THE EFFECT OF TOLBUTAMIDE AND GLIBENCLAMIDE ON THE INCORPORATION OF [^{3}H]LEUCINE AND ON THE CONVERSION OF PROINSULIN TO INSULIN IN ISOLATED PANCREATIC ISLETS

H. SCHATZ*, V. MAIER, M. HINZ, C. NIERLE and E.F. PFEIFFER**

Department of Endocrinology and Metabolism, Center of Internal Medicine and Pediatrics, University of Ulm, Ulm/Donau, Germany

Received 19 May 1972

1. Introduction

Tolbutamide†, which is known for its stimulatory action on insulin secretion [8] fails to increase the biosynthesis of insulin as judged by the incorporation of radioactive leucine into the insulin fraction of pancreatic slices or isolated islets [13, 11]. Tanese et al. [12] even described a dose-related inhibition of the leucine incorporation into proinsulin and insulin, effected by tolbutamide at low glucose concentrations. On the other hand, at 300 mg% glucose a stimulation of insulin biosynthesis in the presence of tolbutamide was reported by these authors.

Glibenclamide [2], a new potent sulphonylurea, exhibited kinetics of insulin secretion differing from those induced by tolbutamide when examined at certain glucose concentrations [9, 1]. Therefore we decided to study the effect of both tolbutamide and glibenclamide on incorporation of [^{3}H]leucine into the proinsulin and insulin fraction of isolated pancreatic islets at various concentrations of glucose.

2. Methods

Pancreatic islets were isolated both from fed male Swiss albino mice, strain NMRI, weighing about 30 g and from fed male rats, strain FW 49 Kirchb.-Lemgo-Bib., weighing about 250 g. Collagenase, which was purchased from Serva, Heidelberg, was used for the digestion of pancreatic tissue, as described by Lacy and Kostianovsky [4].

2.1. *Studies of leucine incorporation*

Batches of 40 islets of comparable size were incubated in 1 ml of Krebs–Ringer bicarbonate buffer, pH 7.4, supplemented with 17 naturally occurring amino acids (20 μg/ml, leucine excluded), bovine serum albumin (2 mg /ml, Behringwerke, Marburg) and a protease inhibitor (1000 KIU/ml Trasylol$^{(R)}$, Bayer Leverkusen) in the presence of [4, 5-^{3}H]L-leucine (50 μCi, 20 Ci/mmole, Amersham).

Glucose was present at concentrations of 100 or 300 mg%, as were tolbutamide and glibenclamide (obtained from Farbwerke Hoechst AG, Germany) at 100, 250 and 500 μg/ml and 2.5, 5.0 and 10 μg/ml, respectively. Incubations were carried out at 37° for 3 hr in an atmosphere of O_2:CO_2 (95:5%, v/v).

After the incubations, both the islets and the media were quickly frozen together to −28°. After thawing, trichloroacetic acid was added, yielding a final concentration of 10% w/v, followed by ultrasonic disintegration. The precipitated proteins were washed with 5% trichloroacetic acid, dissolved in 1 M acetic acid and separated on a calibrated Sephadex G-50 fine column, 1.2 × 55 cm, using 1 M acetic acid as eluent at a flow-rate of 10 ml/hr. Radioactivity and immunological measurable insulin [6] were determined in each fraction of the column and in the pooled

* Fellow of the Alexander von Humboldt Foundation.

** Supported by Deutsche Forschungsgemeinschaft Bonn-Bad Godesberg, SFB 87 Ulm, Proj. 11.

† *N*-(4-methyl-phenylsulfonyl)-*N'*-butyl-urea.

†† *N*-[4-(β⟨5-chloro-2-methoxy-benzamido⟩-ethyl-phenylsulfonyl]-*N'*-cyclohexyl-urea.

peaks. Due to the large number and careful selection of the islets in each sample no differences were found in the insulin content amongst the batches of each 40 islets. Thus, direct comparison was made between the radioactivity curves obtained from the eluates of the islet proteins, based on equal insulin content resp. equal number of islets as frequently reported in literature.

The *secretion* of immunologically measurable *insulin* was estimated hourly during 3 hr of incubation in aliquots of the incubation medium.

2.2. *Pulse labelling studies*

The conversion of proinsulin to insulin was also studied by pulse labelling the islet proteins. After preincubation of 40 islets in 1 ml buffer each at 300 mg% glucose for 1 hr, a pulse of [^{3}H]leucine was given for 10, 20 and 30 min. Subsequently, the islets

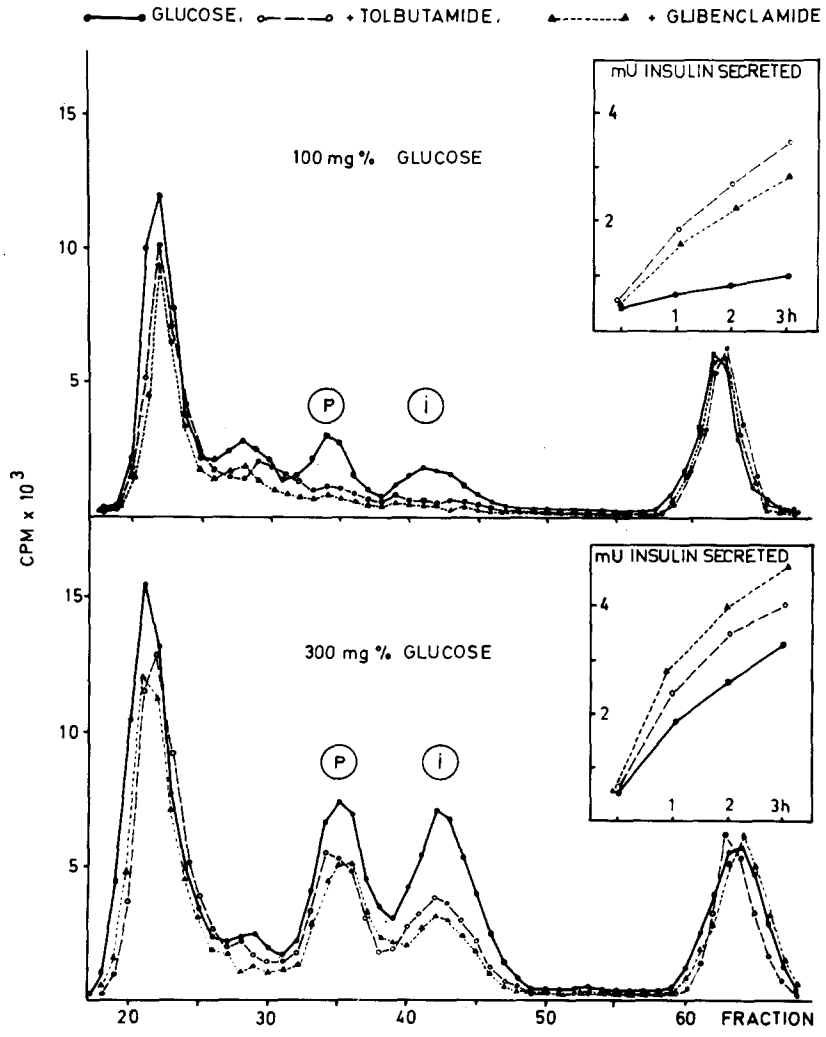

Fig. 1. Incorporation of [^{3}H]leucine into the proinsulin (P) and insulin (I) fraction of 40 isolated pancreatic mouse islets after incubation of the islets for 3 hr in the presence of [^{3}H]leucine at 100 or 300 mg% glucose. Tolbutamide and glibenclamide were present at concentrations of 100, 250 and 500 μg/ml and 2.5, 5 and 10 μg/ml, respectively. After precipitation with trichloroacetic acid the islet proteins were fractionated on Sephadex G-50 fine. The first peak eluting with the void volume represents other islet proteins excluded from the gel, the last peak free [^{3}H]leucine. Secretion of insulin into the incubation medium is also shown.

were washed with the buffer and incubated for a further 2 hr without radioactive and without non radioactive leucine in the presence of tolbutamide (250 μg/ml) or glibenclamide (5 μg/ml) and/or 0, 100 or 300 mg% glucose. Non radioactive leucine was not added on account of the stimulatory action of leucine itself on insulin secretion.

A detailed description of the methods used including the procedures for identification of the individual peaks eluting from the Sephadex column will be given elsewhere [10].

3. Results

The *incorporation of [^{3}H]leucine* into proinsulin and insulin was depressed at 100 mg% glucose, when tolbutamide or glibenclamide was added to the incubation medium. At 300 mg% glucose, the presence of these sulphonylureas reduced the [^{3}H]leucine incorporation into proinsulin and insulin to about two thirds and the half, respectively, as compared with the results in the absence of these drugs (fig. 1). In none of our 14 experiments was an increase of leucine incorporation observed in the presence of sulphonylureas at any glucose concentration. The amount of inhibition did not differ between the concentrations of tolbutamide or glibenclamide which were tested (cf. [12]).

Insulin secretion was increased by sulphonylureas in all experiments. At 300 mg% glucose, stimulation of insulin secretion was greater with glibenclamide (2.5, 5 and 10 μg/ml) than with tolbutamide (100, 250 and 500 μg/ml, respectively,) unlike the findings at 100 mg% glucose (fig. 1).

Essentially identical findings concerning both leucine incorporation and insulin secretion were obtained in experiments with islets of mice (n = 11) and rats (n = 3).

Studies with *pulse labelling* of the islets for 20 and 30 min (fig. 2) showed that conversion of proinsulin to insulin was affected by neither drug. Glucose also had no influence on the conversion of proinsulin to insulin per se. After a pulse of radioactive leucine for 10 min, only very small amounts of radioactivity were found incorporated; no differences were apparent between the individual experiments.

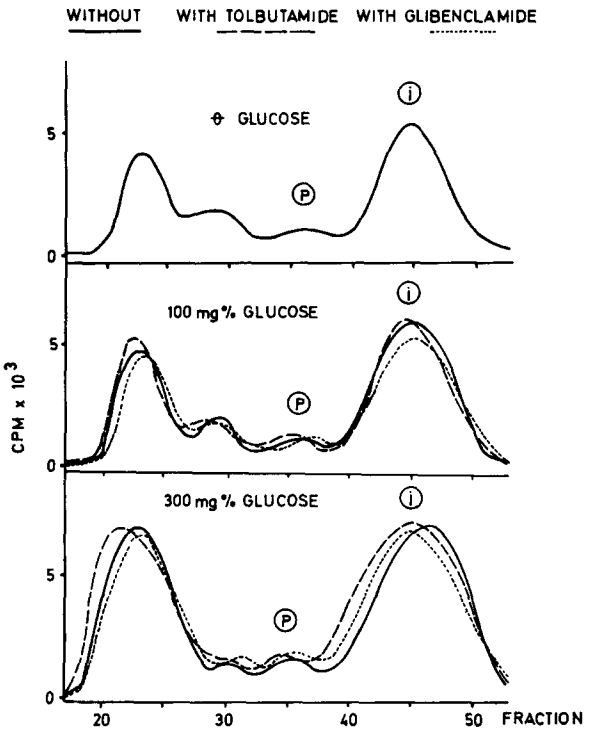

Fig. 2. Incorporation of [^{3}H]leucine into the proinsulin (P) and insulin (I) fraction of each 40 isolated pancreatic mouse islets after pulse labelling of the islet proteins with [^{3}H]leucine for 20 min at a glucose concentration of 300 mg%. Prior to the pulse, the islets were preincubated for 1 hr at 300 mg% glucose. After the pulse, the islets were washed and further incubated for 2 hr in fresh medium containing 0, 100 or 300 mg% glucose. Tolbutamide (250 μg/ml) or glibenclamide (5 μg/ml) was added after the pulse. Fractionation of the islet proteins was on Sephadex G-50 fine. Essentially the same findings were obtained after a pulse with [^{3}H]leucine for 30 min.

4. Discussion

Incorporation of [^{3}H]leucine into the proinsulin and insulin fraction of isolated pancreatic islets of mice and rats was inhibited in the presence of both tolbutamide and glibenclamide. This inhibition was also found but to a relatively lesser extent, at high concentrations of glucose, in contrast to Tanese et al. [12].

Sulphonylureas are potent stimulators of insulin secretion and widely used in the control of diabetes mellitus. Therefore, one hesitates to interpret the in-

hibition of leucine incorporation into proinsulin and insulin *in vitro* as being a true inhibition of insulin biosynthesis effected by tolbutamide or glibenclamide. A report concerning the inhibition of insulin secretion after exposure of pancreatic tissue to tolbutamide [5], however, would be in accordance with this explanation. The clinically known refractory states of the endocrine pancreas following tolbutamide application [7] should also be mentioned in this context. Our present observations were restricted to a period of 3 hr. Hence, no conclusions can be reached from our experiments concerning the action of sulphonylureas on insulin biosynthesis after this period of time.

Inhibition of label uptake by the islets in presence of sulphonylureas would be another explanation for reduced incorporation of leucine. According to Hellman et al. [2], however, glibenclamide does not affect the net leucine uptake of isolated pancreatic islets.

The rate of leucine oxidation within pancreatic islets was found to be reduced at high glucose concentrations compared to results at low concentrations or in the absence of glucose [3]. This fact should be considered when interpreting results of leucine incorporation into proinsulin and insulin achieved at different glucose concentrations.

An inhibitory action of sulphonylureas on the conversion of proinsulin to insulin seems to be unlikely from our pulse labelling experiments. Furthermore, it also appears that proinsulin is converted to insulin, irrespective of the absence or the concentration of glucose. Thus the site of action of tolbutamide and glibenclamide in our *in vitro* model of biosynthesis would seem to be located before the conversion of proinsulin and after the entrance of leucine into the B-cell.

References

[1] R.D. Fussgänger, R. Goberna, M. Hinz, P. Jaros, C. Carsten, E.F. Pfeiffer and S. Raptis, Horm. Metab. Res. Suppl. ad Vol. 1 (1969) 34–40.
[2] B. Hellman, J. Sehlin and I. Täljedal, Endocrinology 89 (1971) 1432–1439.
[3] B. Hellman, J. Sehlin and I. Täljedal, Biochem. J. 123 (1971) 513–521.
[4] P. Lacy and Y.M. Kostianovsky, Diabetes 16 (1967) 35–39.
[5] W.J. Malaisse, F. Malaisse-Lagae, D.A. Mayhew and P.H. Wright, in: Tolbutamide... after ten years, eds. W.J.H. Butterfield and W. van Westering (Excerpta Medica Foundation, 1967) pp. 49–60.
[6] F. Melani, H. Ditschuneit, K. Bartelt, H. Friederichs and E.F. Pfeiffer, Klin. Wscht. 43 (1965) 1000–1007.
[7] E.F. Pfeiffer, H. Ditschuneit and R. Ziegler, Klin. Wschr. 39 (1961) 415–426.
[8] E.F. Pfeiffer, H. Steigerwald, W. Sandritter, A. Bänder, A. Mager, U. Becker and K. Retiene, Dtsch. med. Wschr. 82 (1957) 1568–1574/1560.
[9] S. Raptis, R.M. Rau, K.E. Schröder, J.D. Faulhaber and E.F. Pfeiffer, Horm. Metab. Res. Suppl. ad Vol. 1 (1969) 65–72.
[10] H. Schatz, Y. Abdel Rahman, M. Hinz, H.L. Fehm, C. Nierle and E.F. Pfeiffer, in preparation.
[11] D. Steiner, Acta Diabet. Lat. 6 (Suppl. 1) (1969) 435–472.
[12] T. Tanese, N.R. Lazarus, S. Devrim and L. Recant, J. Clin. Invest. 49 (1970) 1394–1404.
[13] K.W. Taylor and D.G. Parry, J. Endocr. 39 (1967) 457–458.