A STUDY OF SOME ASPECTS OF PLASMA FREE INSULIN-LIKE ACTIVITY

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Leubuer (10) reported that tolbutamide gives more gratifying results when given in single daily doses than when given in divided doses. This was in confirmation of the conclusions of Pfeiffer *et al.* (13) that following tolbutamide, degranulatory phase in the β-cells of Langerhans was followed by the refractory phase during which time the cells were resistant to further stimuli.

The method of Randle (15) for the assay of plasma insulin-like activity has been well established in our laboratory (9, 11, 12). The advantage of this method is that insulin antagonists which bind materials with insulin-like activity can be released by dilution. Moreover, the method estimates free insulin-like activity (FILA). An attempt was, therefore, made to study the effect of tolbutamide and/or glucose on FILA of plasma in mild diabetics. The effect of a second dose of tolbutamide given 12 hr after the first dose was also examined.

Since phenformin has no action in normal subjects but lowers blood glucose level in diabetic subjects (16), an attempt was made to examine the influence of phenformin on the blood glucose level of relatives of proved diabetics.

Another object of the present study though not related to the above was to see if plasma FILA was affected by pregnancy.

MATERIALS AND METHODS

The plasma free insulin-like activity (FILA) was determined in normal persons, diabetic patients, relatives of diabetic patients, in pregnant women and following delivery. The diabetic patients were selected from the Diabetic Clinic, S. S. G. Hospital, Baroda. Pregnant women (10 days before expected delivery) were selected from the Antenatal Clinic of S. S. G. Hospital, Baroda.

Diabetic patients were admitted to the wards. These patients were either of medium or obese build which was assessed according to the criteria of the Life Insurance Corporation of India. They were either recent diabetics or had not taken any type of treatment. In case of doubt about previous medication, 4 days were allowed before withdrawing blood sample.

The venous blood was collected in sterilized, heparinised syringes and the plasma was separated. Then plasma glucose level was estimated by the method of Folin-Malmros (7). To the

plasma, glucose (Analar) was added to make a final concentration of 300 *mg*% if plasma glucos level was less than 300 *mg* and then it was stored at 4°C. Plasma free insuline-like activity was esti mated by the rat diaphragm method of Randle (15) as described earlier (9, 11, 12).

Crystalline insulin powder (Boots Pure Drug Co., Bombay) was used as the standard solution for the insulin assay.

Details of drug administration are given at appropriate places under results.

The rats used for the assay of plasma FILA were obtained from Hindustan Antibiotics Ltd., Pimpri and were all of the same strain.

RESULTS

Effect of glucose and tolbutamide on plasma glucose and plasma FILA of diabetic patients:

Table I shows plasma free insulin-like activity (FILA) and plasma glucose level in 10 mild obese diabetic patients given glucose and tolbutamide. The mean plasma glucose level and plasma FILA two hr after oral glucose load (100 *g*) were higher than the corresponding mean fasting values but the rise was not significant ($p>0.05$). The patients were given tolbutamide (2 *g*) orally after withdrawing the postprandial blood sample. Three hr later there was a significant ($p<0.05$) fall in plasma glucose level. There was a rise in plasma FILA but the rise was not significant ($p>0.05$). However, the value of mean plasma FILA was significantly ($p<0.05$) higher than the fasting value.

TABLE I: *Effects of tolbutamide and/or glucose on the plasma glucose and plasma free insulin-like activity of diabetic patients.*

Procedure (s)			*Mean plasma glucose level (mg% ± S.D.)*	*Mean plasma free insulin-like activity (μU/ml ± S.D.)*
(a)	(i)	Fasting	127.8±48.0	743.5±551.0
	(ii)	Two hr following 100 *g* glucose orally	236.0±82 p>0.05	1224.5±716.5 p>0.05
	(iii)	Three hr following tolbutamide administered orally immediately after post-prandial withdrawal of blood sample	88.0±35.5 p<0.05	1776.0±728.0 p>0.05
(b)	(i)	Fasting	109.0±35.0	861.0±611.0
	(ii)	Two hr following 100 g glucose orally	238.0±83.5 p<0.05	1294.0±738.0 p>0.05
	(iii)	One hr following 1 *g* tolbutamide administered (i/v) immediately after post-prandial withdrawal of blood sample	140.0±58.0 p<0.05	1772.0±739.0 p>0.05
(c)	(i)	Fasting	104.4±37.0	830.0±638.0
	(ii)	One hr following 1 *g* tolbutamide administered (i/v) on the morning of 10th day immediately after collecting the fasting blood sample.	83.9±26.5 p>0.05	1276.0±630.5 p>0.05
	(iii)	Immediately after the withdrawal of blood sample, the patient were given 100 g glucose orally and blood samples withdrawn 2 hr later	171.6±67.5 p<0.05	1905.0±892.5 p>0.05

The experiments were begun at (a). Following this, the patients were given tolbutamide (2 *g*) orally daily in the evening for 10 days. The procedures described under (b) were carried out on the 8th day and those described under (c) were carried out on the 10th day.

The patients were given tolbutamide (2*g*) orally daily in the evening for 10 days. On the 8th day the mean plasma glucose level 2 hr after a 100 *g* glucose load orally was significantly ($p<0.05$) higher than the mean fasting plasma glucose level; but the mean plasma FILA though apparently higher was not significantly ($p>0.05$) raised. These subjects received 1*g* tolbutamide intravenously immediately after withdrawing the postprandial blood sample. There was a significant ($p<0.05$) fall in mean plasma glucose level but the mean plasma FILA was not significantly ($p>0.05$) raised. However, the rise in plasma FILA was significant ($p<0.05$) in relation to the fasting level.

On the 10th day patients received tolbutamide (1 *g*) intravenously. One hr after the administration of tolbutamide there was a fall in mean plasma glucose level and a rise in mean plasma FILA . However, the fall and rise in mean plasma glucose level and mean plasma FILA respectively were not statistically significant ($p>0.05$). Immediately after withdrawing blood, the patients were given 100 *g* glucose orally. Two hr after glucose load there was a significant ($p < 0.05$) rise in mean plasma glucose level but the rise in mean plasma FILA was not significant ($p<0.05$). However, the rise in plasma FILA was significant in ($p<0.05$) in relation to the fasting level.

The rise in mean plasma FILA 1 hr after tolbutamide intravenously and 2 hr after glucose subsequently was 54% and 49% respectively.

The rise in mean plasma FILA 2 hr after glucose load and 1 hr after tolbutamide intravenously was 50% and 54% respectively.

After glucose load on day 1, day 8 and day 10, the rise in mean plasma glucose level was 118%, 84% and 65% respectively.

Effect of phenformin on plasma glucose and plasma FILA in normal subjects:

The effect of phenformin on plasma glucose and plasma FILA was examined in 10 normal subjects before and after the administration of glucose intravenously (50 *ml*, 50%). The subjects received phenformin (100 *mg*) 24 hr after the control test. Three hr after the administration of phenformin the fasting mean plasma glucose level and mean plasma FILA were not significantly different ($p>0.05$) from the respective control mean fasting values obtained 24 hr earlier. Similarly the post-glucose mean plasma glucose level and mean plasma FILA were not significantly different ($p>0.05$) from the corresponding mean post-glucose control values obtained 24 hr earlier. The results are summarized in Table II.

Effect of phenformin on plasma glucose and plasma FILA in the relatives of diabetic patients:

The effect of phenformin on the plasma glucose level and plasma FILA was examined in 10 relatives of diabetic patients. The fasting and post-glucose plasma, glucose and FILA were esti-

mated as outlined above for the normal subjects. The subjects received phenformin 100 *mg* orally 24 hr after the control test. Three hr after the administration of the phenformin the fasting mean plasma gucose level and mean plasma FILA were not significantly different ($p>0.05$) from the corresponding fasting mean control values obtained 24 hr earlier. Similarly following phenformin the post-glucose mean plasma glucose level and mean plasma FILA were not significantly different ($p>0.05$) from the respective mean control post-glucose values obtained 24 hr earlier. The results are summarized in Table II.

TABLE II: *Effects of phenformin and/or glucose on the plasma glucose and plasma free insulin-like activity.*

		Normal subjects		*Relatives of proved diabetic subjects*	
Procedure (s)		*Mean plasma glucose level (mg% ± S.D.)*	*Mean plasma free insulin-like activity (μU/ml ± S.D.)*	*Mean plasma glucose level (mg% ± S.D.)*	*Mean plasma free insulin-like activity (μU/ml ± S.D.)*
(i)	Fasting	94.8 ± 17.3	1660.5 ± 650.5	86.3 ± 14.0	1740 ± 565.5
(ii)	One hr following glucose (50 *ml*, 5% iv)	104.7 ± 13.8 p>0.05	2518.8 ± 650.5 p>0.05	97.0 ± 22.5 p>0.05	2673.8 ± 563.0 p>0.05
(iii)	Three hr following phenformin (100 *mg* orally) administered next morning	94.0 ± 13. 3 p>0.05	1679.0 ± 662.5 p>0.05	87.5 ± 11.5 p>0.05	1742.3 ± 546.5 p>0.05
(iv)	Immediately following withdrawal of blood as described under (iii), glucose (50 *ml*, 50% iv) was given and blood sample withdrawn one hr later	102.0 ± 13.4 p>0.05	2508.5 ± 607.0 p>0.05	101.8 ± 18.5 p>0.05	2576.7 ± 562.0 p>0.05

It is however, very interesting to note that in two subjects the plasma glucose levels were reduced by a single dose of phenformin. The fasting plasma glucose levels before phenformin in these two subjects were 95% and 120% and those follwing phenformin were 72% and 80% respectively.

Effect of pregnancy on plasma glucose and plasma FILA:

The fasting plasma glucose level and plasma FILA were estimated in 10 pregnant women 10 days before expected delivery and 10 days after delivery. The fasting mean plasma glucose level (87.9 ± 11.0 *mg%*) and mean plasma FILA (1544.6 ± 652.0 *μU/ml*) after delivery were not significantly different ($p > 0.05$) from the corresponding mean blood glucose 93.7 ± 9.5 *mg%*) and mean plasma FILA (1695.5 ± 673.0 *μU/ml* levels) obtained during the pregnancy period.

DISCUSSION

The fasting mean plasma FILA of maturity onset diabetic subjects (743.5 ± 551 *μU/ml*)

was lower than that of normal control subjects (1660.0 ± 650 $\mu U/ml$). This agrees with the data reported earlier from our laboratory (12) and by other workers (5, 18).

Oral glucose load (100 g) increased the plasma FILA by 64% in our diabetic subjects (1224.0 ± 776.0 $\mu U/ml$); however, this was still less than the control FILA (1660.0 ± 650.0 $\mu U/ml$).

Reports on the post-glucose rise in FILA in the normal subjects show marked variations in the response. The variations could be attributed to differences in absorption and the interval between the injection of glucose and the time of blood collection. A 20% post-glucose rise in FILA of normal subjects at 2 hr (from 2575.8 ± 761 to 3213 ± 1146.3) was reported previously from this laboratory (11). Bajaj and Yadav (4) described a 50% rise. Others (5,8) have reported to 10-fold rise.

The administration of tolbutamide orally following glucose orally (immediately after withdrawal of postprandial blood sample) on the first day, was effective in increasing further the plasma FILA. This rise in FILA was similar to that reported by Pfeiffer *et al* (13) in diabetic patients.

The rise in plasma FILA after glucose was approximately equal to that produced by tolbutamide. A combination of glucose and tolbutamide produced a statistically significant ($p<0.05$) rise in plasma FILA (from 743.5 ± 551 to 1776 ± 728). A similar response was obtained on the eighth day and the tenth day. It is clear that although glucose or tolbutamide singly produced an inadequate insulinogenic response, the two together produced an adequate response in the sense that the FILA now was within the range observed in normal subjects.

Despite increased blood glucose level, insulin appears to circulate primarily as an inactive complex in the blood of diabetic subjects (1). Since insulin is secreted from the pancreas in the "free" active state, some transformation to the "bound" form must occur in the blood and tissues. Liver, adipose and other tissues may participate both in catalyzing the binding of free insulin and in dissociating the insulin complex according to the metabolic demands (2). Malfunction of this mechanism could, therefore, result in lack or in increase of either from circulating insulin. This may be associated with excessive binding of free insulin or inability in dissociating and utilising the insulin complex (3) and could explain the low plasma FILA observed in the present study.

The administration of tolbutamide could affect the relative amount of free insulin and insulin complexes in the blood of diabetic patients (3). Thus since tolbutamide has a dual action, i.e. sitmulates the release of insulin from the pancreas and also increases the conversion of inactive form to the active form, the increased plasma FILA observed in the present study becomes immediately understandable.

Following the first intravenous test dose of tolbutamide, the patients were given the drug daily orally for 10 days. On the 8th day, the patients were given a glucose load followed by tolbutamide intravenously. The rise in plasma FILA was not different from that on the first day, indicating that even after 12 hr of the previous dose, the β-cells could be stimulated. On the 10th

day the procedure of the 8th day was repeated, but the order of administration was reversed. Again, there was a rise in plasma FILA, further strengthening the conclusion that the β-cells are capable of being stimulated. The effect on the blood sugar level after the administration of tolbutamide provides additional support. However, the conclusions are in contrast to those of Pfeiffer *et al.* (14) who found that the degranulatory phase was followed by a refractory phase during which time the β-cells did not respond to any further stimuli. Leubner (10) also supports the conclusions of Pfeiffer *et al.* (14). Leubner (10) concluded that tolbutamide gives more gratifying results when given in single daily doses than when administered in divided doses.

In normal subjects the plasma FILA and blood glucose level after the administration of phenformin were not different from those of the control values. This would indicate that in normal persons phenformin is ineffective either to release insulin from the pancreas or to lower the blood sugar level by other possible mechanisms. It may be mentioned here that Creutzfeldt and Moench (6) have suggested that phenformin does not stimulate β-cells. Searle and Cavalieri (16) have reported that the glucose level was unaffected in the normal subject after phenformin, though the drug increases the turnover rate of blood glucose and glucose pool significantly by anerobic glycolysis. In normal subjects the increased peripheral utilization of glucose by phenformin is counter-balanced by an equal increase in the rate of glucose release by the liver (16). Thus the blood glucose level of the normal person is unaffected by the administration of phenformin. In diabetic subjects glucose production is near maximal and increased glucose utilization can cause hypoglycaemia during phenformin therapy (16). This prompted us to examine the effect of phenformin in the relatives of diabetics.

Eight of the 10 relatives of diabetic patients showed a pattern similar to that of the normal subjects. However, two subjects showed hypoglycaemic response after the administration of phenformin. There was no change in plasma FILA in these two subjects. This hypoglycaemic response induced by phenformin in the relatives of the diabetic patients suggests that phenformin could be used as a tool to detect the pre-diabetic state. Study in large number of relatives of diabetic patients and the follow up of the "suspects" would necessarily be a prelude to the general acceptibility of the test.

The blood sugar level and plasma FILA of the pregnant women were reduced after delivery, but the reduction was not significant ($p > 0.05$). Welsh and Sims (17) have reported that insulin level is increased during pregnancy and that it comes down to normal level after the termination of pregnancy.

SUMMARY

(1) The mean plasma glucose level and the mean plasma free insulin like activity (FILA) two hr after oral glucose load (100 *g*) were raised in 10 mild obese diabetic patients, but the rise was not significant ($p > 0.05$). The patients were given tolbutamide (2 *g*) orally after withdrawing the postprandial blood sample. Three hr later there was a significant ($p < 0.05$) fall in plasma glucose level. There was a rise in plasma FILA but the rise was not significant ($p > 0.05$). The patients

were given tolbutamide once daily for 10 days. On the 8th day the mean plasma glucose level 2 hr after a 100 *g* glucose load orally was significantly ($p < 0.05$) higher than the mean fasting level but the mean plasma FILA though apparently higher, was not significantly ($p > 0.05$) raised. These subjects received 1 g tolbutamide intravenously immediately after withdrawing the blood sample. There was a significant ($p < 0.05$) fall in mean plasma glucose level but the mean plasma FILA was not significantly ($p > 0.05$) raised. On the 10th day one hr after tolbutamide 1 *g* intravenously, there was a fall in mean plasma glucose level and rise in mean plasma FILA. However, both the fall and rise were not significant ($p > 0.05$). Immediately after withdrawing blood, glucose (100 *g*) was given orally. Two hr later there was a significant ($p > 0.05$) rise in mean plasma glucose level and a rise in mean plasma FILA which was however not significant ($p > 0.05$).

The effect of phenformin was examined on plasma glucose and plasma FILA in 10 normals subjects and 8 relatives of diabetic subjects before and after the administration of glucose intravenously (50 *ml.*, 50%). Phenformin had no effect on mean plasma glucose and mean plasma FILA of normal subjects as well as relatives of diabetics both before and after the administration of glucose. However, in two of the relatives of diabetics, plasma glucose levels were reduced by phenformin.

The fasting mean plasma glucose level and mean plasma FILA in pregnant women 10 days before expected delivery and 10 days after delivery were not significantly different ($p > 0.05$) from each other.

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