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In vitro metabolic effects of gliclazide and glibenclamide in the rat

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The actions of gliclazide and glibenclamide on glucose uptake and glycogen deposition by rat hemidiaphragm and glucose utilization by rat epididymal adipose tissue have been examined *in vitro*. Neither drug exerted an insulin-like action on muscle or adipose tissue with respect to glucose uptake, glycogen deposition or glucose utilization. Neither gliclazide nor glibenclamide augmented the stimulating action of insulin on glucose uptake or glycogen deposition in the rat hemidiaphragm. In a high concentration (1 μg ml^{-1}), but not in lower concentrations, glibenclamide enhanced the action of insulin in stimulating glucose utilization by rat epididymal adipose tissue. Gliclazide was without any significant effect. Twenty eight day oral treatment with gliclazide did not increase basal or insulin-stimulated glucose uptake or glycogen deposition in rat hemidiaphragm muscle or glucose utilization by epididymal adipose tissue. It is concluded that in normal rats sulphonylureas do not exert important insulin-like actions or insulin potentiating effects.

It is generally believed that hypoglycaemic sulphonylurea drugs act primarily by stimulating the secretion of insulin. However, the failure in some studies to detect elevated insulin concentrations during chronic administration of sulphonylureas despite a continued amelioration of the symptoms of diabetes mellitus had led to an examination of the possible role of extrapancreatic actions in the production of the therapeutic effect of these agents (Reaven & Dray 1967; Feldman & Lebovitz 1971; Duckworth et al 1972).

In a previous study (Furman & Musbah 1977) we found neither gliclazide nor glibenclamide to produce hypoglycaemia in the absence of functional β-cells. Moreover, neither drug was found to augment the metabolic effects of insulin. In view of previous findings that glibenclamide and other sulphonylureas could enhance glucose uptake by skeletal muscle (Feldman & Lebovitz 1969; Standing & Foy 1970; Beyer et al 1972) it was considered that the *in vivo* experiments used may have lacked sensitivity in allowing the demonstration of extrapancreatic actions. Therefore, we have examined the possible in vitro effects of gliclazide and glibenclamide on glucose uptake and glycogen deposition by skeletal muscle and glucose utilization by adipose tissue. Some of these experiments were carried out additionally using tissues removed from animals treated for 28 days with gliclazide.

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MATERIALS AND METHODS

Animals. Male Wistar rats (200 g–300 g) maintained on a standard diet (Oxoid 41B) were fasted overnight, killed by a blow on the head and bled.

Glucose uptake by hemidiaphragm. The diaphragms were excised and treated as described by Vallence-Owen & Hurlock (1954). The hemidiaphragms were placed in 25 ml conical flasks with 2 ml Krebs-Henseleit buffer solution (pH 7·3 containing 2 mg ml^{-1} glucose) gassed with O_2/CO_2 and incubated for 90 min at 37 °C with continuous shaking (60 min^{-1}). One hemidiaphragm from each rat was used as a control and the other as a test tissue. Gelatin (2 mg ml^{-1}) was used to prevent insulin from being adsorbed on to the walls of the vessel (Guidox et al 1974). In each experiment some flasks containing buffer solution were incubated without tissue to make allowances for any degradation of glucose in solution not attributable to tissue uptake. After incubation, the glucose concentration of the medium was measured enzymatically (Beckman Glucose analyzer) and the amount of glucose taken up by the tissues was calculated.

Glucose oxidation by epididymal fat. The method was based on that described by Snyder & Godfrey (1961). Pieces of epididymal fat pad were placed in modified Warburg flasks containing 2 ml buffer solution with 2 mg ml^{-1} glucose and 0·1 μCi ml^{-1} [D-U-^{14}C] glucose (Radiochemical Centre, Amersham) and incubated as for diaphragms. Then 0·4 ml of 6 M sulphuric acid was injected through

the rubber enclosure into the medium and 0·4 ml of hyamine hydroxide was injected into the central well of each flask. The flasks were shaken for a further 3 h after which the hyamine was transferred quantitatively into counting vials and the radioactivity was determined by liquid scintillation counting using a Packard Tricarb Liquid Scintillation Spectrometer. Counting efficiency (internal standards) was about 60% and did not vary among the different samples. Results are therefore expressed as counts min^{-1}.

[^{14}C]Glycogen deposition by hemidiaphragm. Hemidiaphragms were incubated in 2 ml buffer solution (1 mg ml^{-1} glucose and 0·1 μCi ml^{-1} [D-U-^{14}C]-glucose) as above. They were then transferred into separate Nessler's tubes containing 2 ml of 30% potassium hydroxide solution and kept at 100 °C for 15–20 min with periodic shaking. Ethanol (4 ml of 95%) was added, the tubes allowed to stand for 15–20 min, centrifuged for 20 min (MSE bench centrifuge) and after the supernatants had been poured off the tubes were inverted and drained for 10 min. The glycogen residues (Wardlaw & Moloney 1961) were transferred into scintillation vials and the radioactivity determined as above.

28 day administration of gliclazide. Gliclazide (10 mg kg^{-1}) or vehicle (alkaline 0·9% sodium chloride solution pH 10) was given orally daily to 2 groups of rats with the aid of a stomach tube for a period of 28 days. No pair feeding or force feeding technique was used but the animals were weighed daily. Tissues from these animals were used as described above.

Statistical methods. All results were expressed as mean ± s.e.m. Statistical significance was determined using Student's *t*-test for unpaired observations. Differences were considered significant where $P < 0.05$.

Drugs. Glibenclamide and gliclazide (generous gifts from Hoechst Pharmaceuticals and Servier Laboratories respectively) were dissolved in alkaline saline and diluted in the Krebs-Henseleit buffer. Wellcome insulin was used.

RESULTS

Effects of insulin. Insulin (100–5000 μU ml^{-1}) produced a dose-dependent enhancement of basal glucose uptake and glycogen deposition by rat hemidiaphragm and increase in the production of $^{14}CO_2$ from [^{14}C]glucose by epididymal adipose tissue (Fig. 1). Epididymal adipose tissue appeared to be more sensitive to insulin than was the diaphragm.

Effects of sulphonylureas. Neither gliclazide (2–50 μg ml^{-1}) nor glibenclamide (0·1–10 μg ml^{-1}) produced any direct effect on glucose uptake or [^{14}C]glycogen deposition by hemidiaphragms or [^{14}C]glucose oxidation to $^{14}CO_2$ by epididymal adipose tissue relative to respective control values of 3·7 ± 0·48 mg g^{-1}, 4452 ± 452 counts min^{-1} g^{-1}, and 8313 ± 964 counts min^{-1} g^{-1}. Additionally, neither drug augmented the effects of a submaximal dose of insulin (500 μU ml^{-1}) in stimulating glucose uptake or [^{14}C]glycogen deposition in diaphragm muscle. The combination of sulphonylurea with insulin consistently produced a larger effect than

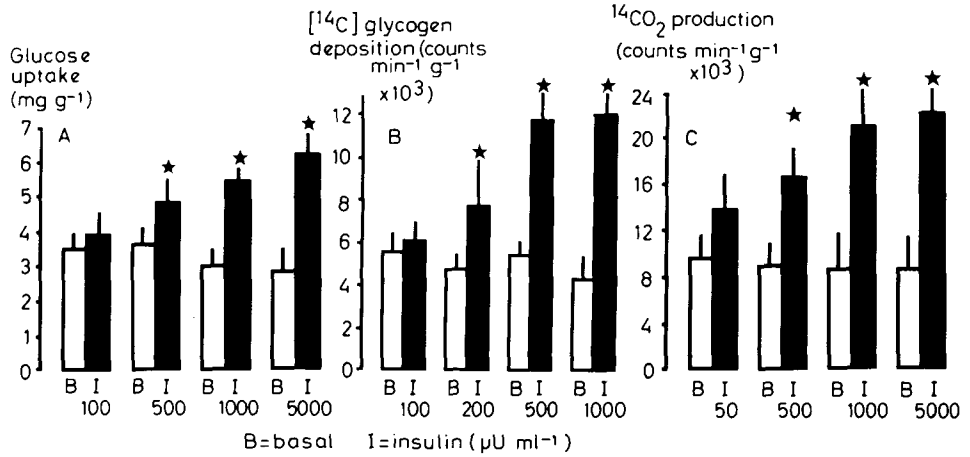

FIG. 1. Effect of insulin on basal glucose uptake and [^{14}C]glycogen deposition by rat hemidiaphragm and glucose utilization ($^{14}CO_2$ production from [^{14}C]glucose) by rat epididymal adipose tissue. Each column represents the mean ± s.e.m. of 6–10 observations.

insulin alone on $^{14}CO_2$ production from [^{14}C]glucose in adipose tissue. However, this difference achieved statistical significance only with glibenclamide 1 μg ml^{-1} (P <0·05 >0·025) and not with other doses of glibenclamide, or with gliclazide (Table 1).

Studies using tissues from rats treated for 28 days with gliclazide. Basal or insulin stimulated values for glucose uptake and [^{14}C]glycogen deposition by hemidiaphragm tissue or $^{14}CO_2$ production from [^{14}C]glucose in epididymal adipose tissue removed from gliclazide-treated rats were not significantly different from corresponding values measured in control tissues. Values for tissues removed from control animals were, for glucose uptake 3·73 ± 0·16 mg g^{-1} (basal) and 5·00 ± 0·16 mg g^{-1} (insulin stimulated) (P <0·02) for glycogen deposition 4404 ± 661 counts min^{-1} g^{-1} (basal) and 9982 ± 1908 (insulin stimulated) (P <0·02), for $^{14}CO_2$ production in adipose tissue, 9256 ± 1251 counts min^{-1} g^{-1} (basal) and 16 015 ± 1522 counts min^{-1} g^{-1} (insulin stimulated) (P <0·02).

Gliclazide- and control-treated rats showed identical weight gains over the treatment period.

DISCUSSION

Although it is difficult to explain the therapeutic efficacy of sulphonylurea drugs solely in terms of their ability to increase insulin secretion there is no general agreement about which additional mechanisms are involved. Several studies have shown various sulphonylureas to have a direct effect in stimulating glucose uptake by rat or mouse diaphragm muscle and adipose tissue in vitro (Quijada et al 1962; Standing & Foy 1970; Beyer et al 1972) and to augment the actions of insulin on these tissues (Feldman & Lebovitz 1969; Standing & Foy 1970; Beyer et al 1972). On the other hand these findings have not been confirmed universally (Cahill et al 1957; Fry & Wright 1957; Vallence-Owen et al 1959). Our findings do not confirm earlier reports in which glibenclamide was found to stimulate glucose uptake by rat and mouse diaphragm muscle (Standing & Foy 1970; Beyer et al 1972). Moreover, we failed to demonstrate any effect of this drug on glycogen deposition in rat diaphragm or glucose utilization by rat epididymal adipose tissue. Similar negative findings were obtained using gliclazide, a sulphonylurea that has not previously been studied in these in vitro systems. The muscle and adipose tissue used here showed a sensitivity to insulin comparable with that found in other studies yet did not respond to sulphonylureas. The range of concentrations of drugs include those concentrations known to stimulate insulin secretion in vitro (Malaisse & Leclercq-Meyer 1972) and for glibenclamide were identical to those used in other studies with muscle in vitro. There is thus no

Table 1. Effects of insulin, alone or in combination with gliclazide or glibenclamide on glucose uptake or [^{14}C]glycogen deposition by rat hemidiaphragm or glucose utilization ($^{14}CO_2$ production) by rat epididymal adipose tissue. The numbers in parentheses refer to the number of observations. Each value is the mean ± s.e.m. The concentration of insulin in the glucose uptake experiments (A) was 500 μU ml^{-1} and in the glycogen deposition (B) and glucose utilization (C) experiments was 250 μU ml^{-1}. The low concentration of gliclazide (gliclazide$_{LOW}$) in (A) was 2 μg ml^{-1} and in (B) and (C) was 5 μg ml^{-1}. The high concentration of gliclazide (gliclazide$_{HIGH}$) was 50 μg ml^{-1} in all experiments. The low concentration of glibenclamide (glibenclamide$_{LOW}$) was 0·05 μg ml^{-1} in (A) and 0·1 μg ml^{-1} in (B) and (C). The high concentration of glibenclamide was 1 μg ml^{-1} throughout.

	A Glucose uptake mg g^{-1} wet wt diaphragm (n = 10)	B [^{14}C]Glycogen deposition counts min^{-1} g^{-1} wet wt diaphragm (n = 8)	C $^{14}CO_2$ production, counts per min^{-1} g^{-1} wet wt adipose tissue (n = 10)
Basal	3·69 ± 0·16	4316 ± 421	5301 ± 1204
Insulin	4·62 ± 0·26*	7895 ± 1158*	16 144 ± 1928***
Basal	3·68 ± 0·16	5789 ± 737	6144 ± 723
Insulin + gliclazide$_{LOW}$	4·62 ± 0·28*	10 211 ± 1368**	22 169 ± 2651***
Basal	3·32 ± 0·16	5684 ± 748	6024 + 964
Insulin + gliclazide$_{HIGH}$	4·26 ± 0·21*	9789 ± 1368*	19 759 ± 2630***
Basal	3·74 ± 0·16	4947 ± 632	5301 ± 722
Insulin + glibenclamide$_{LOW}$	4·62 ± 0·21*	8631 ± 1395*	18 554 ± 2167***
Basal	3·38 ± 0·26	5053 ± 750	5311 ± 482
Insulin + glibenclamide$_{HIGH}$	4·41 ± 0·26*	9474 ± 947**	23 614 ± 2409***†

* Indicates a significant difference between insulin stimulated and basal values (*P<0·05, **<0·01, ***<0·001).
† Indicates a significant difference between the combination of insulin and drug and insulin alone P <0·025.
* Indicates a statistically significant difference from the appropriate control basal value (represented by the open columns) (P <0·05).

obvious reason for the difference between our findings and those of others in relation to glibenclamide. On the other hand, the present results are fully compatible with our unpublished findings that neither gliclazide nor glibenclamide lowered blood glucose or augmented the actions of insulin in eviscerated rats (Furman & Musbah, in preparation). It thus seems unlikely that the acute hypoglycaemic effects of these drugs is mediated by any important direct action on muscle or adipose tissue. Butterfield et al (1962) could not show any effect of tolbutamide on forearm glucose uptake in normal and diabetic man. The tendency of either sulphonylurea to enhance the effect of insulin in stimulating glucose utilization in adipose tissue in the present experiments is interesting although the importance of the effect is doubtful in view of the achievement of statistical significance only with a relatively high dose of glibenclamide.

The failure to demonstrate acute extrapancreatic effects of these drugs may indicate that chronic exposure of the tissues to sulphonylureas may be necessary for such effects to become evident. Feldman & Lebovitz (1969) found tolbutamide to augment the action of insulin in stimulating 2-deoxyglucose transport into mouse diaphragm muscle only after a minimum of 24 h treatment with the drug. However, we found 28 day treatment with maximal doses of gliclazide did not increase basal or insulin stimulated glucose uptake or glycogen deposition in rat diaphragm or glucose oxidation by epididymal adipose tissue.

These experiments do not exclude the possibility of other extrapancreatic mechanisms (inhibition of amino acid release from muscle, inhibition of hepatic glucose production, inhibition of lipolysis). Additionally, they do not exclude the possibility that extrapancreatic mechanisms may operate in diabetic tissues in which the sulphonylureas may interact specifically with some mechanism contributing to the impaired metabolism of these tissues (Reaven et al 1976). Lebovitz et al (1977) have shown gliclazide to augment insulin stimulated glucose disposal in diabetic patients after 4–6 weeks of treatment. Recent work has shown sulphonylurea drugs to increase the number of insulin receptors on mononuclear leucocytes from diabetic patients and on liver cell membranes from ob/ob mice (Olefsky & Reaven 1976; Greenstein 1979).

Finally, the importance of extrapancreatic actions may differ among various members of the sulphonylurea group of drugs although there appears to be no uniformity of findings among different researchers in relation to any one particular drug.

REFERENCES

Beyer, J., Gather, W., Schoffling, K. (1972) Arzneim-Forsch. 22: 2179–2182

Butterfield, W. J. H., Arab, M. M. H., Buckle, A. L. J., Chlouverakis, C., Hanley, T., Mahler, R. F., Wichelow, M. J. (1962) Diabetes Suppl. 11: 43

Cahill, G. F., Hasting, A. B., Ashmore, J. (1957) Diabetes 6: 26–27

Duckworth, W. C., Solomon, S. S., Kitabchi, A. (1972) J. Clin. Endocrinol. Metab. 35: 585–591

Feldman, J. M., Lebovitz, H. E. (1969) Diabetes 18: 84

Feldman, J. M., Lebovitz, H. E. (1971) Ibid. 20: 745–755

Fry, I. K., Wright, P. H. (1957) Br. J. Pharmacol. 12: 350–357

Furman, B. L., Musbah, M. O. (1977) J. Pharm. Pharmacol. 29 Suppl. 55P

Greenstein, B. D. (1979) Br. J. Pharmacol. 66: 317–322

Guidox, R., Mohren, G., Peters, G. (1974) Naunyn-Schmiedeberg's Arch. Pharmacol. 283: 37–52

Lebovitz, H. E., Feinglos, M. N., Bucholtz, H. K., Lebovitz, F. L. (1977) J. Clin. Endocrinol. Metab. 45: 601–604

Malaisse, W. J., Leclercq-Meyer, V. (1972) Eur. J. Clin. Biol. Res. 17: 310–314

Olefsky, J. M., Reaven, G. M. (1976) Am. J. Med. 60: 89–95

Quijada, C., Candilla, R. R., Candilla, J. L. R. (1962) Med. Exp. 6: 65–71

Reaven, G., Dray, J. (1967) Diabetes 16: 487–492

Reaven, G. M., Bernstein, R., Davis, B., Olefsky, J. M. (1976) Am. J. Med. 60: 80–88

Snyder, F., Godfrey, P. (1961) J. Lipid Res. 2: 195

Standing, V. F., Foy, J. M. (1970) Postgrad. Med. J. Dec. Suppl. 16–20

Vallence-Owen, J., Hurlock, B. (1954) Lancet 1: 68

Vallence-Owen, J., Joblin, G. F., Fraser, P. (1959) Lancet 2: 584–586

Wardlaw, A. C., Moloney, P. J. (1961) Can. J. Biochem. 39: 695–712