Biochemical Pharmacology, Vol. 24, pp. 235–239. Pergamon Press, 1975. Printed in Great Britain.

THE STIMULUS-SECRETION COUPLING OF GLUCOSE-INDUCED INSULIN RELEASE—XVII

EFFECTS OF SULFONYLUREAS AND DIAZOXIDE ON INSULAR BIOSYNTHETIC ACTIVITY

JOSEPH LEVY and WILLY J. MALAISSE

Laboratory of Experimental Medicine, Brussels University School of Medicine, Brussels, Belgium

(*Received* 5 *April* 1974; *accepted* 11 *July* 1974)

Abstract—Tolbutamide (0·2 mg/ml), gliclazide (0·02 mg/ml), glipizide (0·005 mg/ml) and diazoxide (0·2 mg/ml) were found to inhibit glucose-induced proinsulin synthesis in isolated rat islets. The effect of these pharmacological agents was characterized by a preferential inhibition of proinsulin as distinct from other protein synthesis, and was associated with a reduced oxidation of glucose by the islets. The inhibitory effects were more marked at low (1·0 mg/ml) than at high (3·0 mg/ml) glucose concentration. These results indicate that the influence of sulfonylureas upon insular function cannot be ascribed merely to a facilitation of glucose metabolism or a cellular accumulation of adenosine-3′,5′-cyclic monophosphate.

Although prolonged inhibition of protein synthesis in insular tissue is reported to cause depressed insulin secretion [1, 2], a number of drugs are known to affect differentially the respective rate of hormonal synthesis and release by the pancreatic β-cell during short-term experiments. In previous work from this laboratory [3–7], we have taken advantage of such dissociated effects to scrutinize the participation of glucose, adenosine-3′,5′-cyclic monophosphate (cAMP), cations and a microtubular-microfilamentous system in the sequence of cellular events leading to insulin release. The present report extends this study to sulfonylureas and diazoxide which are currently used as pharmacological modifiers of insulin secretion.

MATERIALS AND METHODS

Insular biosynthetic activity. The method used for the assessment of insular biosynthetic activity in isolated rat islets is identical to that described in a previous report [7]. Briefly, after incubation for 90 min in media containing [4,5-^{3}H]leucine, the islets were homogenized in acetic acid (2 M), and the homogenate submitted to polyacrylamide gel chromatography. Acid–alcohol extracts of the incubation media were also submitted to polyacrylamide gel filtration, in order to follow the release of newly synthesized hormonal peptides. The total synthesis of proinsulin was calculated as the radioactivity recovered in the proinsulin and insulin peaks in both the insular homogenate and incubation medium. The synthesis of non-hormonal peptides was assumed to correspond to the incorporation of [^{3}H]leucine in insular material eluting prior to the proinsulin peak. The amount of proinsulin converted to insulin and *C*-peptide, which in the present system elutes together with insulin [5], was expressed in per cent of the total amount of proinsulin synthesized. The fractional release of newly synthesized hormonal peptides was also expressed relative to the total amount of proinsulin synthesized. In order to evaluate the effect of various drugs on insular biosynthetic activity, each parameter was expressed in per cent of the control value found within the same experiment in a paired group of islets incubated at the same glucose concentration but in the absence of the agent under study (see Table 2).

Glucose oxidation. For the measurement of glucose oxidation, groups of 15 islets each were incubated for 120 min in bicarbonate-buffered medium (0·2 ml) contained in a small suspended plastic cup itself placed in a scintillation counting vial. The incubation medium contained [^{14}C](U)-glucose (10 μCi/ml; The Radiochemical Centre, Amersham). After incubation, 0·1 ml of HCl (0·2 N) and 0·2 ml of hyamine hydroxide (Packard, Downers Grove) were injected through the rubber stopper, respectively, into the plastic cup containing the incubation medium and into the counting vial. After gentle shaking for 30 min at 4°C, 10 ml of scintillation fluid (Instagel; Packard, Downers Grove) were added to the hyamine. After substraction of the mean amount of radioactivity recovered from identical media incubated in the absence of islets, the rate of glucose oxidation was expressed as pmole/islet per 120 min.

Drugs. Tolbutamide (0·2 mg/ml; Hoechst A.G., Frankfurt (Main), Germany), gliclazide (0·02 mg/ml; Laboratoires Servier, Neuilly-sur-Seine) and glipizide (0·005 mg/ml; Pfizer, Brussels) were used at concentrations known to evoke a maximal insulin secretory response in incubated rat pancreatic tissue [8–10]. Diazoxide (0·2 mg/ml; Schering Corp., Bloomfield,

Table 1. Effect of glucose on insular biosynthetic activity

Glucose (mg/ml)	Proinsulin synthesis (c.p.m. 10^{-3}/25 islets)	Proinsulin synthesis (% of total synthesis)	Proinsulin conversion (%)	N
Nil	19·2 ± 2·0	13·6 ± 1·4	34·8 ± 3·6	5
1·0	104·7 ± 16·4	42·2 ± 5·3	32·5 ± 4·7	6
3·0	169·1 ± 18·4	52·4 ± 4·4	36·9 ± 5·0	6

Mean values (±S.E.M.) are shown together with the number of individual observations (N).

N.J., U.S.A.) was used at a concentration two to three times higher than that required to abolish glucose-induced insulin release [11].

RESULTS

Effect of glucose on proinsulin synthesis. Table 1 summarizes the control data obtained at the three glucose concentrations used in the present experiments. As the glucose concentration of the incubation medium was raised, the synthesis of proinsulin was much more markedly stimulated than that of non-hormonal peptides, the ratio of hormonal to total protein synthesis increasing from a basal value of 13·6 ± 1·4 to 52·4 ± 4·4 per cent at the highest glucose level (3·0 mg/ml). The conversion of proinsulin to insulin and *C*-peptide was not significantly affected by the glucose concentration of the incubation medium. These findings confirm previous observations collected with the same system [5, 7].

Effect of sulfonylureas and diazoxide on insular biosynthetic activity. In the absence of glucose, tolbutamide, gliclazide and glipizide failed to significantly affect the various parameters of insular biosynthetic activity (Table 2). The results of the paired comparisons were rather variable; it should be kept in mind, however, that [^{3}H]leucine incorporation in hormonal peptides normally occurs at a very low rate in the absence of glucose, so that a minor fluctuation in the absolute rate of proinsulin synthesis leads to a rather large per cent change relative to the appropriate control value.

At an intermediate glucose level (1·0 mg/ml), all sulfonylureas significantly reduced [^{3}H]leucine incorporation in hormonal peptides. This inhibitory effect was less pronounced for the non-hormonal peptides, so that the ratio of proinsulin to total protein synthesis was invariably decreased by the sulfonylureas in all of the nine individual paired comparisons. Diazoxide also invariably reduced, although to a somewhat lesser degree, both [^{3}H]leucine incorporation into proinsulin and the ratio of hormonal to total protein synthesis.

At high glucose concentration (3·0 mg/ml), a reduction of both proinsulin synthesis and the ratio of hormonal to total protein synthesis was again observed in ten out of twelve paired comparisons dealing with the effect of either sulfonylureas or diazoxide. However, as a rule, the inhibitory effect of the pharmacological agents was less pronounced (Fig. 1) and failed to achieve statistical significance at this high glucose level (Table 2).

None of the drugs under study exerted any obvious effect upon the conversion of proinsulin to insulin, whatever the glucose concentration of the incubation medium. At the most, there might have been a trend, at the low glucose concentration (1·0 mg/ml), for the

Table 2. Effect of sulfonylureas and diazoxide on insular biosynthetic activity

Glucose (mg/ml)	Other drug (mg/ml)	Proinsulin synthesis	Proinsulin/total synthesis	Proninsulin conversion	N
Nil	Tolbutamide (0·2)	96·2 ± 18·8	108·8 ± 33·3	88·9 ± 24·9	3
Nil	Gliclazide (0·02)	115·6 ± 25·3	109·0 ± 18·4	100·9 ± 9·3	5
Nil	Glipizide (0·005)	94·4 ± 11·4	84·5 ± 11·0	128·6 ± 24·6	4
1·0	Tolbutamide (0·2)	35·8 ± 13·0†	59·8 ± 8·4†	103·1 ± 25·8	3
1·0	Gliclazide (0·02)	49·9 ± 1·7‡	89·7 ± 3·2*	113·7 ± 17·9	3
1·0	Glipizide (0·005)	58·9 ± 7·1†	74·5 ± 6·3*	105·0 ± 1·0	3
1·0	Diazoxide (0·2)	62·2 ± 12·4*	75·3 ± 13·0	123·8 ± 14·7	3
3·0	Tolbutamide (0·2)	69·5 ± 11·0	89·1 ± 9·3	97·5 ± 4·0	3
3·0	Gliclazide (0·02)	72·7 ± 14·7	93·4 ± 17·7	97·9 ± 9·5	3
3·0	Glipizide (0·005)	75·9 ± 16·8	89·7 ± 5·2	104·8 ± 10·4	3
3·0	Diazoxide (0·2)	96·3 ± 5·1	87·3 ± 7·8	99·5 ± 1·0	3

Mean data (±S.E.M.) are expressed in per cent of the paired value found, within the same experiment, in a control group of islets incubated at the same glucose concentration but in the absence of any added drug. Also shown are the number of paired comparisons (N) and the statistical significance of differences between experimental and control values (* $P < 0·10$; † $P < 0·05$; ‡ $P < 0·001$). Absolute values for proinsulin synthesis are illustrated in Fig. 1.

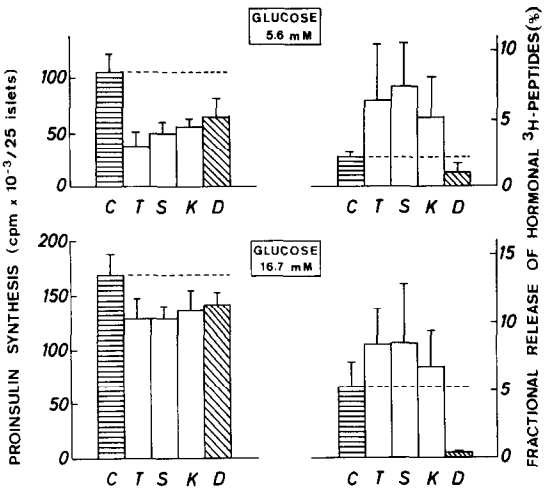

Fig. 1. Effect of sulfonylureas and diazoxide upon the synthesis of hormonal ^{3}H-peptides and their release by isolated islets. Control values found in the presence of glucose alone (C) are compared with experimental data found in the concomitant presence of glucose and either sulfonylureas (T = tolbutamide, S = gliclazide, K = glipizide) or diazoxide (D). Mean values (±S.E.M.) refer to 3 or 6 individual measurements.

pharmacological agents to enhance the fractional conversion of proinsulin which, for the group as whole, averaged 111·4 ± 7·8 per cent of the corresponding paired control values.

Effect of sulfonylureas and diazoxide on the release of newly synthesized hormonal peptides. As illustrated in Fig. 1, the fractional release of newly synthesized hormonal peptides increased as the glucose concentration was raised from 1·0 to 3·0 mg/ml. Tolbutamide, gliclazide and glipizide further enhanced the fractional release of the newly synthesized hormonal peptides, the enhancing action of the sulfonylureas being more marked at low than at high glucose concentration. On the contrary, diazoxide markedly inhibited the glucose-induced release of [^{3}H]peptides. These data indicate that the secretory reponse of the islets to the various drugs occurred according to its classical pattern [8–11].

Incidentally, in the present series of experiments, about half (43·0 ± 3·9%; n = 26) of the secreted [^{3}H]peptides was released as insulin and *C*-peptide, the other half being secreted as proinsulin. This finding confirms prior observations [5, 7].

Effect of tolbutamide and diazoxide on glucose oxidation. As judged by the production of $^{14}CO_2$ from [^{14}C][U]-glucose, the rate of glucose oxidation was higher ($P < 0.025$) at high (3·0 mg/ml) than at low (1·0 mg/ml) glucose concentration (Table 3). At the low glucose level, both tolbutamide and diazoxide, which were used at the same high concentration (0·2 mg/ml), inhibited glucose oxidation by 40–50 per cent. The inhibitory effect of these drugs upon glucose oxidation was less marked at the high glucose level.

DISCUSSION

The present results concerning the effect of sulfonylureas upon insular biosynthetic activity are in good agreement with existing data [2, 12–15]. Several authors found that the inhibitory effect of tolbutamide is relatively more marked at low (0·5 to 1·0 mg/ml) than high (3·0 mg/ml) glucose concentration [12–14]. Inhibition of proinsulin synthesis was also observed in the presence of glibenclamide, although the latter drug was used at much lower concentrations (0·002 to 0·01 mg/ml) than tolbutamide (0·1 to 0·5 mg/ml) [14]. Preferential inhibition by sulfonylureas of proinsulin synthesis as distinct from that of other insular peptides can be inferred from the data reported by Schatz *et al.* [14]. Lastly, Puchinger and Wacker [15] observed that inhibition of insular protein synthesis by tolbutamide is associated with a reduced incorporation of [^{3}H]uridine into islets RNA, especially at low glucose concentration (0·5 mg/ml).

Also in good agreement with the present data, Lin and Haist [16] reported that diazoxide (0·15 mg/ml) causes a modest and insignificant reduction in proinsulin synthesis at high glucose concentration (3·0 mg/ml). These authors did not investigate the effect of diazoxide at lower glucose levels. Although diazoxide was here used at a concentration purposely much higher than that required to abolish glucose-induced insulin release [11], there was a trend for the inhibitory effect of this drug upon proinsulin biosynthesis to be less marked than that of the sulfonylureas (Table 2). Even so, the analogy between the respective effects of sulfonylureas and diazoxide upon glucose-induced proinsulin biosynthesis clearly contrast with the well-known opposite effects of these drugs upon insulin

Table 3. Glucose oxidation by isolated islets

Added drug	Glucose 1·0 mg/ml	Glucose 3·0 mg/ml
Nil	38·7 ± 4·0 (11)	59·8 ± 7·3 (12)
Tolbutamide (0·2 mg/ml)	22·5 ± 2·4† (15)	50·9 ± 5·8 (17)
Diazoxide (0·2 mg/ml)	19·7 ± 1·8‡ (14)	36·0 ± 4·9* (17)

Mean values (±S.E.M.) are expressed as pmole/islet per 120 min and are shown together with the number of individual observations (in parentheses) and the statistical significance of differences between control and experimental values (* $P < 0.01$; † $P < 0.005$; ‡ $P < 0.001$).

release [17, 18]. It is conceivable, therefore, that the inhibition of insular biosynthetic activity is related to a structural configuration common to the four tested molecules and yet not identical to the molecular determinant responsible for their positive or negative insulinotropic action. It is noteworthy that the urinary excretion product of tolbutamide, which lacks hypoglycemic property, is at least as effective as tolbutamide itself in inhibiting the respiratory rate of isolated islets incubated at high glucose concentration [19].

There is yet no satisfactory explanation to account for the inhibitory effect of sulfonylureas upon proinsulin synthesis. It is unlikely that such an inhibition is due to reduced leucine uptake by the islets. According to Hellman *et al.* [20], neither glibenclamide (0·05 mg/ml) nor diazoxide (0·125 mg/ml) affect leucine uptake by isolated islets. Moreover, a reduction in leucine uptake would not account for the preferential inhibition of [^{3}H]leucine incorporation in proinsulin.

A second possibility would be that the inhibition of protein synthesis results from the lowering effect of sulfonylureas upon ATP concentration in the β-cell [21–22]. We do not wish to rule out such a mechanism, especially in view of the fact that the sulfonylurea-induced fall in ATP content of isolated islets is also more marked at low (0·6 mg/ml) than high (3·0 mg/ml) glucose concentration [21]. However, the shortage of ATP is once again unlikely to account for the preferential inhibition of proinsulin synthesis, since dinitrophenol does not exert such a preferential effect [5].

A third hypothesis to be considered is that the preferential inhibition of hormonal synthesis is the result of some kind of abnormality in the process of glucose recognition and/or metabolism in the β-cell. The present finding of an inhibitory effect of tolbutamide and diazoxide upon glucose oxidation is compatible with such a view. In previous reports, such inhibitory effects were not disclosed, but the length of incubation (60 instead of 120 min) and the concentration of diazoxide (0·12 instead of 0·2 mg/ml) were different from those used in the present study [23, 24]. Moreover, a sulfonylurea-induced inhibition of glucose oxidation is compatible with the finding [19] that tolbutamide, which increases the rate of endogenous substrates oxidation measured in the absence of glucose, inhibitis oxygen uptake by isolated islets at high glucose concentration. Incidentally, in comparing the rates of glucose oxidation and those of insulin biosynthesis, the following limitations should be kept in mind: (i) the rate of glucose oxidation, as calculated here, could be influenced by isotopic dilution, especially if sulfonylureas indeed increase the oxidation of endogenous substrates; (ii) in the range of glucose concentrations between 1·5 and 3·0 mg/ml, the rate of proinsulin biosynthesis increases less than that of insulin release [7, 25], so that a fractional reduction in glucose metabolism at the highest glucose level (3·0 mg/ml) may not cause a marked inhibition of proinsulin synthesis; and (iii) the inhibitory effect of the pharmacological agents could be a progressive phenomenon, so that glucose oxidation, measured over 120 min incubation, would appear more markedly inhibited than proinsulin synthesis which was assessed over only 90 min incubation.

Lastly, in view of the frequently quoted suggestion that sulfonylureas may stimulate insulin release by raising the level of cAMP in the β-cells, it should be stressed that the effect of sulfonylureas upon insular biosynthetic activity is vastly different from that of either glucagon, dibutyryl-cAMP or theophylline upon the same process [7, 12, 26].

In conclusion, therefore, the contrasting effect of sulfonylureas on insulin synthesis and release, respectively, confirms previous claims [27–29] that, despite a number of analogies between glucose- and sulfonylurea-induced insulin release [11], and despite the documented effect of sulfonylureas on cAMP synthesis and breakdown [30–33], the action of these hypoglycemic compounds upon insular function cannot be merely ascribed to either a facilitation of glucose metabolism or an accumulation of cAMP in the β-cell.

Acknowledgements—The authors wish to thank Dr. D. G. Pipeleers for his participation in some preliminary experiments, Mrs. M. Mahy and J. Schoonheydt for skilful technical assistance, and Miss B. Vincke for secretarial help. This work was supported in part by grants from the F.R.S.M. (Brussels), a contract of the Ministère de la Politique Scientifique within the framework of the association Euratom-Universities of Brussels and Pisa, and grants-in-aid from Les Laboratoires Servier (Neuilly-sur-Seine) and Pfizer-Europe (Brussels).

REFERENCES

1. D. L. Curry, L. L. Bennett and G. M. Grodsky, *Endocrinology* **83,** 572 (1968).
2. G. E. Morris and A. Korner, *Biochim. biophys. Acta* **208,** 404 (1970).
3. W. J. Malaisse, F. Malaisse-Lagae, M. O. Walker and P. E. Lacy, *Diabetes* **20,** 257 (1971).
4. W. J. Malaisse, D. L. Hager and L. Orci, *Diabetes* **21,** 594 (1972).
5. D. G. Pipeleers, M. Marichal and W. J. Malaisse, *Endocrinology* **93,** 1001 (1973).
6. D. G. Pipeleers, M. Marichal and W. J. Malaisse, *Endocrinology* **93,** 1012 (1973).
7. W. J. Malaisse, D. G. Pipeleers and J. Levy, *Biochim. biophys. Acta* **362,** 121 (1974).
8. 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), p. 61. Excerpta Medica Foundation, Amsterdam (1967).
9. W. J. Malaisse and V. Leclercq-Meyer, *Rev. europ. étud. clin. biol.* **17,** 310 (1972).
10. A. Herchuelz and W. J. Malaisse, *Diabetologia* **9,** 309 (1973).
11. G. R. Brisson and W. J. Malaisse, *Canad. J. Physiol. Pharmacol.* **49,** 536 (1971).
12. T. Tanese, N. R. Lazarus, S. Devrim and L. Recant, *J. Clin. Invest.* **49,** 1394 (1970).
13. T. O. Tjioe and A. Wacker, *Arzneim.-Forsch.* **22,** 1890 (1972).
14. H. Schatz, V. Maier, C. Nierle and E. F. Pfeiffer, *FEBS Lett.* **26,** 237 (1972).

15. H. Puchinger and A. Wacker, *FEBS Lett.* **21,** 14 (1972).
16. B. J. Lin and R. E. Haist, *Endocrinology* **92,** 735 (1973).
17. A. Loubatieres, M. M. Mariani, R. Alric and J. Chapal, in *Tolbutamide...After Ten Years* (Eds. W. J. H. Butterfield and W. Van Westering), p. 100. Excerpta Medica Foundation, Amsterdam (1967).
18. H. S. Seltzer and R. J. Crout, in *Tolbutamide...After Ten Years* (Eds. W. J. H. Butterfield and W. Van Westering), p. 114. Excerpta Medica Foundation, Amsterdam (1967).
19. H. Stork, F. H. Schmidt, S. Westman and C. Hellerström, *Diabetologia* **5,** 279 (1969).
20. B. Hellman, J. Sehlin and I.-B. Täljedal, *Endocrinology* **89,** 1432 (1971).
21. B. Hellman and L.-Å. Idahl, *Acta diabet. lat.* **6** (Suppl. 1), 597 (1969).
22. S. J. H. Ashcroft, L. Chatra, C. Weerasinghe and P. J. Randle, *Biochem. J.* **132,** 223 (1971).
23. S. Westman and C. Hellerström, *Postgrad. Med. J.* (Suppl. December 1970), 28 (1970).
24. S. J. H. Ashcroft and P. J. Randle, *Acta diabet. lat.* **6** (Suppl. 1), 538 (1969).
25. W. J. Malaisse, F. Malaisse-Lagae and P. H. Wright, *Endocrinology* **80,** 99 (1967).
26. H. Schatz, V. Maier, C. Nierle and E. F. Pfeiffer, *Diabetes* **22,** 443 (1973).
27. W. J. Malaisse and F. Malaisse-Lagae, *Eur. J. Pharmac.* **9,** 93 (1970).
28. W. J. Malaisse, F. Malaisse-Lagae and G. Brisson, in *Recent Hypoglycemic Sulfonylureas* (Eds. U. C. Dubach and A. Bückert), p. 114. Hans Huber, Bern (1971).
29. W. J. Malaisse, D. G. Pipeleers and M. Mahy, *Diabetologia* **9,** 1 (1973).
30. G. S. Levey, W. M. I. Schmidt and D. H. Mintz, *Metabolism* **21,** 93 (1972).
31. W.-N. Kuo, D. S. Hodgins and J. F. Kuo, *J. biol. Chem.* **248,** 2705 (1973).
32. I. D. Goldfine, R. Perlman and J. Roth, *Nature, Lond.* **234,** 295 (1971).
33. D. J. Sams and W. Montague, *Biochem. J.* **129,** 945 (1972).