Sensing by intrahepatic muscarinic nerves of a portal-arterial glucose concentration gradient as a signal for insulin-dependent glucose uptake in the perfused rat liver

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Abstract In vivo, insulin increases net hepatic glucose uptake efficiently only in the presence of a portal-arterial glucose gradient. In isolated perfused rat livers supplied with a glucose gradient (portal 10 mM/arterial 5 mM) insulin-induced glucose uptake was blocked by atropine; in livers not supplied with the gradient (portal = arterial 5 mM) insulin-dependent glucose uptake was elicited by acetylcholine. Apparently, the gradient was sensed and transformed into a metabolic signal by intrahepatic nerves, releasing acetylcholine to muscarinic receptors.

Key words: Liver metabolism; Insulin; Bivascular liver perfusion; Muscarinic nerve

1. Introduction

The liver is the glucostat of the organism; it takes up ingested carbohydrates and stores them as glycogen in the absorptive state and releases glucose to maintain euglycemia in the postabsorptive state. Insulin is generally accepted to be the hormone inducing net hepatic glucose uptake and storage into glycogen [1]. However, when glucose was applied via a peripheral vein, even high insulin concentrations failed to induce substantial net hepatic glucose uptake in man [2] as well as in dogs [3]. When instead glucose was given orally, infusion of insulin induced a marked net hepatic glucose uptake in man [4] and in dogs [3,5]. An explanation for this lack of effect of insulin on hepatic glucose metabolism with peripheral glucose infusion could not be given but it was argued that a glucose gradient between the portal vein and the hepatic artery rather than the absolute concentration of glucose might be involved in the regulation of hepatic glucose metabolism. This was demonstrated in vivo with the so called pancreatic clamp in dogs [3] and in vitro with isolated rat liver bivascularly perfused via both the portal vein and hepatic artery [6]. When a glucose concentration gradient was established with a higher concentration in the portal vein, insulin was able to induce net hepatic glucose uptake. When an inverse gradient with a higher glucose concentration in the hepatic artery was offered, insulin was unable to elicit a net hepatic glucose uptake [6]. However, the mechanisms of sensing a portal-arterial glucose gradient and of transmitting a metabolic signal to the hepatocytes have remained unknown so far.

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Abbreviations: PV, portal vein; HA, hepatic artery; IVC, inferior vena cava

Since the liver is richly innervated [7–9] and glucose receptors have been demonstrated electrophysiologically in the portal region of guinea pig liver [10], the hepatic nervous system might be involved in the possible signal chain. This was supported by in vivo data in dogs which had undergone surgical hepatic denervation: insulin no longer stimulated net hepatic glucose uptake [11].

Therefore, it was the aim of the present investigation to examine the possible role of hepatic nerves in sensing a portal arterial glucose concentration gradient and in rendering hepatic glucose uptake insulin-sensitive with isolated, bivascularly perfused rat liver.

2. Materials and methods

2.1. Materials and animals

All chemicals were of reagent grade and from commercial sources. Enzymes and bovine serum albumin were purchased from Boehringer (Mannheim, Germany). Insulin, atropine and acetylcholine were obtained from Sigma (Munich, Germany). Male Wistar rats were kept on a day-night rhythm (7 a.m.–7 p.m.) with ad lib feeding.

2.2. Liver perfusion

All perfusion experiments were started at 9 a.m. The liver was perfused in situ without recirculation via both the portal vein with low pressure (10 mm Hg [1.33 kPa], 80–60% of total flow) and the hepatic artery with high pressure (60–70 mm Hg [7.89–9.31 kPa], 20–40% of total flow) resulting in a total flow of about 4 ml min^{-1} g organ $weight^{-1}$ as described in detail elsewhere [6]. The basic perfusion medium was an erythrocyte-free Krebs-Henseleit-bicarbonate buffer containing 5 mmol/l glucose, 2 mmol/l lactate, 0.2 mmol/l pyruvate and 0.5% bovine serum albumin. As indicated, in some experiments the glucose concentration of the portal and/or arterial perfusion medium was 10 mmol/l. The medium was equilibrated with O_2:CO_2 (19:1).

Experiments were started following a pre-perfusion period of 20 min. Perfusion buffer samples were taken consecutively at 1 min intervals and directly cooled on ice. Metabolite concentrations were measured with standard enzymatic techniques as described before [6]. Total perfusion flow was quantified by fractionating the effluate into calibrated tubes. The portal flow was measured with an SMS-302 electromagnetic flow meter (Hellige, Freiburg, Germany) placed around the portal inflow. The arterial flow was calculated as the difference between total and portal flow.

2.3. Infusion of insulin, atropine and acetylcholine

Insulin was infused into the portal vein diluted in the perfusion buffer containing 0.5% bovine serum albumin to the indicated final sinusoidal concentrations. Atropine and acetylcholine were infused via the portal vein or hepatic artery diluted to the appropriate sinusoidal concentrations indicated.

3. Results

Isolated livers were perfused with medium containing a high glucose concentration (10 mmol/l) via both the portal vein

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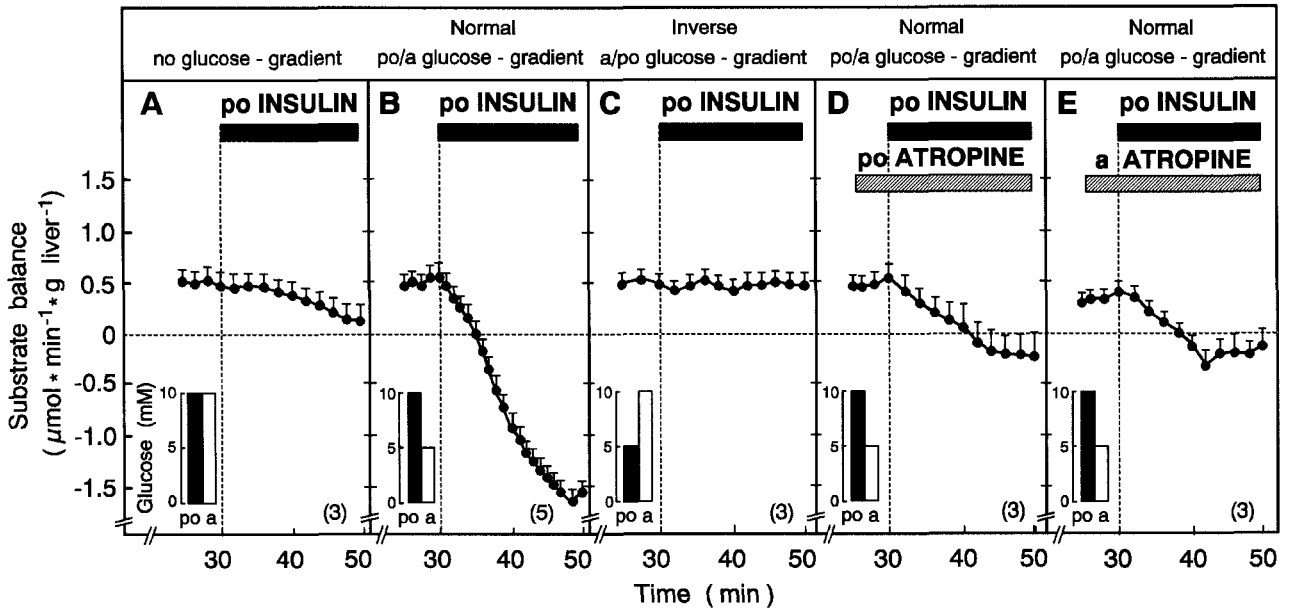

Fig. 1. The insulin-stimulated net hepatic glucose uptake in the presence of a portal-arterial glucose concentration gradient can be blocked by portal or arterial atropine. Livers were perfused via both the portal vein and hepatic artery without recirculation with a Krebs-Henseleit bicarbonate buffer containing 5 or 10 mmol/l glucose as indicated in the lower left inset in each panel, 2 mmol/l lactate, 0.2 mmol/l pyruvate and 0.5% bovine serum albumin. Insulin (100 nmol/l) was infused via the portal vein and atropine (1 nmol/l) via the portal vein or hepatic artery when indicated. Values are means ± S.E.M. of the number of experiments given in parentheses. po, portal; a, arterial.

(PV) and the hepatic artery (HA), i.e. a glucose load of 40 µmol min^{-1} g^{-1}. During the pre-perfusion period without portal insulin infusion the hepatic glucose balance remained unchanged at a basal glucose output of about 0.5 µmol min^{-1} g^{-1} organ weight (Fig. 1A). From min 31 onwards, insulin (100 nmol/l) infused into the PV caused a slight decrease in basal glucose output but did not stimulate net hepatic glucose uptake (Fig. 1A). However, when a portal-arterial glucose concentration gradient was established first (PV 10 mmol/l, HA 5 mmol/l) resulting in a glucose load of 33 µmol min^{-1} g^{-1}, portal insulin induced a marked hepatic glucose uptake (Fig. 1B). Basal glucose output shifted from 0.5 µmol min^{-1} g^{-1} to uptake of about 1.5 µmol min^{-1} g^{-1}; the maximum of the effect was reached within 20 min.

When as a control livers were perfused with an inverse concentration gradient (PV 5 mmol/l, HA 10 mmol/l glucose)

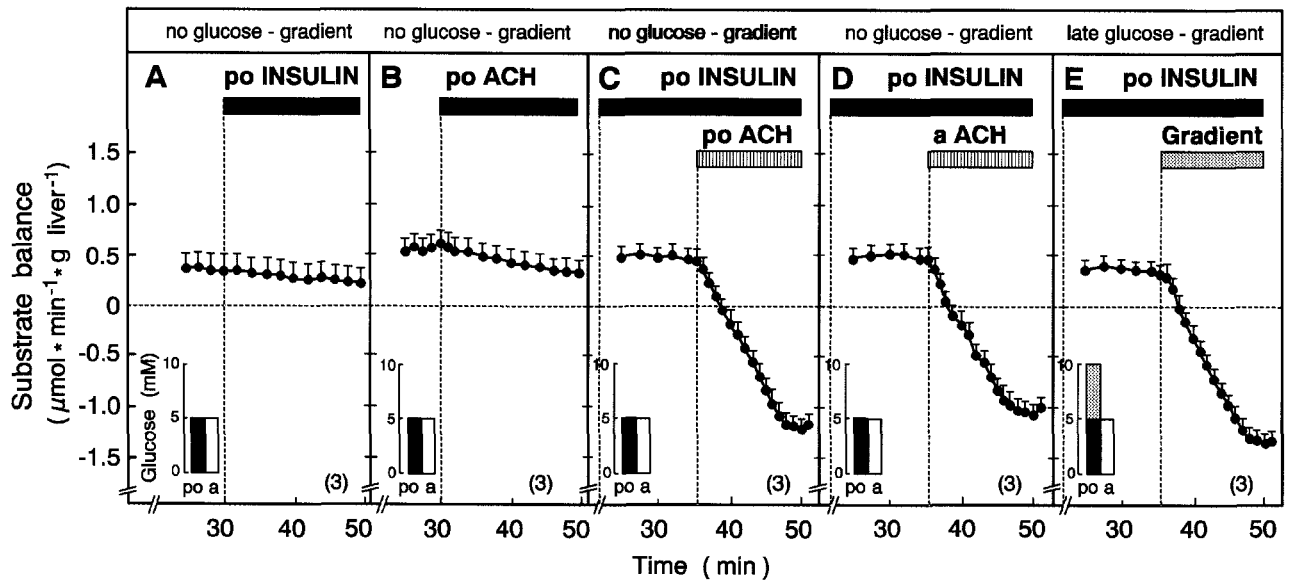

Fig. 2. The portal-arterial glucose concentration gradient is mimicked by portal or arterial acetylcholine. Livers were perfused via both the portal vein and hepatic artery without recirculation with a Krebs-Henseleit bicarbonate buffer containing 5 mmol/l glucose as indicated in the lower left inset in each panel, 2 mmol/l lactate, 0.2 mmol/l pyruvate and 0.5% bovine serum albumin. The glucose concentration in the portal vein was raised from 5 to 10 mmol/l in series E from min 36 onwards. Insulin (100 nmol/l) was infused via the portal vein and acetylcholine (1 µmol/l) via the portal vein or hepatic artery when indicated. Values are means ± S.E.M. of the number of experiments given in parentheses. po, portal; a, arterial.

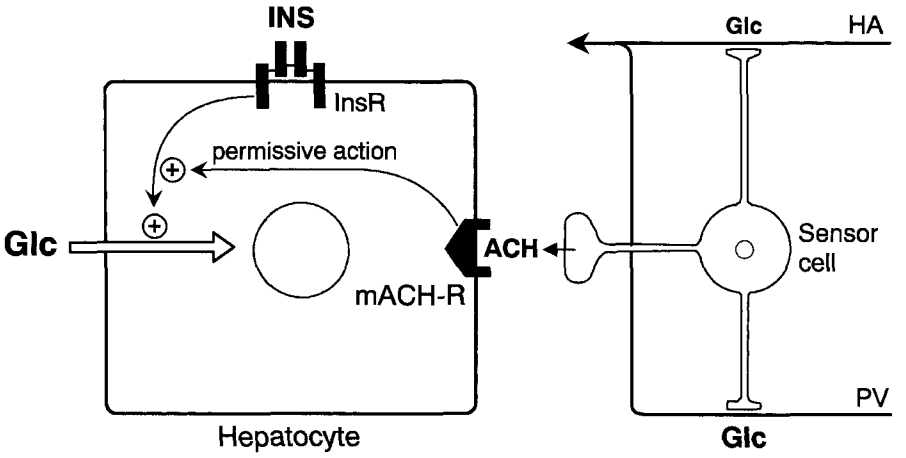

Fig. 3. Hypothetical scheme of the regulatory circuit of sensing and transmitting a glucose concentration gradient to the hepatocytes. If a nerve cell senses a glucose concentration gradient between the portal vein and hepatic artery it releases acetylcholine to a muscarinic receptor. This signal is permissive for insulin action on glucose uptake. The function of sensing the gradient is to distinguish between exogenous glucose from food (po > a) and endogenous glucose (po ≈ a) generated mainly within the liver itself. Ins, insulin; InsR, insulin receptor; mACH-R, muscarinic receptor; ACH, acetylcholine; HA, hepatic artery; PV, portal vein.

resulting in a glucose load of 27 μmol min^{-1} g^{-1}, basal glucose output equalled that of livers offered the higher glucose load without a gradient or the sightly higher glucose load with the normal portal-arterial glucose gradient; with the inverse glucose gradient portal insulin did not cause any change in hepatic glucose balance even after 20 min (Fig. 1C).

To evaluate the role of hepatic nerves livers were perfused with the normal glucose gradient and the normal load of 33 μmol min^{-1} g^{-1}. A portal infusion of atropine (1 nmol/l) was then started 5 min prior to the portal infusion of insulin. Within these 5 min atropine did not significantly alter basal hepatic glucose output. However, it significantly decreased the insulin-induced net hepatic glucose uptake from a maximum of 1.5 to only 0.25 μmol min^{-1} g^{-1} (Fig. 1D). Infusion of atropine into the hepatic artery caused a virtually identical inhibition of net hepatic glucose uptake (Fig. 1E). Apparently, muscarinic cholinergic nerves were involved in sensing the glucose concentration gradient and transforming it into a metabolic signal for the hepatocytes.

If this were the case, it should be possible to mimic the glucose concentration gradient at least in part by infusing acetylcholine into the portal vein or the hepatic artery. With a low glucose load of 20 μmol min^{-1} g^{-1} offered without a gradient (PV 5 mmol/l, HA 5 mmol/l), basal hepatic glucose output was not significantly different from that observed with the higher glucose load without a glucose gradient (PV 10 mmol/l, HA 10 mmol/l) or with the normal glucose load with a normal glucose gradient (PV 10 mmol/l, HA 5 mmol/l); it amounted to about 0.4 μmol min^{-1} g^{-1} (Fig. 2A vs. Fig. 1A,B). With the infusion of portal insulin no significant reduction in basal glucose output could be detected (Fig. 2A). Portal infusion of acetylcholine (1 μmol/l) alone did not cause a significant alteration in basal hepatic glucose output either, even after 20 min (Fig. 2B). However, portal infusion of acetylcholine in the presence of portal insulin infusion resulted in a shift to net hepatic glucose uptake with a maximum of 1.2 μmol min^{-1} g^{-1} (Fig. 2C). Arterial infusion of acetylcholine resulted in an identical shift to net hepatic glucose uptake when compared with portal acetylcholine (Fig. 2D). When in the presence of portal insulin the normal portal-arterial glucose concentration gradient was established instead of a portal acetylcholine infusion by elevating the portal glucose concentration to 10 mmol/l, again a shift to net hepatic glucose uptake of about 1.3 μmol min^{-1} g^{-1} was observed (Fig. 2E).

4. Discussion

4.1. Importance of a portal arterial glucose concentration gradient for net hepatic glucose uptake

In the present investigation it was shown that in the isolated hemoglobin-free perfused rat liver insulin stimulated net hepatic glucose uptake only in the presence of a portal-arterial glucose concentration gradient (with the higher glucose concentration in the PV) (Fig. 1B). These data are in accord with the results of a previous study in which the importance of a portal-arterial glucose concentration gradient for insulin-dependent net hepatic glucose uptake was demonstrated in the isolated rat liver perfused with erythrocyte-containing medium; the amount of glucose taken up was approximately the same [6]. This is in agreement with in vivo experiments in dogs in which the combination of experimental hyperglycemia without a portal-arterial glucose concentration gradient (induced by peripheral glucose infusion) and hyperinsulinemia caused only a weak net hepatic glucose uptake [3,11]. However, net hepatic glucose uptake more than doubled in humans [2] and conscious dogs [3] when hyperglycemia with a portal-arterial glucose concentration gradient and hyperinsulinemia were combined. In addition, it was demonstrated that the magnitude of net hepatic glucose uptake correlated with the magnitude of the portal-arterial glucose concentration gradient [11]. Thus, the importance of a portal-arterial glucose concentration gradient for an insulin-stimulated net hepatic glucose uptake is well established.

4.2. Involvement of intrahepatic nerves

The question remained how hepatic glucose uptake by the hepatocytes was rendered insulin-sensitive, since the cells are positioned in the sinusoidal blood composed of both portal and arterial blood. They are therefore unable to differentiate between glucose that was delivered by the portal vein or the hepatic artery. Thus, the glucose concentration in the portal

vein and for comparison in the hepatic artery (or elsewhere in any other artery) must be sensed and the difference must be transformed into a metabolic signal transmitted to the hepatocytes. One possible mechanism could involve the hepatic nervous system. In the present investigation with isolated perfused rat liver the insulin-stimulated glucose uptake at a normal postprandial glucose load (33 μmol min^{-1} g^{-1}) in the presence of a portal-arterial glucose concentration gradient was markedly reduced by either portal or arterial atropine (Fig. 1D,E). Moreover, when at a lower postabsorptive glucose load (20 μmol min^{-1} g^{-1}) without a glucose gradient insulin was infused together with either portal or arterial acetylcholine, a substantial net hepatic glucose uptake was elicited (Fig. 2C,D). Thus, a portal-arterial glucose gradient could be mimicked by portal or arterial acetylcholine. These two observations strongly indicated that intrahepatic nerves of the hepatoportal area were involved in allowing the stimulation of net hepatic glucose uptake by insulin. On the assumption that intrahepatic nerves deteriorate following liver denervation, the present results are in accord with previous in vivo studies in which dogs were subjected to surgical hepatic denervation and the insulin-dependent response to a portal glucose delivery was completely lost [11]. Moreover, the present observations are in line with a general link between insulin action and cholinergic nerves as shown recently in cats in vivo using the euglycemic clamp technique: portal atropine caused insulin resistance [12]. Finally, the present findings explain the observation that in the isolated liver perfused classically via the portal vein only infusion of insulin and acetylcholine but not of insulin or acetylcholine alone resulted in an increase in liver glycogen content [13].

The liver and portal vein are richly innervated by sympathetic and parasympathetic, efferent and afferent nerves [7–9]; 90% of the nerve fibers of the hepatic vagus were estimated to be afferent, indicating important sensory functions of the hepatoportal area. Glucose receptors have been demonstrated electrophysiologically in the portal vein [10]. Although functional results indicate the existence of muscarinic receptors in the liver [14,15], they have not been shown directly on liver parenchymal or non-parenchymal cells. However, the proposed muscarinic receptor must be located downstream of the confluence of portal and arterial blood since portal as well as arterial acetylcholine could mimic the portal-arterial glucose concentration gradient. The mechanism of sensing the glucose gradient and generating a metabolic signal for the hepatocytes was still functional in isolated rat liver preparations [6], therefore it is clear that these systems contained at least the minimum of elements required for the signal chain. A hypothetical scheme of the regulatory circuit can be proposed (Fig. 3): a sensory nerve cell monitors the glucose concentrations in the portal vein and hepatic artery; when a concentration gradient with the higher concentration in the portal vein is measured, a signal to the hepatocytes is generated and transmitted via muscarinic nerves.

4.3. Possible physiological role

The current opinion is that the liver in the postabsorptive state releases glucose if required by extrahepatic organs and in the absorptive state removes a substantial part of the excess glucose following the intake of a carbohydrate-rich meal. As a result of the absorption of glucose the concentration in the draining vessel (the portal vein) would rise and a portal-arterial glucose concentration gradient would be established. With the sensing of a portal-arterial glucose gradient the liver would be able to distinguish between endogenous and exogenous glucose and glucose uptake by the hepatocytes would be rendered insulin-sensitive only when a concentration gradient signals the appearance of exogenous glucose.

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