which represents additive or antagonistic effects of two sigmoid dose–response curves. The parameters estimated by the equation were as follows: $C_{50_1} = 15.8\,\mu M$; $C_{50_2} = 4.9\,\mu M$; $P_1 = 1.9$; $P_2 = 1.5$; $W_1 = 136.5\%$; $W_2 = -84.5\%$ where W_1 and W_2 represent the maximal effect achieved; C represents the concentration of drug; C_{50_1} and C_{50_2} represent the IC_{50} of the drug; and P_1 and P_2 represent the slope of the dose–response curve.

The W_2 parameter is negative indicating an antagonistic effect. The contribution of the second term in the equation on the overall effect becomes less pronounced with increasing concentration of 2,2′-bipyridyl.

In summary it can be concluded from the finding that non-iron saturated 2,2′-bipyridyl combined with desferrioxamine resulted in an antagonistic effect that other mechanisms of action of iron chelators besides iron deprivation inhibit the growth of the parasites.

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Effects of glimepiride and glibenclamide on insulin and glucagon secretion by the perfused rat pancreas

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Hypoglycemic sulfonylureas stimulate insulin release. Conflicting data were reported, however, on their direct effect upon glucagon release (see Ref. 1 for review). Moreover, various sulfonylureas may differ from one another in terms of their glucagonotropic effect [2]. The present study aims at comparing the effects of glimepiride and glibenclamide upon both insulin and glucagon secretion.

Materials and Methods

The present study was conducted on 20 fed female Wistar rats (body weight: 244 ± 3 g). The perfusion of the pancreas [3, 4] and measurement of insulin and glucagon output [5, 6] were performed as previously described. The flow rate averaged 1.36 ± 0.03 mL/min and the perfusion pressure 30 ± 1 mmHg. The glucose concentration of the perfusate was increased from 5.6 to 16.7 mM at the 86th min. Glimepiride and glibenclamide (final concentration 0.1 μM) were first dissolved in dimethyl sulfoxide (DMSO) (final concentration 0.01%, v/v) and administered from the 41st to 60th min. After perfusion, the weight of the pancreas averaged 634 ± 29 mg, its insulin content 183 ± 15 μg/g and its glucagon content 10 ± 1 μg/g (N = 20 in all cases). All results are expressed as the mean value ± SEM. Statistical comparisons were conducted using the two-tailed non-paired *t*-test.

Results

Both glimepiride and glibenclamide caused a biphasic increase of insulin release, which between the 55th and 60th min was about five times higher than basal output (Fig. 1). The stimulation of insulin release was not reversed upon the removal of the sulfonylureas. The later rise in D-glucose concentration provoked a marked and biphasic increase in insulin output. Between the 87th and 100th min, the output of insulin was higher ($P < 0.01$) in the pancreases first exposed to glimepiride or glibenclamide than in the control experiments. The glucose-induced increment in insulin output (min 87–100 vs min 65–85) was not significantly higher, however, in the glimepiride group (+ 46.3 ± 4.3 ng/min) or glibenclamide group (+ 50.6 ± 8.1 ng/min) than in the control group (+ 34.8 ± 3.2 ng/min).

The infusion of the sulfonylureas induced a small but significant fall in glucagon output (Fig. 2). Thus, the paired

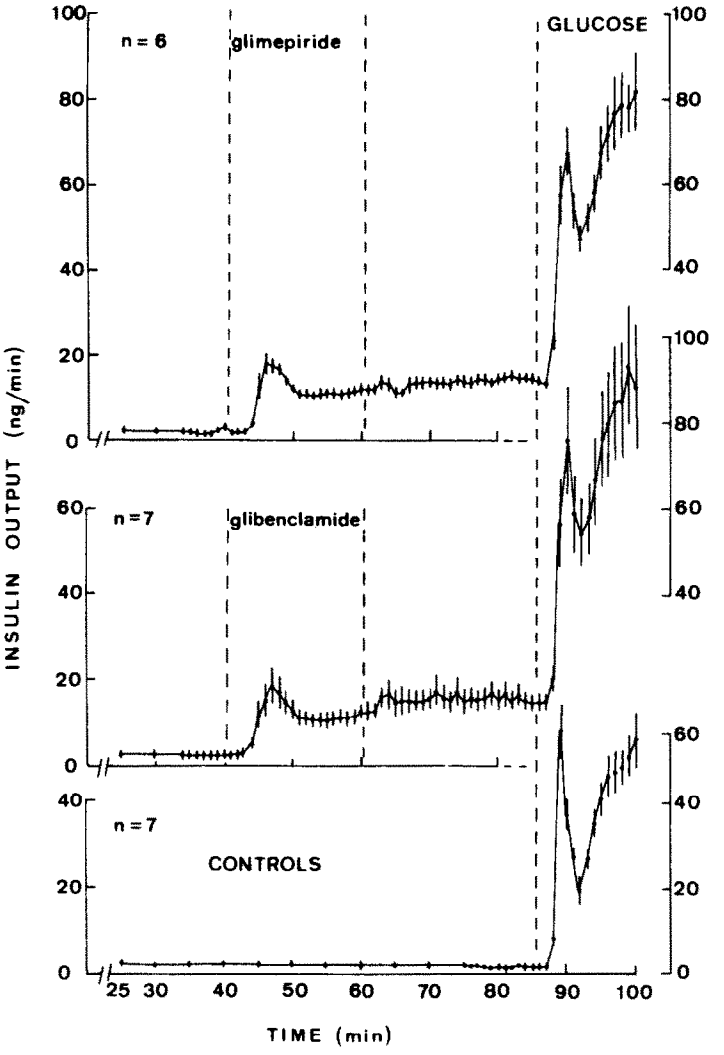

Fig. 1. Effects of glimepiride (0.1 μM, top panel, N = 6) and glibenclamide (0.1 μM, middle panel, N = 7) upon insulin release from the rat pancreas perfused in the presence of 5.6 mM glucose. The sulfonylureas were administered for 20 min as indicated by the vertical dotted lines, no correction being introduced for the dead space of the perfusion device. The control experiments were conducted until min 85 in the sole presence of 5.6 mM glucose (bottom panel, N = 7). A 16.7 mM glucose stimulus was applied at the end of all the experiments (vertical dotted line at min 86).

comparison of glucagon output between min 41–44 and min 46–40 indicated a reduction of 20 ± 4% in the glimepiride group and 21 ± 7% in the glibenclamide group as distinct ($P < 0.05$ or less) from a modest fall of 6 ± 2% in the control experiments. There was no relief from inhibition upon the removal of the sulfonylureas. Moreover, the later rise in glucose concentration failed to affect the already low glucagon secretory rate. This contrasted ($P < 0.01$) with a paired decrease in glucagon output of 29 ± 5% attributable to the rise in glucose concentration in the control experiments.

Discussion

The present results confirm [2] that both glimepiride and glibenclamide cause an immediate and sustained stimulation of insulin release from pancreases exposed to a glucose concentration comparable to that found in the plasma of fasted rats. The insulinotropic action of these drugs persisted after their removal from the perfusate, in fair agreement with data collected in perifused islets [7]. Even so, a further rise in D-glucose concentration augmented insulin output to the same absolute extent as recorded in experiments conducted throughout in the absence of hypoglycemic sulfonylureas. There was therefore no evidence, within the limits of these experiments, for any unfavourable late effect of these drugs upon the B-cell secretory responsiveness to D-glucose.

Under the present experimental conditions, both glimepiride and glibenclamide failed to stimulate glucagon release and, on the contrary, caused a modest inhibition of glucagon secretion. Prior exposure of the pancreas to these drugs also prevented the fall in glucagon output otherwise resulting from a rise in D-glucose concentration. This could conceivably be due to the fact that glucagon

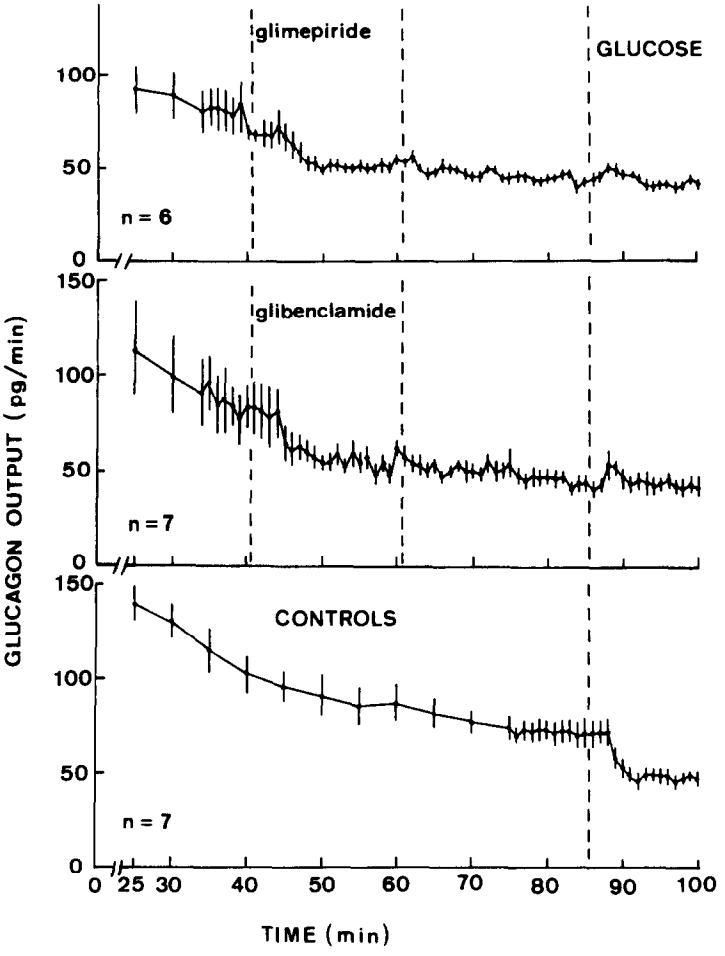

Fig. 2. Effects of glimepiride and glibenclamide upon glucagon release from the perfused rat pancreas. Description of the experiments and graph as in Fig. 1.

release was already inhibited, as a result of the prior exposure to the sulfonylurea.

A current hypothesis ascribes the insulinotropic action of hypoglycemic sulfonylureas to the closure of ATP-responsive K^+ channels [7] and the proposal was made that glucagon-producing cells might be devoid of such channels [8, 9]. If so, the influence of glimepiride and glibenclamide upon glucagon release, as disclosed in the present study, would reinforce the view that all secretory effects of these drugs are not solely attributable to their interaction with the ATP-sensitive K^+ channels [10]. It could be argued, however, that the sulfonylurea-induced changes in glucagon release result from a paracrine effect [11, 12].

In summary, glimepiride and glibenclamide act apparently in a closely comparable manner upon both insulin and glucagon release. Except for the decreased efficiency of D-glucose in suppressing glucagon release after a prior exposure of the pancreas to the hypoglycemic sulfonylureas, no evidence was obtained to suggest that a positive glucagonotropic action of the latter drugs would counteract their hypoglycemic action, as mainly attributable to stimulation of insulin release.

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Organic aciduria in fasted rats caused by 2-[6-(4-chlorophenoxy)hexyl]oxirane-2-carboxylate (etomoxir)

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Impairment of hepatic mitochondrial β-oxidation by disease or inhibitors is often associated with a massive organic aciduria [1–3]. Long-chain fatty acids that cannot be oxidized by the mitochondria undergo β-oxidation to form long-chain dicarboxylic acids in the endoplasmic reticulum [4]. These are then converted to their mono-acyl-CoA esters by an acyl-CoA synthase in the endoplasmic reticulum, which then undergo partial β-oxidation in the peroxisomes to medium-chain and short-chain decarboxylic acids [5]. 2-[6-(4-Chlorophenoxy)hexyl]oxirane-2-carboxylate (etomoxir) is hypoglycaemic in fasted animals and its CoA ester strongly inhibits mitochondrial β-oxidation at the stage of carnitine palmitoyltransferase I [6, 7]. It was therefore of interest to determine the effects of etomoxir on the excretion of organic acids in fasted rats.

Male Wistar rats from a local strain (200–250 g) were maintained on a 13 hr light/11 hr dark cycle. Food was withdrawn at 9.00 a.m. and they were put individually in metabolic cages and injected intraperitoneally with a solution of the sodium salt of *RS*-etomoxir (50 μmol/kg body wt) or 0.14 M NaCl at 3.00 p.m. Urine was collected for 18 hr. The rats were then killed by cervical dislocation at 9.00 a.m. the next day. Urinary organic acids were extracted and analysed by gas-liquid chromatography and their identities were confirmed by mass spectrometry [8]. Creatinine was determined by the standard alkaline picrate method. Rat livers were gently homogenized in 250 mM sucrose, 2 mM Hepes, 2 mM EGTA, pH 7.2, 5 mL of buffer for each gram of liver, and the oxidation of [1-^{14}C]-palmitate and [9,10-^{3}H]palmitate was determined in the homogenates. The homogenate (50 μL) containing 1–2 mg of protein was added to 950 μL of medium at pH 7 and 30° containing 110 mM KCl, 10 mM Hepes, 5 mM $MgCl_2$, 10 mM potassium phosphate, 5 mM ATP, 1 mM L-carnitine, 1 mM EGTA and 0.2 mg cytochrome *c*, in plastic vials in a shaking water bath (170 strokes/min). After a 5 min preincubation 0.12 mM palmitate bound to bovine serum albumin in a molar ratio of 5:1 containing 22 kBq [9,10-^{3}H]palmitate and 2 kBq [1-^{14}C]palmitate was added. At appropriate times the reaction was stopped with 200 μL of 5 M $HClO_4$, to precipitate unchanged and esterified palmitate, then centrifuged (100,000 g_{min}) and the supernatant was divided into three aliquots. One aliquot was counted to give the total acid-soluble radioactivity, the second passed down a 0.5 mL Dowex 1 column (Cl^- form, 200 mesh) and the column washed with 2 mL H_2O to give an eluate containing $^{3}H_2O$, [1-^{14}C]acetyl-carnitine and [2-^{3}H]-acetyl-carnitine, and the third adjusted to pH 12 with KOH and kept at 25° for 45 min to hydrolyse acetyl-carnitine and then passed down a Dowex 1 column and washed with 2 mL H_2O so that the eluate only contained $^{3}H_2O$ released from [9,10-^{3}H]palmitate. The amount of $^{14}CO_2$ formed from [1-^{14}C]palmitate by liver homogenates is small and so was not measured [9].

Administration of etomoxir caused a marked organic aciduria with the excretion of large amounts of the dicarboxylic acids hexanedioic acid, heptanedioic acid, octanedioic acid, decanedioic acid, undecanedioic acid and hexadecanedioic acid (Table 1). The excretion of other organic acids was not significantly different from the controls (Table 1).

The rates of palmitate oxidation measured using [9,10-^{3}H]palmitate or [1-^{14}C]palmitate agreed within 10%. About 70% of the ^{3}H released from [9,10-^{3}H]palmitate was as