

Insulin-dependent inhibition of hepatic glycogenolysis by gastric inhibitory polypeptide (GIP) in perfused rat liver

H. Hartmann, R. Ebert and W. Creutzfeldt

Division of Gastroenterology and Metabolism, Department of Medicine, University of Göttingen, Federal Republic of Germany

Summary. The effect of porcine gastric inhibitory polypeptide on hepatic glycogen metabolism was investigated in the isolated in situ perfused rat liver. Glycogenolysis was stimulated by infusion of glucagon into the portal vein (half maximal effective portal vein concentration ~ 30 pmol/l). When glucagon was infused at a final portal vein concentration of 0.5 nmol/l, simultaneous addition of insulin inhibited the glucagon-dependent glycogenolysis in a dose-dependent way (half maximal effective concentration for insulin about 2 nmol/l). Gastric inhibitory polypeptide alone at a concentration of 1 nmol/l reduced glucagon-dependent glycogenolysis only

slightly. However, when infused simultaneously at low insulin concentrations (0.1 nmol/l), gastric inhibitory polypeptide suppressed hepatic glucose production dose-dependently up to 70%. The data suggest that gastric inhibitory polypeptide exerts direct metabolic effects on hepatic glycogen metabolism predominantly in a situation where insulin is simultaneously present, e.g. following ingestion of glucose.

Key words: hepatic glycogenolysis, gastric inhibitory polypeptide (GIP), insulin, glucagon.

The role of gastric inhibitory polypeptide (GIP) – a gastrointestinal hormone released upon oral ingestion of glucose and fat – in modulating pancreatic insulin secretion has been well established [1, 2]. GIP is regarded as a component of the entero-insular axis [3].

Direct metabolic effects of GIP have not been demonstrated conclusively. Previous work in isolated rat fat cells has suggested a competitive antagonism towards the lipolytic action of glucagon [4], apparently mediated by decreased intracellular cyclic AMP levels [5]. In addition, GIP has been shown to stimulate lipoprotein lipase activity in cultured preadipocytes [6] and to increase uptake of triglycerides during infusion of chylomicrons in dogs [7].

The data on direct effects of GIP on liver carbohydrate metabolism are contradictory. It has been claimed that GIP exerts glucagon-like effects on hepatic glucose metabolism by increasing glucose production in the perfused rat liver [8]. On the other hand, insulin-dependent suppression of glucagon-induced glucose output by GIP has also been found using the isolated perfused rat liver [9]. Similarly, it has been demonstrated that GIP suppressed glucagon-dependent hepatic glucose production in the presence of insulin using the euglycaemic clamp technique in conscious dogs [10]. The aim of the present work was to study direct metabolic effects of GIP on hepatic glycogen metabolism in vitro using the perfused rat liver.

Materials and methods

Materials

Chemicals were reagent grade and from commercial sources. Enzymes were purchased from Boehringer Mannheim GmbH, Mannheim, FRG; bovine serum albumin, bovine glucagon and bovine insulin

from Serva, Heidelberg, FRG. Porcine GIP was obtained from J.C. Brown, Vancouver, Canada.

Animals

Male Wistar rats (150–200 g) were kept on a 12 h day/night rhythm (dark period from 20.00 h to 08.00 h) with free access to standard Altromin® diet, Altromin, Lage, FRG.

Liver perfusion

All experiments were started at 09.30 h when the animals were just at the beginning of the postabsorptive phase with an average hepatic glycogen content of $400 \mu\text{mol glycosyl units/g liver}^{-1}$ [11]. After anaesthesia by intraperitoneal injection of pentobarbital ($60 \text{ mg} \times \text{kg body weight}^{-1}$) the experiments were performed in a 37°C perfusion chamber (Krannich, Göttingen, FRG). Livers were perfused via the

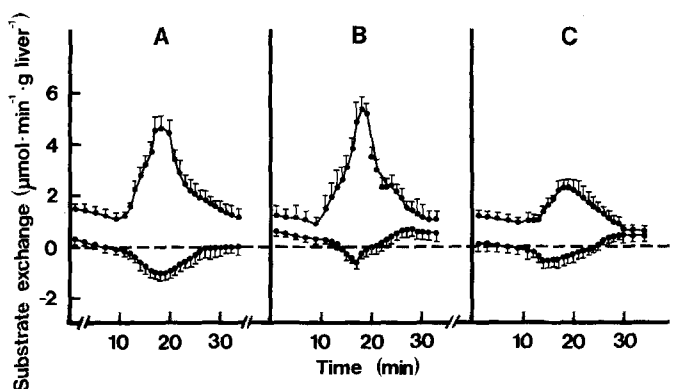


Fig. 1A–C. Suppression of glucagon-dependent stimulation of glycogenolysis in the in situ perfused rat liver by gastric inhibitory polypeptide (GIP) in the presence of insulin. In **A** only glucagon (0.5 nmol/l), in **B** glucagon and insulin (0.1 nmol/l), in **C** glucagon, insulin and GIP (1 nmol/l) were infused simultaneously into the portal vein. The bars indicate infusion periods of 5 min (11–15 min). The upper curves depict glucose, the lower curves lactate exchange. Values are means \pm SEM of 6 experiments for **A** and of 5 and 4 experiments for **B** and **C** respectively

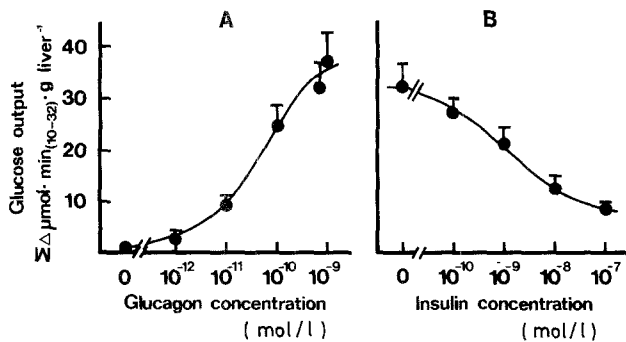


Fig. 2 A and B. Dose response curves for **A** stimulative action of glucagon on hepatic glycogenolysis and **B** suppressive action of insulin on glucagon (0.5 nmol/l)-dependent glycogenolysis. Livers were perfused as depicted in Fig. 1. Glucose output was calculated by integrating the area under the curve for glucose output from the beginning of hormone infusion to min 32 and subtracting the preinfusion values. In **A** various concentrations of glucagon, in **B** simultaneous infusion of a fixed concentration of glucagon (0.5 nmol/l) and various concentrations of insulin were infused. Values are means \pm SEM for **A** of 3 (10^{-12} , 10^{-11} , 10^{-9}), 4 (10^{-10}) experiments, for **B** of 4 (10^{-7} , 10^{-8} , 10^{-9}), 5 (10^{-10}) and 6 (0) experiments

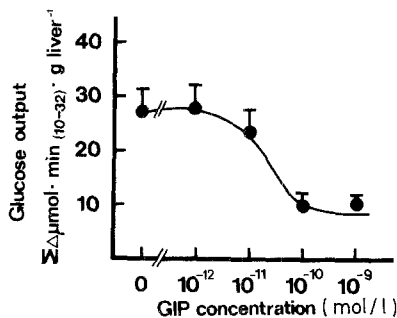


Fig. 3. Dose response curve for the suppressive action of GIP on glucagon (0.5 nmol/l)-dependent glycogenolysis in the presence of insulin (0.1 nmol/l). Hormones were infused as indicated in Fig. 1, glucose output calculated as described in Fig. 2. Values are means \pm SEM of 3 (10^{-10} , 10^{-12}), 4 (10^{-9} , 10^{-11}) and 5 (0) experiments

portal vein without recirculation and hepatic venous samples were collected every minute as described previously [12]. Substrate balances across the liver, e. g. glucose output and lactate uptake, were calculated by subtracting the portal input from the hepatic output (given by portal and hepatic vein substrate concentrations and flow of the medium).

Perfusion media consisted of 30% (v/v) washed bovine red cells suspended in Krebs-Ringer bicarbonate buffer containing 2% (w/v) of bovine serum albumin. Medium glucose and lactate concentrations were adjusted to 5 mmol/l and 2 mmol/l respectively. The flow rate was kept at a rate of 1.8 to 2.0 ml · min⁻¹ · g liver⁻¹, thus resembling physiological flow rates [13, 14]. After an equilibration period of 45 min, samples were collected and hormones were infused into the portal vein for the indicated time periods. Glucose and lactate concentrations were measured photometrically with enzymatic methods using hexokinase plus glucose-6-phosphate dehydrogenase and lactate dehydrogenase [15].

Results

Stimulation of glycogenolysis by glucagon

When glucagon (0.5 nmol/l) was infused into the portal vein, an increase of hepatic glucose output and an in-

Table 1. Effect of gastric inhibitory polypeptide (GIP) on glycogenolysis in the perfused rat liver

| Condition | Hormone concentration | Change in glucose output [ΣΔ μmol · min ⁻¹ · g liver ⁻¹] |
|-------------|-----------------------|--|
| | nmol/l | |
| 1. GIP | 1.0 | 4.9 \pm 0.4 |
| 2. Glucagon | 0.5 | 31.5 \pm 5.3 |
| 3. Glucagon | 0.5 | 26.8 \pm 4.6 |
| Insulin | 0.1 | |
| 4. Glucagon | 0.5 | 21.9 \pm 3.6 |
| GIP | 1.0 | |
| 5. Glucagon | 0.5 | 10.7 \pm 1.7* |
| Insulin | 0.1 | |
| GIP | 1.0 | |

* = $p < 0.01$ from 3 (Student's *t*-test)

Hormones were infused into the portal vein as indicated in Fig. 1. Glucose output was calculated as described in Fig. 2. Values are means \pm SEM of 3, 6, 4, 4 experiments for data group 1, 2, 3, 4, 5 respectively

crease of lactate uptake was observed (Fig. 1A). Since hepatic lactate uptake increased much less (at the peak in C₆ units $\sim 0.5 \mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g liver}^{-1}$) than glucose output ($\sim 4 \mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g liver}^{-1}$), increased glucose production resulted predominantly from glycogenolysis. Upon cessation of hormone infusion, glucose output and lactate uptake returned to preinfusion values. The increase of glucose production by the liver following glucagon infusion was dose-dependent with a half maximal effective portal vein concentration of ~ 30 pmol/l (Fig. 2A).

Suppression of the glucagon-dependent stimulation of glycogenolysis by insulin

Simultaneous infusion of insulin and glucagon (0.5 nmol/l) resulted in a dose-dependent suppression of glucagon-stimulated glycogenolysis with a half maximal effective insulin concentration of about 2 nmol/l (Fig. 2B). At a portal vein concentration of 0.1 nmol/l, insulin did not alter glucagon-stimulated glucose output (Fig. 1B).

Effect of GIP on hepatic glucose output

Infusion of GIP alone (1 nmol/l) did not change hepatic glucose output appreciably compared to preinfusion values (Table 1). Simultaneous infusion of glucagon (0.5 nmol/l) and GIP at high concentration (1 nmol/l) resulted in a slight reduction of glucose output (Table 1).

When GIP was infused simultaneously with insulin (0.1 nmol/l) a dose-dependent inhibition of glucagon-stimulated glucose production was observed (Fig. 3). A maximum suppression of up to 70% was achieved (Fig. 1C). This could already be demonstrated at a physiological hormone concentration of 0.1 nmol/l (Fig. 3).

Discussion

Regulation of hepatic carbohydrate metabolism is achieved by a variety of hormones, especially insulin and glucagon [16, 17], and by the autonomic nervous system [12, 18]. Since it has been demonstrated that GIP as a component of the entero-insular axis modulates insulin secretion from the endocrine pancreas [1, 2], an indirect insulin-mediated contribution to the regulation of hepatic glucose metabolism is well established. In contrast, direct metabolic effects of GIP on the liver have received little attention; moreover, results of *in vitro* and *in vivo* studies yielded contradictory data [8–10]. A consistent effect of insulin on hepatic carbohydrate metabolism in the perfused liver is its antagonistic action towards glucagon-stimulated glycogenolysis [19].

In the present study it was shown that GIP also exerts a direct inhibitory action on glucagon-stimulated glycogenolysis in the presence of insulin. The infusion of GIP alone into the portal vein did not change glucose balance (Table 1). This observation is at variance with data reported by Hanks et al. [8] which demonstrated an increase of glucose production following infusion of GIP (~1 nmol/l) in perfused livers from fasted rats. However, a more recent study by these authors also found that GIP alone does not alter hepatic glucose balance under normo- and hyperglycaemic conditions in perfused livers from fed as well as from fasted animals [20]. In the present study a non-recirculating medium with a high content of erythrocytes (30% v/v) has been used which allows physiological flow rates [13, 14]. This experimental approach has been shown to reliably detect minor changes in metabolite balances across the liver [21].

Simultaneous infusion of glucagon and GIP resulted only in a slight reduction of glucagon-stimulated glycogenolysis at high GIP concentration. However, when insulin was present in the medium at a low concentration which by itself did not significantly affect glucose output, GIP suppressed glucagon-stimulated glycogenolysis in a dose-dependent way. These data are in accordance with earlier findings from our laboratory [9], and with *in vivo* studies reported recently by Andersen et al. who showed an insulin-dependent inhibition of glucagon-stimulated hepatic glucose production by GIP in conscious dogs [10]. The underlying molecular events at the hepatocytes have not been elucidated.

The physiological significance of this insulin-dependent but direct metabolic effect of GIP is not clear. However, it is of interest that not only pancreatic but also gut hormones directly participate in the regulation of hepatic carbohydrate metabolism. Taken together with the finding that GIP also directly stimulates triglyceride uptake [7], this gut peptide seems to act as an anabolic hormone.

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Prof. Dr. W. Creutzfeldt
Medizinische Universitätsklinik
Robert-Koch-Strasse 40
D-3400 Göttingen
FRG