973). Insulin interaction with its receptors: experimental evidence for negative cooperativity. *Biochem. Biophys. Res. Commun.* **55**, 154-161.
- DeMeyts, P. and J. Ross (1975). Cooperativity in ligand binding: A new graphic analysis. *Biochem. Biophys. Res. Commun.* **66**, 1118-1126.
- DeMeyts, P., A. R. Bianco and J. Roth (1976). Site-site interaction among insulin receptors. *J. Biol. Chem.* **251**, 1877-1888.
- Desbuquois, B. and G. D. Aurbach (1971). Use of polyethylene glycol to separate free and antibody bound peptide hormones in radioimmunoassays. *J. Clin. Endocrinol. Metab.* **33**, 732-738.
- Gorden, P., J. Carpentier, M. L. Moule, C. C. Yip and L. Orci (1982). Direct demonstration of insulin receptor internalization; a quantitative electron microscopic study of covalently bound ^{125}I -photoreactive insulin incubated with isolated hepatocytes. *Diabetes* **31**, 659-662.
- Haigler, H. T., F. R. Maxfield, M. C. Willingham and I. Pastan (1980). Dansylcadaverine inhibits internalization of I-epidermal growth factor in BALB 3T3 cells. *J. Biol. Chem.* **255**, 1239-1241.
- Hammons, G. T., R. M. Smith and L. Jarett (1982). Inhibition by bacitracin of rat adipocyte plasma membrane degradation of ^{125}I -insulin is associated with an increase in plasma membrane bound insulin and a potentiation of glucose oxidation by adipocytes. *J. Biol. Chem.* **257**, 11563-11570.
- Kobayashi, M. and M. Olefsky (1979). Effects of streptozotocin-induced diabetes on insulin binding, glucose transport and intracellular glucose metabolism in isolated adipocytes. *Diabetes* **28**, 87-95.
- Lowry, D. H., W. J. Rosebrough, A. L. Farr and R. J. Randall (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265-275.
- Muggeo, M., R. S. Bar and J. Roth (1977). Change in affinity of insulin receptor following oral glucose in normal adults. *J. Clin. Endocrinol. Metab.* **44**, 1206-1209.
- Neville, D. M. (1968). Isolation of an organ specific protein antigen from cell-surface membrane of rat liver. *Biochem. Biophys. Acta* **154**, 540-552.
- Okamoto, M., H. Kuzuya, Po-Feng Kuo and H. Imura (1982). Insulin receptors on erythrocytes and hepatocytes from streptozotocin induced diabetic rats. *Endocrinol. Japon.* **29**(5), 623-630.
- Olefsky, J. M., J. Johnson, F. Liu, P. Edwards and S. Baur (1975). Comparison of ^{125}I -insulin binding and degradation to isolated rat hepatocytes and liver membranes. *Diabetes* **24**, 801-810.
- Olefsky, J. M. and M. Kobayashi (1978). Mechanisms of the fasting-induced increase in insulin binding to rat adipocytes. *J. Clin. Invest.* **61**, 329-338.
- Olefsky, J. M., and M. Kao (1982). Surface binding and rates of internalization of ^{125}I -insulin in adipocytes and IM-9 lymphocytes. *J. Biol. Chem.* **257**, 8667-8673.
- Ronnett, G. V., G. Tennekoon, V. P. Knutson and M. D. Lane (1983). Kinetics of insulin receptor transit to and removal from the plasma membrane. *J. Biol. Chem.* **258**, 283-290.
- Scatchard, G. (1949). The attractions of protein for small molecules and ions. *Ann. N. Y. Acad. Sci.* **51**, 660-672.