Insulin Response to Glucagon with or without Tolbutamide in Normal and Diabetic Subjects

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CHIBA, M., OHNEDA, A., SAKAI, T. and GOTO, Y. *Insulin Response to Glucagon with or without Tolbutamide in Normal and Diabetic Subjects*. Tohoku J. exp. Med., 1982, 136 (2), 169-175 — In order to investigate insulin response to glucagon, eight normal subjects and thirty diabetic patients were studied. According to fasting blood glucose, diabetic patients were classified into three groups; mild, moderate and severe. An oral glucose tolerance test, a glucagon test and a tolbutamide-glucagon test were performed at intervals of several days. In the glucose tolerance test, insulin response was reduced in the patients with moderate or severe diabetes. Plasma insulin increased and reached a peak 3 min after glucagon injection (glucagon 1) in the normal controls ($66.1 \pm 9.9 \mu\text{U/ml}$), while plasma insulin response to glucagon 1 was reduced in the three diabetic groups (35.8 ± 7.3 , 33.2 ± 9.7 and $22.9 \pm 5.0 \mu\text{U/ml}$, respectively). In the normal subjects glucagon injected 60 min after tolbutamide (glucagon 2) caused a rise in insulin ($72.0 \pm 4.9 \mu\text{U/ml}$). In the diabetic groups, glucagon 2 caused a reduced response of plasma insulin (38.6 ± 8.9 , 49.5 ± 17.4 and $34.8 \pm 11.3 \mu\text{U/ml}$, respectively). Glucagon with or without tolbutamide produced a far greater maximal response of plasma insulin in all the diabetic groups than in the normal subjects, while glucose produced a not significantly different increment of plasma insulin between the normal subjects and the mild diabetics. The present study demonstrates the glucagon injection with or without tolbutamide could clearly discriminate the insulin response in diabetics from that in normal subjects. ——— insulin response; glucose tolerance test; glucagon test; tolbutamide-glucagon test

Glucose tolerance test has been clinically and widely used, in order to investigate insulin response in diabetes mellitus. However, it is difficult to discriminate diabetics from the normal subjects in view of insulin levels. In 1974, it was reported from our laboratory that the plasma insulin level 30 min after glucose load was significantly decreased in diabetes mellitus compared with the normal controls when glucose was given 60 min after tolbutamide injection (Ohneda et al. 1974). Furthermore, we reported preliminarily a decrease in the maximal insulin response in a few cases of diabetes mellitus when glucagon was administered 60 min after tolbutamide injection (Ohneda et al. 1975). Therefore, the present study was undertaken in order to investigate the responses of plasma

insulin to glucagon with or without tolbutamide and to compare those to glucose in normal and diabetic subjects.

SUBJECTS AND METHODS

In this study, eight healthy students and 30 diabetic subjects were investigated. The age of the subjects ranged from 29 to 69 years. In each group, an oral glucose tolerance test (GTT) was followed by an intravenous glucagon test (GT) and a tolbutamide-glucagon test (TGT) at intervals of several days. In GTT, 50 g glucose was administered orally after an overnight fast and blood samples were drawn at 30 min intervals for 2 hr, as described previously (Goto et al. 1960). In GT, after two baseline samples were drawn, 1 mg of glucagon dissolved in 1 ml of saline solution, which was donated by Novo Institute, was injected into the cubital vein within 10 sec and blood samples were obtained from the antecubital vein at the other side at 1, 3, 6, 10, 15, 20, 30, 45, 60, 90 and 120 min.

In TGT, after two base line samples were drawn, 1 g tolbutamide in 5% solution (Rastinon,* Hoechst Japan) was injected into the antecubital vein for 2 min and blood samples were drawn 3, 6, 10, 20, 30, 45 and 60 min after the start of the injection. Just after drawing blood samples at 60 min, 1 mg of glucagon was injected intravenously and blood samples were obtained at the same protocol as in GT.

A diagnosis for diabetes mellitus was made by the recommendation of Japan Diabetic Society (Kuzuya 1970).

Thirty diabetic patients were divided into three groups according to the fasting levels of blood glucose, mild, moderate and severe diabetes mellitus. The mild diabetes (10) was classified by the fasting blood glucose lower than 120 mg/100 ml, moderate diabetes (10) between 120 and 200 mg/100 ml and severe diabetes (10) higher than 200 mg/100 ml.

Capillary blood was obtained from the ear lobe and blood glucose was determined by the glucose oxidase method (Teller 1956). Blood obtained from the antecubital vein into the heparinized syringes was poured into the glass tubes chilled in an ice box. The blood was centrifuged immediately after the completion of the test and plasma was stored at -20°C until assay. Plasma insulin was determined by the Morgan-Lazarow method (1962).

Data in the study are presented as the mean $\pm$ S.E. and the statistical analyses were performed by Student's *t* test.

RESULTS

Glucose tolerance test

The mean levels of blood glucose and plasma insulin during GTT in the normal subjects and the diabetic patients are presented in Fig. 1. In the normal controls, blood glucose rose from the fasting level of 87.4 ± 1.8 mg/100 ml and reached a peak of 152.4 ± 4.1 mg/100 ml at 30 min. Then blood glucose fell to the initial level at 120 min. Plasma insulin during GTT in the normal subjects increased from the fasting level of 7.5 ± 1.3 U/ml and reached a peak level of 64.9 ± 12.6 U/ml at 60 min, returning to the initial level at 120 min.

The fasting level of blood glucose in the mild, moderate and severe diabetics were 105.1 ± 2.4 , 155.4 ± 4.0 and 220.1 ± 11.4 mg/100 ml, respectively. Blood glucose after oral glucose load was 228.9 ± 13.4 at 60 min in mild diabetes, 310.0 ± 11.6 at 90 min in moderate diabetes and 369.7 ± 14.2 mg/100 ml at 90 min in severe diabetes.

Insulin response to glucose was reduced in the diabetics, as glucose tolerance

Fig. 1

Fig. 1. Blood glucose and plasma insulin during the oral glucose tolerance test in normal and diabetic subjects (mean $\pm$ S.E.). $\bullet$ — $\bullet$, normal subjects ($N=8$); $\circ$ — $\circ$, mild diabetes ($N=10$); Δ — Δ , moderate diabetes ($N=10$); $\times$ — $\times$, severe diabetes ($N=10$).

Fig. 2

Fig. 2. Blood glucose and plasma insulin during the intravenous glucagon test in normal and diabetic subjects (mean $\pm$ S.E.). $\bullet$ — $\bullet$, normal subject ($N=8$); $\circ$ — $\circ$, mild diabetes ($N=10$); Δ — Δ , moderate diabetes ($N=10$); $\times$ — $\times$, severe diabetes ($N=10$).

was decreased. The fasting levels of plasma insulin in the mild, moderate and severe diabetics were 9.8 ± 1.7 , 5.3 ± 1.0 and 8.2 ± 2.6 $\mu\text{U/ml}$, respectively. The maximal levels of plasma insulin during GTT were 42.2 ± 7.2 $\mu\text{U/ml}$ in mild diabetes, 18.2 ± 3.4 $\mu\text{U/ml}$ in moderate diabetes and 14.9 ± 4.4 $\mu\text{U/ml}$ in severe diabetes.

The maximal increment of plasma insulin was 55.5 ± 13.0 $\mu\text{U/ml}$ in the normal subjects and those in mild, moderate and severe diabetes were 39.1 ± 7.9 , 16.4 ± 4.3 and 9.4 ± 2.7 $\mu\text{U/ml}$, respectively.

Glucagon test

The changes in blood glucose and plasma insulin after the intravenous injection of glucagon are shown in Fig. 2. Blood glucose rose from the fasting level of 91.4 ± 1.2 $\text{mg}/100$ ml to a peak level of 143.9 ± 2.2 $\text{mg}/100$ ml at 20 min after glucagon injection in the normal subjects, returning to the initial level at 45 min. Plasma insulin rose from the base line level of 6.6 ± 1.6 $\mu\text{U/ml}$ and reached a peak level of 66.1 ± 9.9 $\mu\text{U/ml}$ at 3 min.

In three groups of the diabetics, blood glucose rose after the glucagon injection and reached a peak later than in the normal subjects. In addition, response of

plasma insulin to glucagon was reduced in all the diabetic groups. In mild diabetes, blood glucose rose from the fasting level of 97.6 ± 9.2 and reached a peak level of 174.9 ± 7.3 mg/100 ml at 30 min. Plasma insulin increased from the fasting level of 9.9 ± 1.8 μ U/ml to a peak of 35.8 ± 7.3 μ U/ml at 3 min. In moderate diabetes, blood glucose rose from the base line of 146.4 ± 4.6 to a peak of 214.3 ± 6.1 mg/100

Fig. 3. Blood glucose and plasma insulin after tolbutamide followed by glucagon in normal and diabetic subjects (mean $\pm$ S.E.). $\bullet$ — $\bullet$, normal subjects ($N=8$); $\circ$ — $\circ$, mild diabetes ($N=10$); Δ — Δ , moderate diabetes ($N=10$); $\times$ — $\times$, severe diabetes ($N=10$).

ml at 30 min. Plasma insulin increased from the base line level of 8.5 ± 1.8 μ U/ml to a peak level of 33.2 ± 9.6 U/ml at 3 min. In severe diabetes, blood glucose rose from the fasting level of 212.7 ± 14.7 mg/100 ml to a peak level of 261.7 ± 15.6 mg/100 ml. The increase in plasma insulin was minimum in the group of severe diabetes, the peak insulin level showing 22.9 ± 5.0 μ U/ml at 3 min.

Tolbutamide-glucagon test

The changes in blood glucose and plasma insulin in TGT are shown in Fig. 3. In the normal controls, blood glucose decreased from the fasting level of 87.9 ± 2.7 mg/100 ml to the nadir of 58.4 ± 3.2 mg/100 ml at 30 min after tolbutamide injection. Plasma insulin rose from the fasting level of 6.3 ± 0.7 μ U/ml to a peak of 69.4 ± 13.1 μ U/ml at 6 min, and then fell to the initial level at 60 min. After 1 mg of glucagon was injected at 60 min, blood glucose rose from the preinjection level of 76.1 ± 5.7

mg/100 ml to a peak of 125.8 ± 2.8 mg/100 ml at 80 min and returned to the initial level at 105 min. Plasma insulin rose after glucagon injection from the initial level of 10.4 ± 0.6 μ U/ml to the peak level of 72.0 ± 4.9 U/ml at 70 min.

In these three diabetic groups, blood glucose continued to fall gradually until the time of 60 min after the tolbutamide injection, and the glucagon injection induced the same increment of blood glucose as that in the normal controls. In mild diabetes, blood glucose decreased from the fasting level of 104.8 ± 4.2 to the nadir of 71.8 ± 3.9 mg/100 ml at 60 min. After the glucagon injection blood glucose rose and reached a peak of 131.8 ± 4.4 mg/100 ml at 80 min. In moderate diabetes, blood glucose fell from the fasting level of 148.0 ± 3.0 to the level of 120.0 ± 3.5 mg/100 ml at 60 min. Blood glucose increased to a peak of 164.8 ± 2.4 mg/100 ml at 75 min after the glucagon injection. In severe diabetes, blood glucose decreased from the fasting level of 239.7 ± 20.9 to the nadir of 210.1 ± 18.2 mg/100 ml at 60 min. After the glucagon injection blood glucose rose and reached 260.2 ± 22.9 mg/100 ml at 90 min.

The responses of plasma insulin to either tolbutamide or glucagon were lowered remarkably in the three diabetic groups. The maximal levels of plasma insulin after tolbutamide injection in mild, moderate and severe diabetes were 29.5 ± 3.6 , 19.7 ± 4.7 and 13.8 ± 2.4 μ U/ml, respectively. The increments of plasma insulin after glucagon were also markedly reduced in all the diabetic groups, compared with the normal subjects. The maximal levels of plasma insulin in mild, moderate and severe diabetes were 38.6 ± 8.9 , 49.5 ± 17.4 and 34.8 ± 11.3 μ U/ml, respectively.

Comparison of maximal response of insulin to various stimuli

The maximal responses of plasma insulin to various stimuli in the normal subjects and the diabetic patients are shown in Fig. 4. The maximal insulin response to glucose decreased in all the diabetic groups but a significant difference

Fig. 4. Comparison of maximal insulin increment after glucose glucagon or tolbutamide in normal and diabetic subjects (mean $\pm$ s.e.).

was observed only in moderate and severe diabetes in comparison with the normal subjects ($p < 0.05$ and $p < 0.05$, respectively).

The maximal levels of plasma insulin after a single injection of glucagon (Glucagon I) decreased significantly in all the diabetic groups compared with the normal controls ($p < 0.05$ at largest). The maximal levels of plasma insulin after the glucagon injection in TGT (Glucagon II) were also significantly decreased in all the diabetic groups ($p < 0.025$ or less).

The maximal levels of plasma insulin after the tolbutamide injection were reduced significantly in all the diabetic groups compared with the normal subjects ($p < 0.025$ or less).

DISCUSSION

Since the immunoassay for insulin was introduced, various stimuli have been proved to promote insulin secretion. However, there is no definite difference between normal subjects and diabetic patients as far as the fasting level of plasma insulin is concerned (Yalow and Berson 1960; Ohneda 1966). In order to discriminate diabetics from normal subjects, various stimuli including glucose have been employed. Insulin response to glucose, however, is not differentiated between normal and diabetic subjects (Yalow and Berson 1960). This is true even when the maximal insulin level and insulinogenic index during glucose tolerance test are compared (Sato 1974).

In the present study, it was observed that the injection of 1 mg of glucagon caused the same increment of plasma insulin as in oral glucose load or tolbutamide injection in the normal subjects. However, the mode of insulin response to glucagon differs from that of the response to other stimuli in diabetic patients. In general, glucagon injection, especially with tolbutamide, induced a larger increment of the plasma insulin than oral glucose load or tolbutamide injection, although this difference was not significant.

As far as the maximal insulin response is concerned, glucose load could not discriminate the mild diabetes from the normal subjects, whereas glucagon (with or without tolbutamide) or tolbutamide could do.

Therefore, glucagon I, glucagon II or tolbutamide is superior to oral glucose in view of the response of plasma insulin in normal and diabetic subjects.

Ryan et al. (1967) proposed the intensive stimulation of B cell of the pancreatic islet, in which 1 mg glucagon and 0.5 g tolbutamide were injected 30 min after oral glucose load. According to their study, an excessive response of plasma insulin was observed in mild diabetes, whereas severe diabetics showed a decreased response. In the test proposed by Ryan et al., glucagon and tolbutamide promote insulin secretion from the B cells in the pancreas, where the synthesis and secretion of insulin are elevated by glucose administered previously. Therefore, their method elucidates the maximal intensive stimulation in both synthesis and release.

To the contrary, the tolbutamide-glucagon test, shown in the present study, consists of the enhanced release of insulin by tolbutamide and glucagon and would elicit the insulin reservoir in the B cells.

At any rate, the present study demonstrates that glucagon with or without tolbutamide could clearly discriminate the insulin response in diabetics from that in normal subjects. Therefore glucagon is a useful tool in the investigation of insulin response in man.

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