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Effects of the Synalbumin Insulin Antagonist in vivo; its Examination with a New Method

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Summary. A new method was elaborated for examination *in vivo* of the synalbumin insulin antagonist. It was shown that the glucose concentration of incubated liver homogenates prepared from untreated rats increased with time. Previous intravenous insulin administration prevented this increase. If insulin was injected together with albumin prepared from the serum of diabetic patients, this effect of insulin was antagonized.

Effets in vivo de l'antagnoniste de l'insuline, la synalbumine; Etude par une méthode nouvelle

Résumé. Un nouvelle méthode à été mise au point pour l'examen *in vivo* de l'effet antagoniste de la synalbumine, vis-à-vis de l'insuline. On a démontré l'augmentation dans le temps de la teneur en glucose des homogénéisats de foie d'une population de rats temoins, tandis que cette augmentation ne se manifeste pas dans les homogénéisats de rats préalablement traités par injection intraveineuse d'insuline. Par contre si l'insuline injectée était mélangée avec de l'albumine extraite du sérum de personnes diabétiques, cet effet de l'insuline était annulé.

In vivo Effekte des Synalbumin Insulin Antagonisten; Untersuchungen mit einer neuen Methode

Zusammenfassung. Zur Untersuchung des *in vivo* Effektes des Synalbumin Insulin Antagonisten wurde eine neue Methode ausgearbeitet. Es wurde nachgewiesen, daß die Glucose-Konzentration des Rattenleberhomogenisates während einer Inkubation zunimmt. Eine der Ratte im voraus gegebene Insulininjektion hemmte diese Zunahme. Wenn aber das Insulin zusammen mit aus dem Serum von Diabetikern gewonnenem Albumin gegeben wurde, war dieser Effekt des Insulins nicht nachweisbar.

Key-words: Antagonism, insulin, *in vivo*, liver homogenate, synalbumin.

Introduction

Several workers have examined the effects *in vivo* of Vallance-Owen's Synalbumin insulin antagonist [11, 12]. Jervell [6] using the intraperitoneal technique of Rafaelson *et al.* [10] showed that this Synalbumin antagonist opposed insulin action *in vivo* on the synthesis of glycogen in rat diaphragm muscle. These results were confirmed with intravenous administration of the Synalbumin, prepared by the trichloroacetic acid-ethanol method of Debro or by the Fernandez method [7]. However, when albumin previously shown to be antagonistic to insulin *in vitro* was infused for three hours into the femoral vein of lightly anaesthetized rats, no antagonism to insulin nor any diabetogenic effect could be demonstrated [2].

A new method has been elaborated for the examination *in vivo* of the Synalbumin insulin antagonist. In this new method the effect of insulin was estimated by its inhibitory action on glycogenolysis in the liver homogenate, and Synalbumin could be tested for its insulin antagonistic effect.

Material and Methods

Albumin was prepared from fresh serum of fasting healthy subjects and of fasting diabetic patients by the method of Fernandez *et al.* [4]. Ordinarily, single preparations of albumin were used, although in some experiments two albumin samples were pooled in order to provide sufficient protein.

80 wistar rats of both sexes weighing 100—120 g were used. Eight animals were investigated in any one experiment, two in each group. Ten sets of experiments were done. The non-starving, unanaesthetized rats were injected in the tail vein.

Group I served as controls and received per 100 g body weight 2.5 ml of Krebs-bicarbonate buffer solution (KRb).

Group II received per 100 g body weight 2.5 ml KRb and 0.4 units of crystalline insulin (Kőbányai Gyógyszerárugyár, Budapest, Hungary).

Group III received per 100 g body weight 2.5 ml KRb, 0.4 units crystalline insulin, and 250 mg albumin prepared from the serum of normal subjects.

Group IV received per 100 g body weight 2.5 ml KRb, 0.4 units crystalline insulin, and 250 mg albumin prepared from the serum of diabetic patients.

This gives at the time of the injection a human albumin concentration of 3.8 g per 100 ml in the plasma of the rat. The concentration of the total blood volume would be 2.8 g per 100 ml.

45 min after the injection, the rats were killed with a blow on the head. The blood sugar before the injection and that before killing were determined using the Somogyi-Nelson method [9]. The liver was taken out quickly, and placed in cold KRb for 10 min. 2.5 g of the liver was then homogenized for 2 min in 12 ml of cold KRb using a Potter homogenizer. The homogenate was centrifuged at 2000 rev/min for 5 min. The supernatant fluid was incubated in a Warburg apparatus at 37.3°C, with a shaking frequency of 20/min. Samples for glucose determination were taken at 0, 150 and 300 min of incubation. The glucose concentration was determined by the method of Somogyi-

Nelson [9]. At the same time the glycogen concentration of the homogenate was determined by the method of Good-Kramer-Somogyi [5].

All albumin samples were tested at a concentration of 1.25 per cent for insulin antagonism *in vitro* [11], those from diabetic patients being antagonistic, those from normal subjects non-antagonistic.

Results

The results of the investigations are shown in the following tables.

Table 1. *Blood sugar values (mg%) of the rats before the injection of 0.4 units of crystalline insulin per 100 g body-weight and 45 min later, before killing.* [*mean: S.E.M.*]

	Group I	Group II	Group III	Group IV
before injection	102±8	104±8	98±6	104±10
before killing	100±9	93±9	100±9	98±9

It can be seen that there were no significant differences in the blood sugar concentrations of the different groups before the injection of this amount of insulin and 45 minutes later, before killing.

Table 2. *Glucose concentration of the liver homogenates in mg%, during the incubation at 37.3° C.* [*mean: S.E.M.*]

	Group I	Group II	Group III	Group IV
0 time	166±20	83±15	110±13	186±27
150 min	347±35	121±14[a]	132±21[a]	354±24
300 min	392±40	133±20[a]	125±20[a]	451±31

[a] = $p < 0.001$

Table 3. *Glycogen concentration of the liver homogenates in mg%, during the incubation at 37.3° C.* [*mean: S.E.M.*]

	Group I	Group II	Group III	Group IV
0 time	130±25	128±25	141±23	137±20
150 min	52±14	112±19[a]	100±24[a]	49±11
300 min	45±10	132±18[a]	116±17[a]	40±11

[a] = $p < 0.001$

Discussion

Insulin antagonistic effect of albumin prepared from the serum of diabetic patients was examined with a new method. The serum albumin samples were prepared by the Fernandez method, which did not involve dialysis and so occasional artefacts of the Visking tubing did not influence the results [8]. The problem of these possible artefacts, which was later discussed by Ensinck *et al.* [3], can be eliminated by using this method of albumin preparation. The increase of glucose concentration in the liver homogenate was caused by the glycogenolysis, and it could be prevented by previous administration of insulin to the rat. The amount of insulin given did not cause a significant fall in the blood sugar of the rats [1], but it was enough to reduce the glycogenolysis in the liver homogenate during the incubation period. With this method it could be shown that intravenous administration of albumin prepared from the serum of diabetic patients inhibited this effect of insulin. According to the results, the method described above can be used for the determination of Synalbumin. On the other hand, it may also prove an activity of Synalbumin *in vivo*.

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