Increases in intestinal glucose absorption and hepatic glucose uptake elicited by luminal but not vascular glutamine in the jointly perfused small intestine and liver of the rat

Andreas GARDEMANN,* Yuji WATANABE, Volker GROBE,† Silvia HESSE and Kurt JUNGERMANN
 Institut für Biochemie, Fachbereich Medizin, Georg-August-Universität, Humboldtallee 23, D-3400 Göttingen, Germany

1. Previous studies have shown that an arterial-to-portal glucose concentration gradient may be an important signal for insulin-dependent net hepatic glucose uptake. It is not known whether intestinal factors also contribute to the regulation of hepatic glucose utilization. This problem was studied in a newly developed model which allows luminal perfusion of the small intestine via the pyloric sphincter and a combined vascular perfusion of the small intestine via the gastroduodenal artery and superior mesenteric artery, and of the liver via the hepatic artery and portal vein. 2. In both the presence and the absence of 1 mM-glutamine in the vascular perfusate, only about 7% of a luminal bolus of 5500 μ mol (1 g) of glucose was absorbed by the small intestine, and nothing was taken up by the liver. 3. With small doses of 75–380 μ mol (11–55 mg) of luminal glutamine, but not with 300 μ mol of alanine, the intestinal absorption of the luminal glucose bolus was increased almost linearly from 7% to a maximum of 40% and the hepatic uptake from 0% to a maximum of 22%. 4. The increase of hepatic glucose uptake caused by luminal glutamine was only observed when the glucose load was applied into the intestinal lumen, rather than into the superior mesenteric artery. 5. The relative hepatic glucose uptake (uptake/portal supply) was enhanced from 0% to 55% with an increase in portal supply by luminal glutamine, whereas with a similar range of portal glucose supply the relative hepatic uptake by the isolated liver, perfused simultaneously via the hepatic artery and portal vein, was slightly decreased, from 20% to 15%. 6. Addition of various amounts of portal glutamine and/or alterations in the Na⁺ content of the portal perfusate failed to mimic the luminal glutamine-dependent activation of hepatic glucose uptake. Therefore the luminal-glutamine-elicited activation of hepatic glucose uptake was apparently not caused by a simple increase in the portal–arterial glucose gradient, by glutamine itself or by Na⁺-dependent alterations in hepatic cell volume. The results suggest that luminal glutamine caused not only an increase in intestinal glucose absorption by unknown mechanisms but also the generation of one or more humoral or nervous ‘hepatotropic’ signals in the small intestine which enhanced the hepatic uptake of absorbed glucose.

INTRODUCTION

The liver, acting as the glucostat of the organism, holds an exceptional anatomical position in that it is supplied with arterial blood via the hepatic artery and simultaneously with venous blood from the stomach, spleen, pancreas and intestine via the portal vein (PV).

Little is known about the regulation of hepatic glucose metabolism by the enterohepatic axis, which comprises substrates (e.g. absorbed nutrients), endocrine factors and neurotransmission. Only the regulation of hepatic glucose metabolism by three gastrointestinal peptides (vasoactive intestinal peptide, gastric inhibitory polypeptide and glucagon-like peptide 1) has been studied in isolated hepatocytes and in the isolated perfused liver [1–4]. With isolated vascular perfusions of the small intestine alone or the liver alone, the possible importance of the enterohepatic axis for the regulation of hepatic glucose metabolism cannot be investigated. *In vitro*, these studies are only possible with a system allowing simultaneous perfusion of the small intestine and the liver, which would allow the investigation of the haemodynamics and metabolism for each organ, as well as the inter-relationships between the small intestine and liver. Only two different combined intestine–liver preparations have been described [5,6]. The first preparation allowed perfusion of the jejunum and ileum exclusively via the superior mesenteric artery

(SMA), and of the liver via the PV only [5]. This model was extended to perfusion of the jejunum and ileum via the SMA and of the liver via the hepatic artery and PV [6]. However, in both systems the duodenum, an important site of glucose absorption, was excluded from the perfusion by ligating the gastroduodenal artery (GDA).

It was the aim of the present investigation to develop a luminal perfusion of the small intestine and a combined vascular perfusion of the small intestine via the GDA and SMA, and of the liver via the hepatic artery and PV, and to investigate whether intestinal glucose absorption and hepatic glucose balance could be modulated by luminal or vascular glutamine. It was found that luminal but not vascular glutamine enhanced the intestinal absorption of glucose by an as yet unknown mechanism, and at the same time enhanced the hepatic uptake of absorbed glucose via the formation of one or more humoral or nervous ‘hepatotropic’ signals by the small intestine. A preliminary report of this work has been published [7].

MATERIALS AND METHODS

Materials

All chemicals were of reagent grade and from commercial sources. Enzymes and BSA were purchased from Boehringer

Abbreviations used: PV, portal vein; SMA, superior mesenteric artery; GDA, gastroduodenal artery; IVC, inferior vena cava; CT, coeliac trunk; PHA, proper hepatic artery.

† Deceased July 17, 1991.

* To whom correspondence and reprint requests should be addressed.

Fig. 1. Scheme showing the combined perfusion of rat small intestine and liver

(Mannheim, Germany), and dextran was from Sigma (München, Germany).

Animals

Male Wistar rats (350–400 g) were obtained from Winkelmann (Borchen, Germany). For at least 1 week before the experiment they were subjected to a 12 h day–night rhythm with free access to food (standard diet from Ssniff, Soest, Germany). All experiments were started at 10:00 h.

Liver perfusion

The animals were anaesthetized by intraperitoneal injection of pentobarbital (200 mg/kg body wt.). The liver was perfused *in situ* without recirculation in a 37 °C cabinet via both the PV and the hepatic artery. The preparation technique has been described in more detail elsewhere [8]. Thus a bivascular perfusion system was established with a total flow rate of about 4 ml/min per g, a low pressure component via the PV (10–15 mmHg; 60–80 % of total flow) and a high pressure component via the hepatic artery (70–90 mmHg; 20–40 % of total flow). The experiments were started after a pre-perfusion period of 30 min.

Analysis of arterial, portal and total flow. Total perfusion flow was quantified by fractionating the effluate into calibrated tubes. The portal flow was measured with an SMS-302 electromagnetic flowmeter from Hellige (Freiburg, Germany) placed into the portal inflow. The arterial flow was obtained as the difference between total and portal flow.

Sampling. Samples were taken every 1 min after an initial period of 30 min and directly cooled on ice.

Combined intestine–liver perfusion

Preparation technique. The animals were anaesthetized by intraperitoneal injection of pentobarbital (200 mg/kg body wt.). The abdomen was opened by a midline incision. Heparin (50 units) was injected into the inferior vena cava (IVC) to avoid coagulation. Without heparin a much delayed and only partial exsanguination of the small intestine and liver was observed. Open ligatures were positioned around the left renal vein, the SMA and around the aorta, caudal and cranial to the vascular origin of the SMA. Rats were killed by injecting an overdose of

pentobarbital (40 mg) into the left renal vein. Immediately afterwards the ligature around the left renal vein was closed, the SMA was cannulated, the IVC was opened by a longitudinal incision and the perfusion of intestine and liver was started, with an initial flow rate of about 30–35 ml/min (diameter of the cannula: 1.4–1.6 mm). The three open ligatures around the aorta and SMA were closed and the cannula was carefully fixed. Then the diaphragm was opened and the ventral part of the ribs removed. Directly afterwards, the coeliac trunk (CT) was cannulated after positioning open ligatures around the CT and around the aorta both cranial and caudal to the vascular origin of the CT (diameter of the cannula: 1.2–1.4 mm). These ligatures were closed and the cannula was carefully fixed. From this stage of the preparation the gastrointestinal organs were perfused at a flow rate of about 40–50 ml/min (Fig. 1).

Next, the spleen, pancreas and stomach were freed from connective and adipose tissue and removed after ligating their arteries and veins. Then the duodenum was cannulated via the pyloric sphincter and the caecum, and the cannulas were fixed. The intestinal content was washed out very carefully with 30–50 ml of warmed saline solution, to prevent intestinal distension and mucosal damage. Afterwards, a cannula was introduced into the right atrium, the tip was pushed forward to the confluence of the hepatic vein and the IVC, and the cannula was fixed with two ligatures. At this stage of the preparation an exclusive perfusion of intestine and liver was established. A flexible catheter (diameter 0.6 mm) was introduced into the PV, the tip was pushed forward to the liver hilus and the catheter was fixed with Superglue at the surface of the portal vein. Then the small intestine and the liver were separated from the rest of the body. This organ block was transferred into a bath filled with 0.9% NaCl (37 °C). Floating the small intestine in this bath was an effective and indispensable method by which to keep vascular perfusion flow and pressure constant. The vascular cannulas of the SMA, CT and IVC and the luminal inflow and outflow cannulas of the small intestine were fixed in their physiological positions. Finally, the abdominal IVC was closed by ligation.

Thus a simultaneous vascular perfusion of the small intestine (including the proximal part of the colon) and of the liver were established via the SMA (65–75 % of total flow) and the CT (25–35 % of total flow). This perfusion system allowed luminal infusion of the small intestine via the pyloric sphincter. The intestine was perfused via the GDA (duodenum) and the branches of the SMA (jejunum, ileum and proximal part of the colon). The liver was perfused via the PV and the proper hepatic artery (PHA) (Fig. 1). The preparations were finished after 50–60 min, and experiments were started after an additional period of 20 min. Total flow rates of about 3.5–4 ml/min per g of intestine for the small intestine and about 4 ml/min per g of liver for the liver were maintained. The intestinal lumen was not perfused. Only 1.5 ml was infused during the 1 min stimulation periods via the pyloric sphincter. Therefore the transit of lumenally applied glucose and/or glutamine was not the result of a constant infusion via the pyloric sphincter, but was due to intestinal peristalsis.

Leakage control. To control leakage, haemoglobin (50 mg/ml) was injected selectively into the inflow cannulas of the CT and SMA after transferring the small intestine and liver into the bath. All leakages were eliminated before the start of the pre-perfusion period. Therefore the measured and calculated flow data represent reliable values for the haemodynamic and metabolic characterization of the small intestine and liver.

Perfusion medium. The perfusion medium used from the beginning of the preparation period until the end of experiment was a Krebs–Henseleit buffer containing 5 mM-glucose, 2 mM-lactate, 0.2 mM-pyruvate, 3 % dextran and 1 % BSA and equili-

brated with O_2/CO_2 (19:1). In the majority of experiments, 1 mM-glutamine was added to the perfusion medium as indicated in the text and Figures.

Analysis of luminal flow and vascular flow and pressure of the small intestine. (i) The luminal outflow (ml/min) was quantified by fractionating the effluente into calibrated tubes. (ii) Fluid secretion into the intestine (ml/min) was defined as the difference between luminal outflow and inflow. (iii) The average fluid secretion (ml/min) was defined as the ratio of the sum of the fractionated fluid secretion and the perfusion time in min. (iv) The vascular flow in the SMA and CT (ml/min) was measured using Hellige SMS-302 flowmeters placed into each inflow. The pressures in the SMA and CT were measured using T-tubes placed into each inflow. (v) The portal flow (ml/min) was defined as the difference between SMA flow and the average net fluid secretion.

Analysis of total, portal and arterial flow of the liver. (i) The total flow in the liver (ml/min) was quantified by fractionating the effluente of the IVC into calibrated tubes. (ii) The portal flow was defined as the flow in the SMA minus the outflow in the portal catheter minus the average fluid secretion into the intestine. The calculated portal flow values were checked by direct measurements of the portal flow with a non-invasive flow electrode positioned around this vessel at the end of some experiments. (iii) Hepatic arterial flow was defined as the difference between total and portal flow.

Sampling. After an initial period of 20 min, samples were taken every 1 min, from: (i) the effluent of the IVC, (ii) the perfusate of the PV, and (iii) the effluent of the small intestine. All samples were cooled on ice directly. Samples of the PV perfusate were collected at a low flow rate of about 0.5 ml/min (compared with a portal flow rate of 25–35 ml/min).

Determination of metabolites

Metabolites were measured using standard enzymic techniques [9]: glucose with glucose dehydrogenase, lactate with lactate dehydrogenase and glutamate pyruvate transaminase, and glutamine with glutamate dehydrogenase and glutaminase. Oxygen was determined with a blood gas analyser ABL 500 from Radiometer (Copenhagen, Denmark).

Evaluation

Metabolic effects were evaluated by calculating the area under the curve of the intestinal and hepatic alterations in glucose balance. When baselines of glucose balance after infusion differed from those of the pre-stimulation period, both baselines were connected for evaluation of the area under the curve. Significance was tested with Student's *t* test for paired or unpaired values, as indicated.

RESULTS

Functional and morphological integrity of the intestine–liver perfusion

Various parameters were used as indexes of the stable functions of the perfused rat intestine–liver preparations. A decrease in flow and a rise in SMA pressure reflected deterioration of the preparation by sensitively indicating tissue oedema, an increase in microvascular resistance and the development of embolism [6,10]. Throughout the experiment the perfusion flow remained nearly constant at about 40–45 ml/min, and the perfusion pressures of the SMA and CT did not exceed the initial values of about 80 mmHg (results not shown). Histological examination of cross-sections taken after 20 min of pre-perfusion and 55 min of perfusion revealed a normal intestinal morphology (results not

shown); tissue oedema and destruction of the mucosal epithelium were not detected. Intestinal peristalsis without spasmodic contractions was maintained throughout the experiment. Under basal conditions, intestinal and hepatic glucose and lactate balance and the lactate/pyruvate ratio did not alter significantly, also indicating the viability of the perfusion system (results not shown). Fluid secretion into the lumen of about 0.5–0.8 ml/min was observed (results not shown).

Oxygen consumption and supply were measured in the presence of vascular glutamine (1 mM) at the beginning and the end of the experiment; data are expressed as means $\pm$ S.D. The oxygen tension was 587 ± 11 mmHg ($828 \pm 16 \mu M$) in the artery, 304 ± 7 mmHg ($429 \pm 10 \mu M$) in the PV and 106 ± 5 mmHg ($149 \pm 7 \mu M$) in the IVC. Thus, with the flow rates maintained (see the Materials and methods section), the oxygen delivery was $2.9 \pm 0.6 \mu mol/min$ per g of intestine and $2.1 \pm 0.1 \mu mol/min$ per g of liver, and the oxygen consumption amounted to $1.4 \pm 0.3 \mu mol/min$ per g of intestine and $1.6 \pm 0.1 \mu mol/min$ per g of liver respectively. The consumption rate for the small intestine compared well with results obtained in intestinal perfusions with red blood cells [6,10–12]. In isolated liver perfusions with erythrocyte-free media the oxygen uptake was between 2 and $2.5 \mu mol/min$ per g [13–18].

Activation of intestinal glucose absorption and hepatic uptake of absorbed glucose by luminal but not vascular glutamine

During the 6th, 31st and 55th min of perfusion, 5500 μmol of glucose (1 g in 1.5 ml of 0.9% NaCl) was infused luminally either without the addition of any other substance or in the presence of 300 μmol (27 mg) of alanine or various amounts of glutamine in

Fig. 2. Effect of luminal and arterial glutamine and of luminal alanine on intestinal glucose absorption and hepatic glucose balance in the combined perfusion of rat small intestine and liver

The perfusion medium was a Krebs–Henseleit buffer containing 5 mM-glucose, 2 mM-lactate, 0.2 mM-pyruvate, 3% dextran and 1% BSA, and was equilibrated with a gas mixture of O_2/CO_2 (19:1). In all experiments glucose was applied intraluminally within 1 min (5500 μmol ; 1 g). Glutamine (Gln) was infused either intraluminally (i.l.) within 1 min (150 μmol ; 22 mg; b) or intra-arterially (i.a.) throughout the experiment (1 mM; c). Alanine was infused intraluminally within 1 min (300 μmol ; 27 mg; d). Intestinal glucose absorption is expressed as portal glucose concentration in mM. Hepatic glucose balance is given by: (concentration in hepatic vein in $\mu mol/ml \times$ flow in ml/min per g) – [(concentration in portal vein in $\mu mol/ml \times$ portal flow in ml/min per g) + (concentration in hepatic artery in $\mu mol/ml \times$ arterial flow in ml/min per g)]. A positive value indicates release; a negative value indicates uptake. Values are means $\pm$ S.E.M. of five (a, b), six (c) or three (d) experiments.

Fig. 3. Hepatic glucose balance after infusion of arterial or portal glucose in rat liver perfused via the hepatic artery and PV

The perfusion medium was a Krebs-Henseleit buffer containing 5 mM-glucose, 2 mM-lactate, 0.2 mM-pyruvate, 1 mM-glutamine, 3% dextran and 1% BSA, and was equilibrated with a gas mixture of O_2/CO_2 (19:1). Glucose was infused via the portal vein (a) to reach a portal concentration of about 10, 15 or 20 mM, or via the hepatic artery (b) to reach a hepatic arterial concentration of 22 or 28 mM. The calculated proximal sinusoidal glucose concentration is given in (c). Hepatic glucose balance (d) is defined in Fig. 2. There was an overshoot in glucose uptake after the sudden increase in portal or arterial glucose concentration due to filling up of the extra- and intra-cellular glucose space, and an overshoot in glucose release after the sudden decrease in portal or arterial glucose concentration due to wash-out from the previously filled up glucose space. Therefore the apparent uptake and release due to filling and emptying of the glucose spaces correspond to the open area under the curve; glucose uptake due to metabolism is shown as the hatched area under the curve. A positive value indicates release; a negative value indicates uptake. Values are means $\pm$ S.E.M. of three experiments.

the range 75–380 μ mol (11–55 mg). Preliminary experiments revealed that the second and third stimulation periods were essentially as effective as the first one (results not shown). The transit time for glucose was 14 ± 2 min.

Only about 7% of the luminal bolus was absorbed by the intestine, and nothing was taken up by the liver (Fig. 2a), when 5500 μ mol of glucose was applied alone. A small dose of 150 μ mol of glutamine, infused luminally with glucose, more than doubled the intestinal glucose absorption and shifted the hepatic glucose balance from output to uptake during the period of intestinal absorption (Fig. 2b). In contrast, intestinal glucose absorption was essentially the same as in the controls and the hepatic glucose balance was not altered when 1 mM-glutamine was added via the SMA and the CT throughout the whole experiment (Fig. 2c). Similar observations were made when 300 μ mol of alanine instead of 150 μ mol of glutamine was applied to the intestinal lumen together with glucose (Fig. 2d). Luminal glutamine (150 and

Fig. 4. Intestinal glucose and glutamine absorption and hepatic glucose balance with increasing luminal glutamine in a combined perfusion of rat small intestine and liver

The perfusion medium described in Fig. 3 was used. Glucose (5500 μ mol; 1 g) and increasing amounts of glutamine [75 (a), 150 (b), 260 (c) and 380 (d) μ mol (11, 22, 38 and 55 mg)] were infused luminally within 1 min. Intestinal glucose and glutamine absorption is expressed as portal glucose concentration in mM. Hepatic glucose balance is defined in Fig. 2; a positive value indicates release; a negative value indicates uptake. Values are means $\pm$ S.E.M. of three (a, c, d) or four (b) experiments.

380 μ mol) did not enhance hepatic glucose uptake when the glucose load was not applied via the intestinal lumen but infused into the SMA, which raised the portal glucose level from 5 to 15 mM (results not shown).

Intestinal and hepatic flow and lactate balance were not significantly altered by luminal glucose in the presence or absence of luminal or vascular glutamine or luminal alanine (results not shown).

An increase in the portal glucose supply as a possible mechanism for the activation of hepatic uptake of absorbed glucose by luminal glutamine

It was observed that luminal glutamine increased intestinal glucose absorption, thereby enhancing the portal glucose supply and increasing the portal-arterial glucose gradient (Fig. 2b versus Fig. 2a). This increased portal glucose supply and enhanced gradient might be the signals for hepatic glucose uptake [8,19]. Therefore the portal glucose supply was varied in the perfusion system of the isolated liver by an infusion pump, and in the combined intestine-liver perfusion by increasing the luminal glutamine content. The calculated relative glucose uptake (uptake/portal supply) of the combined intestine-liver perfusion was compared with that of the isolated liver perfusion. If the relative glucose uptakes of both systems were similar, the activation of hepatic glucose uptake by luminal glutamine would apparently be a result of an increase in the portal glucose supply or an enhanced portal-arterial glucose gradient. All of the following experiments were performed in the presence of 1 mM vascular glutamine, since this amino acid serves as an important respiratory fuel for the intestine [20].

Fig. 5. Dependence of the relative hepatic glucose uptake on the portal glucose supply in the combined perfusion of rat small intestine and liver and in the isolated rat liver perfused via the hepatic artery and PV

Relative hepatic glucose uptake is defined as hepatic glucose uptake ($\mu\text{mol/g}$ of liver) $\times$ 100/portal glucose supply ($\mu\text{mol/g}$ of liver). The data are taken from Figs. 3 and 4. The portal glucose supplies in the combined intestine–liver perfusion corresponded to certain amounts (μmol) of luminal glutamine which are given in parentheses. ●, Isolated liver perfusion; ○, combined intestine–liver perfusion.

Isolated liver perfusion. In the isolated rat liver the basal arterial and portal glucose concentration was 5 mM. During stimulation periods of 5 min the portal glucose concentration was raised to 10, 15 or 20 mM, corresponding to a calculated proximal sinusoidal concentration of 8, 12 or 15 mM. The arterial concentration was raised to 22 or 29 mM, corresponding to a calculated proximal sinusoidal concentration of 12 or 15 mM (Fig. 3). All of these increases in arterial and portal glucose supply resulted in a change from glucose output to uptake. A more pronounced increase in the portal or arterial glucose supply caused a greater enhancement of hepatic glucose uptake. However, the relative hepatic glucose uptake (uptake/portal supply) was slightly decreased (cf. Fig. 5). Surprisingly, arterial application of glucose was as effective as portal infusion in activating hepatic glucose uptake when the calculated proximal sinusoidal glucose concentration was similar during arterial and portal infusion (Fig. 3).

Combined intestine–liver perfusion. In the combined intestine–liver perfusion, 5500 μmol of glucose was infused luminally together with 75, 150, 260 or 380 μmol of glutamine (Fig. 4). The increase in luminal glutamine concentration from 75 to 150 μmol strongly enhanced intestinal glucose absorption and hepatic glucose uptake. A further increase in the luminal glutamine concentration enhanced intestinal glucose absorption only by about 1.7-fold, but hepatic glucose uptake was increased by about 3-fold (Fig. 4). In the presence of luminal glutamine the intestinal transit time of glucose was 14 ± 2 min.

In the intestine–liver preparation the relative hepatic glucose uptake (uptake/supply) was increased from 0% to 55% on increasing the portal supply, whereas in the isolated liver perfusion a similar enhancement of the portal glucose concentration slightly decreased the relative hepatic uptake, from

Fig. 6. Hepatic glucose balance with various portal Na^+ and glutamine concentrations in rat liver perfused via the hepatic artery and PV

The perfusion medium described in Fig. 3 was used. Glucose was infused via the portal vein to reach a portal concentration of 17 mM. Simultaneously, one of the following additions was made via the portal vein: glutamine to reach a portal concentration of 2 mM, Na^+ to raise the portal level from 146 to 156 mM, or a Na^+ -free perfusion medium to lower the portal Na^+ level from 146 to 136 mM. Hepatic glucose balance is defined in Fig. 2. The overshoot in glucose uptake after the start and in glucose release after the end of the enhanced glucose infusion is explained in Fig. 3. A positive value indicates release; a negative value indicates uptake. Values are means $\pm$ S.E.M. of three experiments.

20% to 15% (Fig. 5). Therefore a larger increase in portal glucose supply cannot mediate the luminal glutamine-dependent increase in hepatic glucose uptake; other factors must therefore be involved in this process.

Liver cell swelling as a possible mechanism for the activation of hepatic uptake of absorbed glucose by luminal glutamine

Experimental liver cell swelling has been shown to inhibit transiently the net glycogenolytic and glycolytic flux in perfused rat liver [21] and to stimulate glycogen synthesis in isolated hepatocytes [22]. Liver cell swelling was caused by addition of amino acids such as glutamine [23] and by depleting the portal Na^+ level [22], leading to hypo-osmolarity of the perfusate. It is possible that the activation of hepatic glucose uptake by luminal glutamine was due to hepatocellular swelling. However, the following evidence does not support this hypothesis.

Since luminal application of 150 μmol of glutamine, although causing an activation of hepatic glucose uptake, did not alter the

portal levels of this amino acid (Fig. 4), a glutamine-dependent swelling of hepatocytes [21–24] is unlikely to cause the activation of hepatic glucose uptake (see also Fig. 6). Only luminal infusion of 380 μ mol (55 mg) of glutamine slightly increased the portal level of this amino acid, from 1 mM to about 1.3 mM (Fig. 4). Furthermore, it was shown in the combined intestine–liver perfusion that high levels of portal glutamine (2, 6 and 11 mM) failed to enhance hepatic glucose uptake (results not shown).

In the isolated perfused liver the portal Na⁺ concentration was decreased from 146 mM to a calculated level of about 136 mM, whereas the portal glucose level was raised from 5 to 17 mM. Nevertheless, this decrease in portal Na⁺ did not enhance, but clearly decreased, hepatic glucose uptake during the portal glucose infusion (Fig. 6a versus Fig. 6d). The same observation was made when glutamine was simultaneously infused to raise the portal level of this amino acid from 1 to 2 mM (Fig. 6a versus Fig. 6e).

Since glucose is absorbed by the small intestine together with Na⁺, the increased portal glucose concentration should not be accompanied by a decrease, but rather by an enhancement, in the portal Na⁺ level. This should lead to a transient hyperosmolarity of the portal perfusion medium. It is possible that this enhanced portal Na⁺ level could act as mediator of the luminal glutamine-dependent activation of hepatic glucose uptake. This hypothesis would explain why luminal glutamine failed to enhance the hepatic uptake of vascularly infused glucose. Variations in the glucose and Na⁺ levels were simulated in the isolated liver perfusion by raising the portal concentration of Na⁺ from 146 mM to a calculated level of about 156 mM, and of glucose from 5 mM to 17 mM. This increase in portal Na⁺ did not enhance hepatic glucose uptake during a portal glucose infusion (Fig. 6a versus Fig. 6b). A similar result was obtained when the portal glutamine level was raised from 1 to 2 mM (Fig. 6a versus Fig. 6c).

Therefore variations in the glutamine and Na⁺ levels apparently did not mediate the activation of hepatic glucose uptake by luminal glutamine.

DISCUSSION

Mechanism of activation of intestinal glucose absorption by luminal glutamine

Luminal but not arterial glutamine activated the intestinal absorption of glucose by an as yet unknown mechanism (Fig. 2). Since intestinal metabolism of glutamine carbon was similar after both arterial and luminal administration [20,25,26], it is unlikely that the increase in intestinal absorption of glucose was caused by an intermediate of intestinal glutamine metabolism. Since the transit time of luminal glucose and the fluid secretion of the small intestine were not affected by luminal glutamine (results not shown), a change in these parameters was apparently not responsible for the glutamine-dependent increase in intestinal glucose absorption. It therefore can be speculated that glutamine-dependent receptors, exclusively located at the luminal side of the enterocytes or other intestinal cells, caused the formation, activation or release of signals which enhanced intestinal glucose, absorption, and possibly also the activation of hepatic glucose uptake.

Mechanism of activation of hepatic uptake of absorbed glucose by luminal glutamine

A greater increase in portal glucose supply and an enhanced portal–arterial glucose gradient. The increased portal glucose supply and enhanced portal–arterial glucose gradient, caused by the luminal glutamine-dependent activation of intestinal glucose absorption (Fig. 2), might act as signals for hepatic glucose

uptake. This hypothesis, however, was refuted by the finding that the relative hepatic glucose uptake was greatly increased by luminal glutamine in the combined intestine–liver perfusion, but not by a simple enhancement of the portal glucose supply in the isolated perfused liver.

Liver cell swelling. Glutamine and hypo-osmotic media have been shown to cause an increase in the hepatocellular volume and a transient inhibition of net glycogenolytic and glycolytic flux [21], as well as a stimulation of glycogen synthesis [22]. The present data clearly demonstrated that osmotic alterations to the portal perfusion medium by adding various amounts of glutamine (results not shown) or by increasing or decreasing the Na⁺ content (Fig. 6) failed to enhance hepatic glucose uptake. Therefore changes in portal osmolarity are not likely to be the cause of the glutamine-dependent activation of hepatic glucose uptake.

Since luminal but not arterial glutamine activated the hepatic uptake of luminally applied but not vascularly administered glucose, it is likely that both glutamine and glucose activated the synthesis or release of an intestinal factor which caused the shift of hepatic balance from output to uptake of glucose absorbed by the small intestine.

Intestinal glutamine metabolites. A metabolite, originating from luminal but not from arterial glutamine, and subsequently secreted into the portal vein, might have caused the activation of hepatic glucose uptake. However, intestinal metabolism of glutamine was similar after both arterial and luminal administration [20,25,26], and the quantitative differences in the metabolites were very small. Therefore it seems unlikely that the activation of hepatic glucose uptake was caused by an intermediate of intestinal glutamine metabolism.

Gastrointestinal factors. The activation by luminal glutamine of hepatic uptake of absorbed glucose may be mediated by a specific gastrointestinal factor. While the so-called entero–insular axis has been well characterized [27–30], only a little information is available about the regulation of hepatic carbohydrate metabolism by gastrointestinal hormones [1–4]. Most hormone-producing cells of the gut are of the receptor–secretory type, i.e. a hormone is released by mechanical or chemical stimuli from the luminal side [30,31]. For example, the gastric inhibitory polypeptide, which is specifically released by intestinal absorption of luminal glucose, subsequently interacts with pancreatic β -cells by stimulating insulin secretion [27–30]. Only the regulation by gastric inhibitory peptide [1,3,32], vasoactive intestinal peptide [2] and glucagon-like peptide 1 [4] of hepatic glucose metabolism has been investigated. These peptides are not likely candidates for an intestinal factor enhancing glucose utilization in the liver, since they failed to activate hepatic glucose uptake.

Neurotransmission. Since denervation of the islets significantly decreased the insulin response to an oral glucose load, the entero–insular axis is not fully explained by the existence of humoral factors [27–30]. Therefore it is also possible that the activation of hepatic glucose uptake by luminal glutamine was mediated by neurotransmission, especially since intestinal and hepatic nerve plexuses remained intact in the present combined perfusion of small intestine and liver.

Combined action of more than one factor. The entero–insular axis is a complex system, comprising substrate stimulation by the absorbed nutrients, endocrine factors and neurotransmission [27–30]. Similarly, the enterohepatic axis might also depend on these three factors, which could jointly act as mediators of hepatic glucose uptake by luminal glutamine.

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