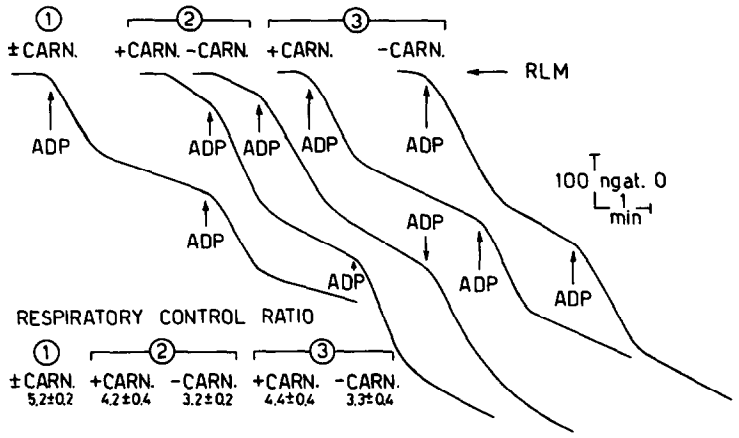

Fig. 2. Oxygen consumption traces of mitochondria isolated from rat livers perfused in the presence and in the absence of L-carnitine. Mitochondria were isolated from rat livers after 10 min perfusion in the presence and in the absence of 0.75 mM L-carnitine. The respiratory rates were measured in medium A (1) and B (2) immediately after the isolation procedure, and in medium B after 6 hr storage at 0° (3). Mitochondria (RLM; 3 mg of mitochondrial proteins) or 150 μM ADP were added when indicated by the arrows. The oxygen traces represent a typical set of experiments and the mean values (±S.D.) of respiratory control ratios of 10 different sets of experiments are also reported (+CARN vs −CARN: $P < 0.01$ for RCR ②, $P < 0.025$ for RCR ③).

damaging action of noxious conditions, like aging. It may be assumed that the observed protective action of L-carnitine is due to the removal, during liver perfusion, of long-chain acyl CoA by conversion to the corresponding long-chain acyl carnitines by the membrane bound carnitine palmitoyl transferases (E.C.2.3.1.21). This removal results in an increased stability of the mitochondrial membrane with higher efficiency of state 3 respiration (Figs 1 and 2). Furthermore the observed accelerated rate of respiration in state 3 might reflect an easier translocation of adenine nucleotides resulting from the activation of adenylate translocase partially inhibited by long-chain fatty acids.

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Glibenclamide induces glucokinase in rat pancreatic islets and liver

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Hypoglycemic sulfonylureas such as glibenclamide initiate insulin secretion through a direct stimulatory effect on pancreatic B-cell plasma membrane receptors in the B-cells [1]. The insulin secretory response of isolated pancreatic islets to hypoglycemic sulfonylureas is considerably exaggerated by the concomitant presence of glucose in the incubation medium [1], particularly when islets have been isolated from fed animals [2]. But through their hypoglycemic action the sulfonylureas diminish the insulin secretory responsiveness of the B-cell. Thus another mechanism is required to maintain the long-term hypoglycemic action of the sulfonylureas *in vivo*. Here we show that this mechanism is the induction of pancreatic islet and liver glucokinase.

Glucokinase is a high K_m glucose phosphorylating and rate limiting enzyme [3], which regulates the amount of glucose to be stored in the liver [3–5] and the flux through the glycolytic pathway in the pancreatic B-cells thereby coupling extracellular glucose concentration and insulin secretion [6]. While glucose regulates the rate of glucose

phosphorylation by glucokinase in pancreatic islet and liver cells, insulin determines whether glucokinase will phosphorylate glucose or not [7].

Experimental

Pancreatic islets from male Wistar rats (220–260 g) were isolated from the pancreas following collagenase digestion. Tissues were homogenized in ice-cold homogenization medium (20 mM Hepes, 210 mM mannitol, 70 mM sucrose, pH 7.4) [8], supplemented with 1 mM dithiothreitol and 5% glycerol. Supernatant was obtained by three successive centrifugation steps at 700 *g*, 8000 *g* and 100,000 *g*, respectively [9]. Glucokinase activity in 100,000 *g* cytosolic fractions was assayed at 37° and pH 7.4 by recording the increase in absorbance at 340 nm of a reaction mixture containing 20 mM Hepes (pH 7.4), 125 mM KCl, 7.5 mM $MgCl_2$, 5 mM ATP, 0.5 mM NADP, 0.7 U/ml glucose-6-P dehydrogenase from Bakers Yeast, 0.01 U/ml 6-P-gluconate dehydrogenase, 100 mM D-glucose and cytoplasmic supernatant in a total volume of 300 µl (pancreatic islets) or 260 µl (liver), respectively [10–12]. Hexokinase activity was assayed at 1 mM D-glucose and subtracted from the total activity recorded at 100 mM D-glucose to give glucokinase activity. One unit of enzyme activity was defined as 1 µmole of glucose-6-P formed from glucose and ATP per min at 37°. All chemicals were purchased from Merck AG, Boehringer Mannheim GmbH, or Sigma Chemical Co.

Results and discussion

Our observation shows that starvation of rats for four days significantly ($P < 0.001$) reduced glucokinase activity in cytoplasmic fractions from pancreatic islets and liver from 7.1 ± 0.3 to 2.3 ± 0.1 mU/mg protein and from 10.2 ± 0.5 to 3.5 ± 0.1 mU/mg protein, respectively. Pancreatic islet and liver glucokinase activities returned to normal after refeeding two-day fasted animals for two days (7.0 ± 0.2 mU/mg protein and 10.8 ± 0.4 mU/mg protein, respectively). Insulin treatment (a single injection of 5 IU/kg b.wt. Novo Ultralente long-acting insulin two days before measurement of glucokinase activity) also normalized pancreatic islet and liver glucokinase activities in four day fasted rats (6.9 ± 0.4 mU/mg protein and 7.7 ± 0.3 mU/mg protein, respectively) (N = 3–18 in each experiment).

We therefore investigated if an induction of pancreatic islet and liver glucokinase in response to glibenclamide treatment could be the explanation for the improved insulin secretory responsiveness of pancreatic B-cells and the improved glucose disposal by the liver from the circulation. Both pancreatic islet and liver glucokinase activities decreased in dependence on the duration of fasting up to four days (Fig. 1). However, treatment of the rats with the hypoglycemic sulfonylurea glibenclamide during two days before the experiment (twice daily 0.4 mg/kg b. wt.) counteracted the starvation-induced decrease in glucokinase activity in cytoplasmic fractions from pancreatic islets and liver (Fig. 1). In fact a two-day glibenclamide treatment of four-day starved rats returned pancreatic islet (5.6 ± 0.6 mU/mg protein) and liver (7.4 ± 0.3 mU/mg protein) glucokinase activities into the range of enzyme activities also found in pancreatic islets or liver from fed, refed, or insulin treated rats (see above).

This glibenclamide-induced induction of glucokinase was dose-dependent (Fig. 2). Both for pancreatic islet and liver glucokinase the half maximally effective dose of glibenclamide was in the range of 0.15 mg/kg b. wt. (Fig. 2). The induction of glucokinase after glibenclamide treatment was not registered four hours after treatment and was half after one day of treatment (Fig. 3). The somewhat faster induction of pancreatic islet glucokinase as compared to liver glucokinase (Fig. 3) may result from earlier availability of increased insulin concentrations to the pancreatic islet cells as compared to liver cells due to the direct insulin secretory action of glibenclamide on the pancreatic B-cells. Addition of glucokinase antibodies [13] or mannoheptulose [4, 5] to the reaction mixture inhibited glibenclamide-induced pancreatic islet and liver glucokinase activities *in vitro* (data not shown). In accordance with earlier studies [5, 6] hexokinase activities in pancreatic islets and liver were only one-third of the glucokinase activities (Fig. 1).

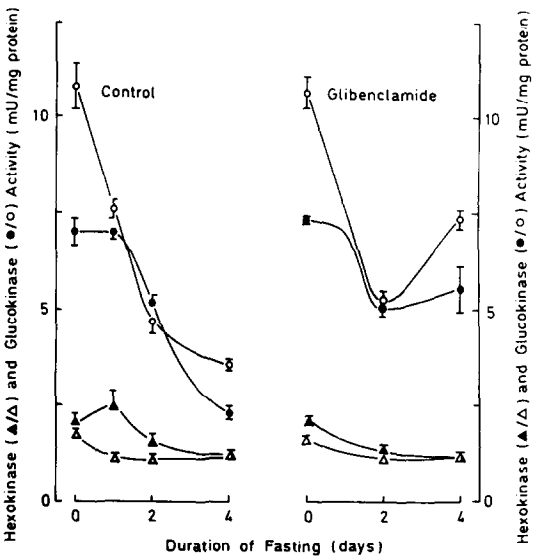

Fig. 1. Starvation-dependent reduction ($P < 0.01$; Anova) of rat pancreatic islet glucokinase (●) and rat liver glucokinase (○) activity (mU/mg protein) (left graph) and the inductive effect of glibenclamide treatment (0.4 mg/kg body weight s.c. twice daily for 2 days) on the starvation-reduced pancreatic islet glucokinase (●) and liver glucokinase (○) (right graph). The glucokinase activity in cytoplasmic fractions from liver from four-day starved glibenclamide-treated rats was slightly higher when compared to two-day starved glibenclamide-treated rats due to a 20% reduction of liver cytoplasmic protein content. A slight starvation-dependent reduction of pancreatic islet (▲) and liver (△) hexokinase (left graph) activity was not affected by glibenclamide treatment (▲ and △) (right graph). Values shown are the means ± S.E. for 3–18 experiments.

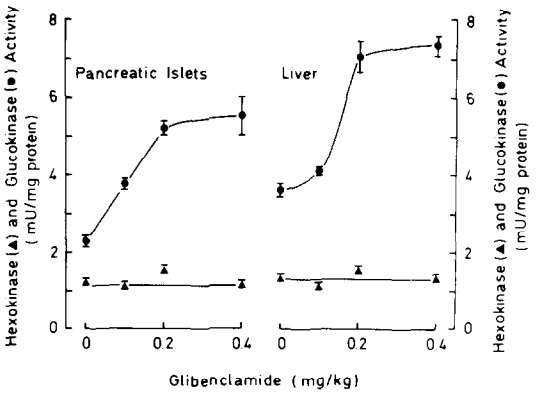

Fig. 2. Dose-dependent induction ($P < 0.05$; Anova) of pancreatic islet and liver glucokinase (●) (mU/mg protein) by glibenclamide treatment (0.1, 0.2, or 0.4 mg/kg body weight s.c. twice daily for 2 days) in four-day starved rats. Pancreatic islet and liver hexokinase (▲) (mU/mg protein) were not affected by glibenclamide treatment. Values shown are the means ± S.E. for 3–18 experiments.

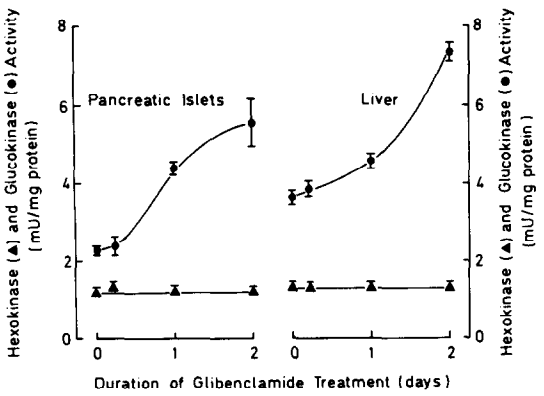

Fig. 3. Time-dependent induction ($P < 0.05$; Anova) of pancreatic islet and liver glucokinase (●) (mU/mg protein) by glibenclamide treatment (once 4 hours, twice daily for 1 day and twice daily for 2 days 0.4 mg/kg body weight s.c. before measurement) in four-day starved rats. Pancreatic islet and liver hexokinase (▲) (mU/mg protein) were not affected by glibenclamide treatment. Values shown are the means ± S.E. for 3–18 experiments.

Hexokinase activities from both tissues were slightly reduced by fasting (Fig. 1) but not affected by glibenclamide treatment in any of the experiments (Figs 1–3).

The inductive effect of glibenclamide treatment on glucokinase is not a direct effect of glibenclamide but rather an effect of the insulin released from the pancreatic B-cells in response to glibenclamide stimulation. This is proven by the fact that fed rats made diabetic (serum glucose concentrations above 15 mM) by injection of alloxan (100 mg/kg b. wt. four days before the experiment) presented liver glucokinase activities in the range of those observed in the liver from fasted non-diabetic rats [14] (4.7 ± 0.4 mU/mg protein; N = 3). These low liver glucokinase activities from diabetic rats were not significantly raised by glibenclamide treatment (6.1 ± 0.1 mU/mg protein N = 4) but normalized by insulin treatment (12.4 ± 1.3 mU/mg protein; N = 6).

Thus our experiments have shown that hypoglycemic sulfonylureas such as glibenclamide can maintain their long-term hypoglycemic action through induction of pancreatic islet and liver glucokinase by insulin. Induction of pancreatic islet glucokinase keeps the glucose recognition system sensitive for the initiation of insulin secretion and biosynthesis by glucose stimulation. This induction of pancreatic islet glucokinase by insulin augments the signal generating flux rate through the glycolytic pathway relative to the actual glucose concentration surrounding the pancreatic B-cell. A sustained insulin secretory response of the pancreatic B-cell to absorptive and post-absorptive glucose keeps liver glucokinase in an induced state. This enables the liver to regulate the blood glucose concentration. Thus the induction of glucokinase *in vivo* represents a link between the pancreatic and extrapancreatic effects of glibenclamide and provides an explanation for the interaction between pancreatic islets and liver in the maintenance of glucose homeostasis [15].

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Uptake and accumulation of gentamicin in the developing inner ear of the mouse *in vitro*

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The sequences of both the bactericidal and the nephrotoxic actions of the aminoglycoside antibiotics include an energy-dependent uptake into the affected cells [1–3]. Whether an analogous step is also part of the ototoxic mechanism is not known. Pharmacokinetic studies relating to the cochlear actions of the aminoglycosides have only provided information on drug disposition in the fluids [4, 5] and the tissues of the inner ear [6, 7]. Questions of transport mechanisms or related issues such as whether the aminoglycosides enter the cells passively along, or actively against, a concentration gradient have not been addressed yet. Electrophysiological studies, however, have suggested that the ototoxic actions of gentamicin require an as yet undefined energy-dependent process [8], and a cellular uptake was postulated in a recent model of aminoglycoside ototoxicity [9].

To determine parameters of uptake, the drug con-