Variations in Synalbumin Insulin Antagonism during Tolbutamide Tolerance Test

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Summary. Albumin was extracted from plasma and serum taken during intravenous Tolbutamide tolerance tests in 18 healthy volunteers and tested at a concentration of 2.5 g per 100 ml for insulin antagonism. A significant rise in insulin antagonism was observed after 30 and 60 min, falling to the initial level at 90 min following Tolbutamide injection. These changes were inversely related to the corresponding blood sugar levels.

Variations dans l'antagonisme de la synalbumine vis-à-vis de l'insuline au cours de tests de tolérance au tolbutamide

Résumé. L'albumine a été extraite du plasma et du sérum prélevé au cours de tests de tolérance au tolbutamide intraveineux chez 18 volontaires en bonne santé, et testée à une concentration de 2.5 g pour 100 ml pour y déceler l'antagonisme à l'insuline. Une augmentation significative de l'antagonisme à l'insuline a été observée

après 30 et 60 min, le taux initial était atteint 90 min après l'injection de tolbutamide. Ces modifications étaient inversement proportionnelles aux taux correspondants de la glycémie.

Schwankungen des Synalbumin-Insulin-Antagonismus während des Tolbutamid-Toleranz-Tests

Zusammenfassung. Bei 18 stoffwechselgesunden Freiwilligen wurde Albumin aus während eines Tolbutamid-Tests entnommenen Plasma- und Serumproben extrahiert und in einer Konzentration von 2,5 g% auf Insulin-Antagonismus getestet. Nach 30 und 60 min fand sich ein signifikanter Anstieg des Insulin-Antagonismus und 90 min nach der Tolbutamidgabe ein Abfall auf die Ausgangswerte. Die Veränderungen verhielten sich umgekehrt proportional zu den jeweiligen Blutzuckerspiegeln.

Key-words: Antagonism, insulin, rat diaphragm, synalbumin, tolbutamide.

Introduction

Patients with 'essential' or idiopathic diabetes mellitus have increased insulin antagonism associated with their plasma albumin — an exaggeration of that also found in the albumin fraction from healthy people [11]. In the physiological range of concentration (3.5—5.5 g per 100 ml), both diabetic and normal albumin completely inhibit the effect of 1000 microunits per ml of insulin added *in vitro* to the incubation medium [13]. At 1.25 g per 100 ml however, the albumin from essential diabetics as well as those constituted as diabetics, is still highly antagonistic (synalbumin positive), whereas albumin prepared from healthy subjects does not exhibit insulin antagonism (synalbumin negative). Recent *in vivo* studies lend further support to these observations [7, 8].

Synalbumin insulin antagonism changes during oral and intravenous glucose tolerance tests, the antagonism being inversely proportional to the glucose levels in the blood [6]; which suggests that the effectiveness of any circulating insulin is modified by alterations in synalbumin antagonism which in turn are mediated through changes in blood sugar. In previous studies the blood glucose rose initially; accordingly we have studied intravenous tolbutamide tolerance tests where the immediate response is a fall in glucose levels.

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Volunteers and Methods

Eighteen healthy doctors and laboratory personnel, aged 20—27, were studied after a twelve hour fast. After withdrawing fasting blood sample in a heparinised syringe, 20 ml of 5% sodium tolbutamide was injected intravenously during a two minute period; the midpoint of injection was considered as zero minute and subsequent blood samples were collected at thirty, sixty and ninety minute intervals. The subjects were rested for the duration of the test. The glucose concentration was estimated in all samples. Albumin was extracted from the plasma by a modification [13] of the Debro method [2].

In two volunteers, blood samples were collected in nonheparinised dry syringes and after taking adequate aliquots for glucose estimation, blood was allowed to clot. Serum was separated and albumin was prepared by the Fernandez method [4] which does not require the use of trichloroacetic acid or dialysis; albumin prepared by this method has previously been found to be equipotent to that prepared by the Debro method, regarding insulin antagonism [18].

Insulin antagonism was determined by the rat-diaphragm procedure [15, 16]. Albumin extracts were dissolved in buffer solution [5] containing glucose (300 mg per 100 ml); insoluble matter was removed by centrifugation. Insulin was added to the buffer alone and to the buffer containing 2.5 g albumin per 100 ml to make a final concentration of 1000 microunits per

ml. This concentration of albumin is the same as that used in the previous study of synalbumin changes during glucose tolerance test [6]. The glucose uptake of the diaphragms was determined in: [1] buffer alone; [2] buffer and insulin; and [3] buffer and 2.5% albumin in the presence of insulin. All the samples from one tolbutamide test were assayed in any one experiment, thereby facilitating the comparison between antagonistic effects of samples taken during the same test.

Fig. 1. Effect of insulin on the glucose uptake of the rat diaphragm in the presence of albumin samples tested at 2.5% taken during 18 intravenous tolbutamide tolerance tests, with the corresponding blood glucose values

Results

Figure 1 shows the mean values for the effect of insulin in the presence of 2.5 g albumin per 100 ml, extracted at various time intervals after tolbutamide administration. In all the volunteers, the insulin effect on the diaphragm showed a significant decrease at 30 min; this coincided with the peak hypoglycaemic response in the subjects. Clinical symptoms of hypoglycaemia were noticed in five individuals; their blood glucose concentration ranged between 27–40 mg% at this point. The increased antagonism at 30 min returned to the pre-tolbutamide levels at 90 min. There was no difference in these results when the albumin was extracted by the Fernandez method, therefore all the results have been given together in the figure.

Discussion

In healthy volunteers, the level of synalbumin insulin antagonism varies considerably and consistently after intravenous administration of sodium tolbutamide; it increases significantly thirty minutes after

the injection and subsequently tends to fall to the initial fasting levels by 90 min.

In contrast to these findings, Devrim *et al.* [3] in six normal subjects found that the increased antagonism at 30 min after tolbutamide continued to rise up to 120 min and suggested that this was due to the rise in plasma insulin following intravenous tolbutamide. However, glucose also causes a rise in plasma insulin but with a fall in antagonism [6]. Thus from this observation and the studies reported here the changes in synalbumin are related inversely to the blood sugar.

There is considerable evidence that the synalbumin antagonist is identical with the B Chain of insulin [12] and liver contains a high concentration of glutathione insulin transhydrogenase which breaks the insulin molecules into its component A and B Chains [9, 10]. Moreover, hypophysectomy [14] and adrenalectomy [17] are known to reduce the level of synalbumin antagonism and growth hormone stimulates glutathione insulin transhydrogenase [1]. Thus the short term variations in the level of synalbumin antagonism after tolbutamide as well as those previously reported after glucose administration could well be due to changes in pituitary-adrenal activity which in turn is governed by acute fluctuations in blood sugar.

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