Pharmacodynamic Aspects of Tolbutamide, Glibenclamide, Glibornuride, and Glisoxepide II. Repeated Administration in Combination with Glucose

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Summary. The dynamics of insulin secretion have been studied in patients with maturity-onset diabetes, after i. v. administration of tolbutamide, glibenclamide, glibornuride (Ro 6-4563) and glisoxepide (BS 4231). The injection of each of the four compounds was combined with glucose, and administered in equipotent doses (ED 30) twice with a 3 h interval. The addition of glucose had no influence on the characteristic differences in insulin secretion after tolbutamide, glibornuride and glisoxepide on the one hand, and glibenclamide on the other. Earlier experiments without glucose had shown an obviously reduced insulin response after the second injection of each of the sulphonylureas studied. This response was modified by the administration of glucose, which led to equal peaks of insulin after the first and second injections. Furthermore, the peak level of insulin was maintained longer if each of the four substances was given together with glucose. These findings are evidence for a differentiation of the β -cytotrophic effect of sulphonylureas and glucose. Thus, the combined administration of both sulphonylureas and glucose, led to an almost additive increase of serum insulin. In contrast to the findings of other authors, even glibenclamide acted in this respect just like the other substances, as long as equipotent doses were compared.

Comparaison des actions pharmacodynamiques de nouvelles substances du type des sulfonyles hypoglycémiantes.

II. Applications répétées aux sujets diabétiques combinées avec glucose

Résumé. Quatre types de sulfonyles hypoglycémiantes — tolbutamide, glibenclamide, glibornuride (Ro 6-4563) et glisoxepide (BS 4231) — ont été appliqués par voie intraveineuse à des diabétiques âgés, deux fois dans un intervalle de trois heures. Les doses qui diminuent la concentration du glucose du sang de 30% (ED 30) ont été examinées en combinaison avec du glucose. Dans des expérimentations précédentes nous avons trouvé des concentrations d'insuline inférieures après la deuxième injection de toutes les sulfonyles hypoglycémiantes, appliquées sans une dose de glucose additionnelle. L'administration combinée stimulait la sécrétion d'insuline à la deuxième injection d'une intensité comparable à la première. Les maximums de la réponse insulinaire n'étaient pas influencés par les effets du glucose, mais

l'insulinémie était plus prolongée. Ce résultat indique qu'il y a un mode différent de la stimulation β -cytotropique entre le glucose et les sulfonyles hypoglycémiantes. L'application combinée ne provoquait qu'une augmentation additionnelle de la sécrétion d'insuline comparée à l'effet de l'injection de glucose seul.

Pharmakodynamik von Tolbutamid, Glibenclamid, Glibornurid und Glisoxepid.

II. Wiederholte Anwendung in Kombination mit Glucose

Zusammenfassung. In der Untersuchung werden die Wirkungen von 4 Sulfonylharnstoffderivaten auf die Insulinsekretion des Erwachsenenendobetikers verglichen. Hierzu wurden äquipotente Mengen (ED 30) von Tolbutamid, Glibenclamid, Glibornurid (Ro 6-4563) und Glisoxepid (BS 4231) zweimal in 3stündigem Abstand in Kombination mit Glucose injiziert. Sogar bei zusätzlicher Glucoseapplikation zeigt sich wiederum der charakteristische Unterschied in der Dynamik der Insulinsekretion nach Tolbutamid, Glibornurid und Glisoxepid einerseits und Glibenclamid andererseits. In früheren Untersuchungen haben wir gezeigt, daß es zu signifikant niedrigeren Insulinsekretionsraten bei der 2. Gabe der 4 Substanzen kommt. Die kombinierte Sulfonylharnstoff-Glucosegabe hebt jedoch diesen Mechanismus auf und führt zu gleich hohen Insulinspiegeln nach beiden Stimulationen. Die maximalen Insulinspiegel nach Sulfonylharnstoffen werden durch eine zusätzliche Glucoseapplikation nicht verstärkt, jedoch wird der übliche schnelle Abfall der Insulinkonzentration verhindert und wirksame Spiegel über eine längere Zeit aufrechterhalten. Aufgrund dieser Resultate wird auf verschiedenartige betacytotrope Wirkungen von Sulfonylharnstoffen und Glucose geschlossen. Die Insulinsekretion auf Glucose hin wurde durch die Kombination mit Sulfonylharnstoffen etwa im Verhältnis 1:1,5 verstärkt. Dies gilt ebenfalls für Glibenclamid. Die in der Literatur beschriebene „Glucosefreundlichkeit“ der Substanz, die sich in der Potenzierung seiner Wirkung in Anwesenheit von Glucose zeigen soll, war bei Verwendung äquipotenter Dosen nicht zu erkennen.

Key words: Tolbutamide, glibenclamide, glibornuride (Ro 6-4563), glisoxepide (BS 4231), i.v. dose-response relations, dynamics of insulin secretion, repeated administration of sulphonylurea compounds, effect of added glucose.

A number of recent publications on the mode of action of the so-called sulphonylureas of the second generation have caused a renewed discussion of the therapeutic use of oral hypoglycaemic agents. Hypoglycaemic incidents following glibenclamide given either in too high dosages or with a wrong indication

[7], draw attention to the need for caution in the therapeutic application of this drug. On the other hand, some new and striking qualities of glibenclamide on insulin secretion have been reported [20, 21, 22, 27]. Thus, some authors advocate the general use of this substance in cases of maturity-onset diabetes [20, 22].

As a matter of fact, a variety of quantitative [2, 17, 23, 24, 25, 26] and qualitative [1, 6, 14, 15, 19] differences from former sulphonylurea derivatives are known. There are undoubted differences between the dynamics of insulin release with glibenclamide and that with tolbutamide and the other compounds [9, 10, 11, 12]. Other qualities of glibenclamide, such as its capacity to give rise to similar rates of insulin output when repeatedly administered [20, 21, 22] seem to be at least dubious [11, 12]. Observations *in vitro* [6] and examinations performed with healthy subjects [21, 22] indicated that insulin secretion is potentiated enormously after additional administration of glucose. Under the same conditions tolbutamide gave a weak and only additive increase of serum insulin levels. All these examinations are handicapped

and glisoxepide (BS 4231)¹ on insulin secretion, when given in equipotent doses combined with glucose.

Materials and Methods

Experimental subjects: The subjects studied were normal healthy volunteers and maturity-onset diabetics with a mild metabolic disorder and fasting blood glucose levels of about 150 mg%. No patient was under any treatment at the time of the study except dietetic regulations. Oral hypoglycaemic agents had been withdrawn for at least 48 h. All persons were of normal weight.

Experimental design: Our previous studies [11, 12] performed on fasted, healthy subjects indicated a

Fig. 1. Variations in blood glucose in 8 maturity-onset diabetics following i.v.-administration of two repeated doses of various sulphonylurea derivatives with a three hour interval combined with glucose. (Mean of starting levels $\pm$ SEM: 158 ± 4.3 mg%)

by comparing effects of various hypoglycaemic sulphonamides without exact dose-response relations.

Some new representatives of the sulphonylurea compounds of the second generation, with a potency for lowering blood glucose similar to that of glibenclamide, are now being introduced into diabetes therapy. The present report will compare the effects of tolbutamide, glibenclamide, glibornuride (Ro 6-4563)¹

30% blood-glucose-lowering effect when using: 7.5 mg tolbutamide, 0.006 mg glibenclamide, 0.12 mg glibornuride and 0.02 mg glisoxepide per kg body weight. This so-called ED 30 as described by Frerichs and Puls [5], has been established to permit the use of equipotent doses. Since drugs lowering blood glucose were studied in this examination, the variations in blood glucose were used to determine the dose-response relations.

Eight non-obese, maturity-onset diabetics received 0.33 g glucose/kg, and afterwards each of the four

¹ We are indebted to the Hoffmann La-Roche Inc. for the gift of glibornuride (Ro 6-4563) and to the Bayer and Schering Inc. for the gift of glisoxepide (BS 4231).

compounds in the dosage corresponding to the ED 30, combined with a glucose load (0.33 g/kg body weight). This was repeated on different days with each of the compounds. Glucose and the sulphonylureas plus glucose were administered i.v. twice with a three hour interval. Samples for determination of blood glucose, free fatty acids and serum insulin were obtained at 0, 5, 10, 20, 40, 60, 90, 120, 180, 185, 190, 200, 220, 240 and 270 min. All tests were randomized and carried out starting at 8 a.m. under resting conditions after an overnight fast.

Analytical procedures: Levels of blood glucose were determined on an autoanalyzer by Hoffman's ferricyanide method [13]; serum insulin by the immunoassay of Meade and Klitgaard [16] in the modification of Melani *et al.* [18]; and free fatty acids by

pounds, the starting levels were generally attained after two hours. The second combined administration of glucose and sulphonylureas depressed blood glucose levels to the baseline already by 1.5 h, whereas the levels were significantly higher after this period with glucose alone ($p < 0.01$). On comparing the four compounds, the blood-glucose-lowering effect was almost the same with no statistical difference. As shown in previous studies [11, 12] dose-response relations seemed to be essential. This proves to be true also in diabetic subjects, both with or without additional glucose.

Response of free fatty acids: Similar observations were obtained with concentrations of free fatty acids (Fig. 2). An immediate initial rise, probably due to a short-lasting release of catecholamines, is followed by

Fig. 2. Variations in free fatty acids (FFA) in 8 maturity-onset diabetics following i.v.-administration of two repeated doses of various sulphonylurea derivatives with a three hour interval combined with glucose. (Mean of starting levels $\pm$ SEM: $887 \pm 26.6 \mu\text{E/l}$)

the procedure of Dole and Meinertz [4]. Both the decrease in blood glucose and that of free fatty acids were expressed in per cent of the initial level. The results were statistically evaluated according to Student's *t*-test.

Results

Blood glucose response after repeated administration: Blood glucose levels increased rapidly to about 115% after the injection of glucose, and did not return to the starting levels before three hours (Fig. 1). After additional administration of the hypoglycaemic com-

a distinct decrease of levels of FFA to about 40%. Maximal antilipolytic activities were generally found after 90 min. The second injection caused a further decrease lasting for only one hour. Depressions of the levels of FFA with the four sulphonylurea compounds did not differ statistically. Even after administration of glucose alone, where a weaker decrease of free fatty acids could be expected, almost equally intense antilipolytic activities were seen. The glucose load of 0.33 g/kg, with its strongly stimulating effect on the β -cell, seemed to produce maximal antilipolysis. Thus, the minor differences after additional application of the hypoglycaemic substances were not evident. Meas-

urement of glycerol levels showed almost similar results.

Response of serum insulin: Glucose (0.33 g/kg) given alone, caused a delayed increase of serum insulin, a plateau-like maximum and a likewise delayed decrease of IRI after the first injection (Fig. 3). In contrast, after the second injection the peak levels of insulin were attained already within 5 min. Comparing the levels 5 min after the two stimulations, IRI was significantly higher ($p < 0.001$) after the repeated injection. The combined administration of glucose and sulphonylurea compounds showed the characteristic difference between tolbutamide, glibornuride and glisoxepide on the one hand, and glibenclamide on the other. The first three substances caused peak concentrations of insulin already 5 min after administra-

tion, whereas glibenclamide reacted in its typical prolonged manner. Generally, maximal insulin levels after the second stimulation were found to be equally intense or even slightly higher than after the initial injection. Effective concentrations of IRI were maintained for at least three hours after the first stimulation. In contrast, the decrease of serum insulin following the peak values after the second stimulation was more rapid. The period of observation, however, was too short to give further details. Comparing the β -cytotropic effects of glucose with the combination of sulphonylureas plus glucose, an almost uniform increase in the levels of IRI was observed. The ratio of the absolute amounts of insulin secreted after glucose and

Discussion

after glucose plus sulphonylurea were 1:1.5 for glibenclamide, 1:1.7 for tolbutamide, 1:1.5 for glibornuride and 1:1.6 for glisoxepide.

Previous data, based on the determination of the i.v. ED 30 in healthy normals, indicated the high blood-glucose-lowering potency of the sulphonylureas of the second generation [11, 12]. Equally intense blood-glucose-lowering activities were seen using 1250 times smaller doses of glibenclamide, and 63 and 375 times smaller doses of glibornuride and glisoxepide, respectively, when compared with tolbutamide. The four sulphonylurea derivatives revealed a uniform

Fig. 3. Variation in immunoreactive insulin (IRI) in 8 maturity-onset diabetics following i.v.-administration of two repeated doses of various sulphonylurea derivatives with a three hour interval combined with glucose

tion, whereas glibenclamide reacted in its typical prolonged manner. Generally, maximal insulin levels after the second stimulation were found to be equally intense or even slightly higher than after the initial injection. Effective concentrations of IRI were maintained for at least three hours after the first stimulation. In contrast, the decrease of serum insulin following the peak values after the second stimulation was more rapid. The period of observation, however, was too short to give further details. Comparing the β -cytotropic effects of glucose with the combination of sulphonylureas plus glucose, an almost uniform increase in the levels of IRI was observed. The ratio of the absolute amounts of insulin secreted after glucose and

and equally strong blood-glucose-lowering effect, indicating that the dose-response relations used in this study are also valid for diabetics and when given in combination with glucose.

Further differences were observed in the dynamics of insulin secretion. Glibornuride and glisoxepide caused a sharp initial increase of serum insulin levels within 5 min of injection. Effective levels of insulin continued for nearly three hours after additional administration of glucose. In contrast to this pattern of insulin secretion characteristic of tolbutamide, glibenclamide showed the well-known, delayed insulin response with its plateau-like maximum. Previous data obtained with the identical group of diabetics

but without additional glucose, indicated a reduced insulin response after the second stimulation with the four sulphonylurea compounds [11, 12]. The additional administration of glucose compensated this mechanism, and led to equally intense or even slightly higher insulin concentrations. The effects of tolbutamide, glibornuride and glisoxepide on insulin secretion, were very short when given without glucose [11, 12]. In diabetics, insulin concentrations dropped to 50% of the peak levels within 10 min, and reached the baseline after 60 min. The additional glucose load led to a delayed decrease in insulin concentration lasting for three hours, but not interfering with the sulphonyl-induced initial insulin peak. The three substances caused the same initial peak levels of insulin whether given with or without glucose. In principle, the same results were seen with glibenclamide. Peak concentrations were not intensified by glucose, but only maintained longer. These results indicate that the β -cytotrophic effects of hypoglycaemic sulphonamides and of glucose are not identical. Apparently, sulphonylureas have an intrinsic, short-lasting β -cytotrophic effect which is almost independent of glucose concentration in the first phase of stimulation. The second phase seems to be a reinforcement of the capacity of glucose itself to stimulate insulin secretion. These findings suggest two different storage systems for insulin. One, readily accessible and easily affected by agents such as sulphonylurea derivatives, and perhaps in a minor way by glucose; and another, less accessible, responding mainly to glucose, an action which in turn is facilitated by sulphonylurea derivatives. As a matter of fact, observations *in vitro* by Curry *et al.* [3] and by Grodsky *et al.* [8] seem to support these findings.

Several investigators reported a potentiating effect of glucose on insulin secretion after glibenclamide which could not be obtained with sulphonylurea derivatives such as tolbutamide [6, 20, 21, 22]. Therapeutic consequences were even derived from this observation. Our present examinations do not confirm these results. With the equipotent doses we used, a uniform increase of serum insulin was seen when comparing the insulinotropic effects of glucose with the combined administration of glucose and sulphonylureas. The former findings are certainly due to the high dosages used in the other studies, and the lack of appropriate dose-response relations. The claim of the very promising qualities of glibenclamide thus requires new substantiated evidence. Whether or not the prolonged action of glibenclamide is due only to its low solubility and its effect on the transport mechanisms is still dubious.

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