Impaired glucose sensing by intrahepatic, muscarinic nerves for an insulin-stimulated hepatic glucose uptake in streptozotocin-diabetic rats

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Abstract Insulin-induced net hepatic glucose uptake depends on the sensing by muscarinic, intrahepatic nerves of a glucose concentration gradient between portal vein and hepatic artery. The function of these intrahepatic nerves was examined in streptozotocin-diabetic rats. In the presence of the glucose gradient insulin induced net glucose uptake in isolated perfused livers from control and acutely diabetic but not from chronically diabetic animals. The neurotransmitter acetylcholine still mimicked the existence of the gradient, excluding a metabolic impairment of livers of chronically diabetic animals. The impairment of the intrahepatic nerves due to diabetic neuropathy could contribute to postprandial hyperglycemia in diabetes mellitus.

Key words: Bivascularly perfused rat liver; Streptozotocin-induced diabetes; Diabetic neuropathy; Hepatic nerve; Glucose metabolism

1. Introduction

Net glucose uptake in the liver and subsequent incorporation into glycogen is under the control of insulin [1]. However, only when glucose was offered via the oral route a substantial net hepatic glucose uptake could be achieved with an infusion of insulin in man [2] as well as in dogs [3,4]. Insulin failed to induce a marked net hepatic glucose uptake when glucose was delivered via a peripheral vein [3,5]. This lack of an effect of insulin on liver metabolism following peripheral glucose infusion was found to be due to the absence of a portal > arterial glucose concentration gradient, which is established following absorption of a normal oral glucose load. The importance of this gradient was demonstrated with conscious, chronically catheterized dogs [3] and with isolated, bivascularly perfused rat livers [6]. Only in the presence of a glucose concentration gradient with the higher concentration in the portal vein was insulin able to induce net hepatic glucose uptake. The gradient is sensed and transmitted to the hepatocytes by intrahepatic, muscarinic nerves [7].

Neuropathy, like microangiopathy and retinopathy, is one of the most common late complications of diabetes mellitus [8,9]. The reported prevalence varies considerably, depending on the criteria used for defining diabetic neuropathy. A large hospital-based study found a prevalence of diabetic peripheral neuropathy of 22.7% in patients with insulin-dependent diabetes mellitus (IDDM) and 32.1% in patients with non-insulin-dependent diabetes mellitus (NIDDM) [10]. The main site of manifestation of diabetic neuropathy is the sensory system, leading to hypoesthesia, hyperesthesia and dysesthesia. Diabetic neuropathy can also affect the autonomous nervous system and, only rarely, the motor nerves. In a multicenter study using two measures of abnormality 22.1% of patients with NIDDM and 16.8% of those with IDDM showed cardiac autonomic dysfunction [11]. In addition, a severe impairment of the sympathetic hepatic nerves could be demonstrated in isolated perfused livers of 3 months streptozotocin-diabetic rats [12].

Therefore, it was the aim of the present investigation to evaluate the possible functional impairment due to a chronically diabetic state of the intrahepatic nerves, which sense the glucose concentration gradient between portal vein and hepatic artery and transmit a corresponding signal to the hepatocytes.

2. Materials and methods

2.1. Materials

All chemicals were of reagent grade and from commercial sources. Enzymes were provided by Boehringer (Mannheim, Germany), insulin, atropine, acetylcholine and streptozotocin were obtained from Sigma (Deisenhofen, Germany) and bovine serum albumin from Appli Chem (Darmstadt, Germany).

2.2. Animals

Male Wistar rats were obtained from Harlan-Winkelmann (Borchen, Germany). The rats were subjected to a 12-h day-night rhythm (7 a.m.–7 p.m.) for at least one week. All rats had free access to food and water (standard diet of Ssniff, Soest, Germany). The perfusion experiments were started between 9 and 10 a.m.

2.3. Induction of diabetes

To induce diabetes mellitus rats (120–140 g in the chronic diabetes group and 250–290 g in the acute diabetes group) were starved for 24 h and streptozotocin (50 mg/kg, 33 g/l dissolved in 50 mmol/l sodium citrate, pH 4.5) was injected intraperitoneally. Thereafter the animals had free access to food and were used in the experiments after 3 months or 48 h of streptozotocin injection.

Treatment of the animals was in accordance with the German Law on the Protection of Animals and was performed with permission of the State Animal Welfare Committee.

2.4. Bivascular liver perfusion

The preparation of the isolated, bivascularly perfused rat liver has been described in detail earlier [13,14]. In brief, it is an in situ perfusion without recirculation via both the portal vein and the hepatic artery. Following anesthesia by an intraperitoneal injection of pentobarbital (40 mg/kg) and a midline laparotomy the rat was killed by an i.v. overdose of pentobarbital. Blood and urine samples were drawn by puncturing of femoral artery and urinary bladder for subsequent determination of the glucose concentrations. Then a catheter was introduced into the portal vein (PV), and the inferior vena cava (IVC) was incised longitudinally; then the liver perfusion was started via the PV. Following splenectomy and gastrectomy the intestine was removed by ligating the radix mesenterii and the common hepatic artery

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Abbreviations: HA, hepatic artery; IVC, inferior vena cava; PV, protal vein; SMA, superior mesenteric artery

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(HA) was directly cannulated. At this stage the additional perfusion via the artery was started and a bivascular liver perfusion was established with a low pressure component via the PV (10 mmHg (1.33 kPa), 80–60% of total flow) and a high pressure component via the HA (60–70 mmHg (7.89–9.31 kPa), 20–40% of total flow) resulting in a total flow of 4 ml/min/g organ weight. An outflow catheter was placed in the IVC at the inflow of the hepatic veins. Finally the thorax was opened and IVC and aorta were ligated. Experiments were started following a preperfusion period of 20 min.

The 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 was changed to 10 mmol/l. The medium was equilibrated with O_2/CO_2 (19:1).

2.5. Infusion of insulin, atropine or acetylcholine

Insulin, atropine or acetylcholine were diluted in the perfusion buffer containing 0.5% bovine serum albumin to the indicated sinusoidal concentrations and infused into the PV.

2.6. Determination of metabolites

Perfusion buffer samples were taken consecutively in 1-min intervals and directly chilled on ice. Glucose was determined with glucose dehydrogenase (Merck glucose system) [15]. Blood and urine glucose concentrations were determined with a Beckman glucose analyzer 2 with the glucose oxidase method [16]. Urine samples were centrifuged and adequately diluted with Beckman dilution solution before glucose determination.

2.7. Measurement of perfusion flow rates

Total perfusion flow was quantified by fractionating the effluate into calibrated tubes. Portal flow was measured using a Research Flowmeter T 106 (Transonic Systems, Ithaca, NY, USA) with the flow probe placed in the portal inflow. The arterial flow was the calculated difference between total and portal flow.

2.8. Statistical analysis

All results were presented as means ± S.E.M. for the indicated number of experiments.

3. Results

3.1. Characterization of streptozotocin-diabetic rats

The intraperitoneal injection of streptozotocin resulted in the development of a diabetic state in all treated rats. The animals showed clinical signs of hyperglycemia (e.g. polydipsia and polyuria) within the following 30–50 h. At the time of the experiments the two streptozotocin-treated groups, the 3-months chronically diabetic rats and the 48-h acutely diabetic rats, had increased blood and urine glucose concentrations (Table 1). In the 3-months diabetic group only rats with blood glucose concentrations of more than 20 mmol/l were used for the experiments. Most of the rats that had been diabetic for 3 months in addition showed signs of severe complications of chronic diabetes, e.g. enlarged bowels and urinary bladders as well as cataracts. No animal died due to the induction of diabetes by streptozotocin or during the diabetic period of 3 months.

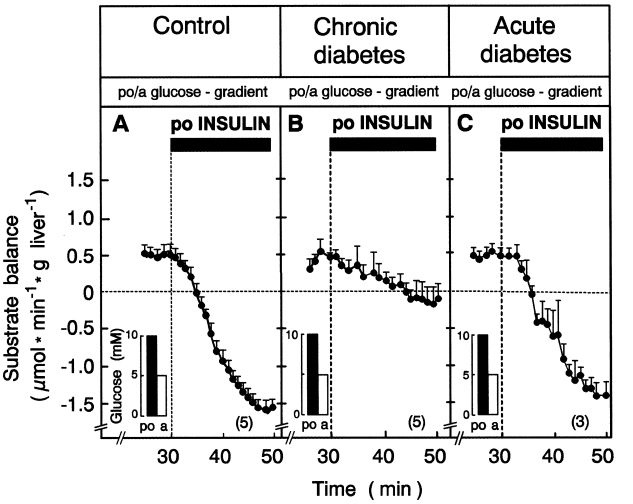

Fig. 1. Impairment by chronic but not acute streptozotocin-induced diabetes of the insulin-stimulated net glucose uptake in the presence of a portal > arterial glucose concentration gradient in the perfused rat liver. Livers were perfused via both the portal vein (PV) and hepatic artery (HA) without recirculation with a Krebs-Henseleit bicarbonate buffer containing 10 mmol/l (PV) or 5 mmol/l (HA) 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. Diabetes was induced by an intraperitoneal injection of streptozotocin (50 mg/kg) and the rats were used for the experiments 3 months (chronic) or 48 h (acute) later. Control rats received no injection. Insulin (100 nmol/l) was infused via the portal vein when indicated. Values are means ± S.E.M. of the number of experiments given in parentheses. po, portal; a, arterial.

3.2. Impaired function of intrahepatic nerves in chronically streptozotocin-diabetic rats

Isolated livers from control rats were supplied with a glucose concentration gradient by infusing medium containing a high glucose concentration (10 mmol/l) into the portal vein (PV) and medium containing a lower glucose concentration (5 mmol/l) into the hepatic artery (HA). During the preperfusion period without infusion of portal insulin hepatic glucose balance remained unchanged at a basal glucose output of about 0.5 μmol/min/g organ weight (Fig. 1A). Infusion of insulin (100 nmol/l) into the PV from the 31st min onwards induced a shift from basal net glucose output to a net uptake of about 1.5 μmol/min/g in livers from control rats. In contrast, in livers from chronically diabetic rats portal insulin infusion caused only a shift to a minor net glucose uptake of about 0.2 μmol/min/g (Fig. 1B). In livers from acutely diabetic animals portal insulin infusion from the 31st min onwards elicited a shift from glucose output to a marked net glucose uptake of about 1.3 μmol/min/g (Fig. 1C). Basal hepatic glu-

Table 1
Characterisation of the 3-months streptozotocin-diabetic and 48-h streptozotocin-diabetic rats by body weight, blood glucose level and urine glucose level

	Control	Diabetes 3 months	Diabetes 48 h
Body weight (g)	286 ± 21	304 ± 35	295 ± 12
Blood glucose (mmol/l)	8.2 ± 1.6	27.4 ± 6.3	22.7 ± 5.2
Urine glucose (mmol/l)	n.d.	56.3 ± 8.2	48.3 ± 4.7

Values are means ± S.E.M. of eight (control), five (chronic diabetes) and three (acute diabetes) experiments.

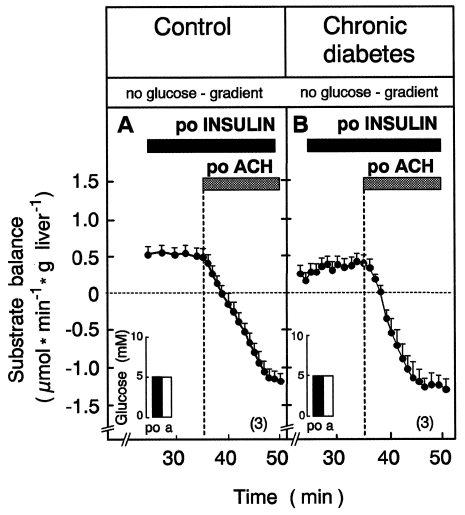

Fig. 2. Lack of impairment by chronic streptozotocin-induced diabetes of the insulin plus acetylcholine-stimulated net glucose uptake in the absence of a portal > arterial glucose concentration gradient in the perfused rat liver. Livers were perfused as described in the legend to Fig. 1. Insulin (100 nmol/l) plus acetylcholine (1 μmol/l) was infused when indicated in the absence of a portal > arterial glucose concentration gradient (PV and HA = 5 mmol/l). Values are means ± S.E.M. of the number of experiments given in parentheses. po, portal; a, arterial.

cose release in the two groups of diabetic rats was not different when compared with control rats.

It was shown previously that the portal > arterial glucose concentration gradient was sensed and transmitted to the hepatocytes via intrahepatic, muscarinic nerves and that it could be mimicked by infusing acetylcholine into the PV [7]. To distinguish between a loss of function of the intrahepatic nerves and a possible functional impairment of the hepatocytes in the chronically diabetic group, the preserved ability of acetylcholine to mimic the gradient was examined. Therefore, as a control, liver perfusions were performed with a portal insulin infusion in the absence of a portal > arterial glucose concentration gradient (PV 5 mmol/l, HA 5 mmol/l). Under these conditions in livers from control rats the portal infusion of insulin did not alter the glucose balance; however, the additional portal infusion of acetylcholine (1 μmol/l) resulted in a shift to a clear net glucose uptake with a maximum of 1.2 μmol/min/g (Fig. 2A). In livers from chronically diabetic animals portal infusion of insulin in the absence of a glucose gradient again did not induce a net glucose uptake but the additional portal infusion of acetylcholine from the 31st min onwards still induced a shift to a marked net glucose uptake of about 1.3 μmol/min/g (Fig. 2B). Apparently, in the chronically diabetic state the hepatocytes were fully functional, only the intrahepatic nerves were impaired.

4. Discussion

In the present study a functional impairment of intrahepatic, muscarinic nerves involved in the sensing of a portal > arterial glucose concentration gradient was found in livers of rats with chronic streptozotocin-induced diabetes mellitus. In livers of acutely 48 h diabetic rats the function of the intrahepatic nerves remained unaltered excluding a possible neurotoxic effect of streptozotocin on the intrahepatic autonomous nerves.

4.1. Impairment of intrahepatic nerve function in chronically diabetic rats

In livers of rats with 3 months of streptozotocin-induced diabetes the insulin-elicited net glucose uptake in the presence of a portal > arterial glucose gradient was clearly reduced when compared with livers from control rats (Fig. 1B vs. Fig. 1A). This lack of the normal effect of insulin in livers of chronically diabetic animals could be due to a functional impairment of the metabolic capacity of the hepatocytes or of the intrahepatic nerves which sense the portal > arterial glucose gradient and transmit a corresponding signal to the hepatocytes. In livers of chronically diabetic animals portal insulin was still able to induce net glucose uptake when the gradient was mimicked by infusion of acetylcholine into the portal vein (Fig. 2B); this finding indicates that the metabolic function of the hepatocytes was not critically altered. In line with this conclusion are the previous observations that in streptozotocin-diabetic rats the activity of glucokinase [17] and glycogen synthase [18] were only reduced but not absent, which may be due to the decreased but still detectable secretion of insulin [17]. Therefore, the lack of the normal insulin effect should be ascribed to a functional loss of the intrahepatic nerves due to diabetic neuropathy.

Diabetic neuropathy is a well known late complication of IDDM as well as NIDDM [8–10]; it can affect the sensory and effectory autonomous nervous system (cf. Section 1). In general, an autonomous neuropathy was found in 17–22% of patients with IDDM or NIDDM [19]. Histological studies in diabetic patients revealed an altered morphology of the vagus nerve including a reduction of the amount of myelinated fibers [20]. Functional studies with streptozotocin-induced diabetes in rats demonstrated an impairment of sodium-potassium-ATPase activity in the vagus nerve [21] and, in addition, of sympathetic hepatic nerves [22].

4.2. Possible pathophysiological significance of the impairment of intrahepatic nerves

In the absorptive state the liver removes a substantial part of the glucose taken up in excess of actual demands and incorporates it into glycogen. The sensing of the portal > arterial glucose concentration gradient enables the liver to distinguish between endogenous and exogenous glucose [7]. Following absorption of normal dietary carbohydrates the glucose concentration in the draining vessel of the small intestine, the portal vein, is increased and thus a glucose concentration gradient is established between the portal vein and hepatic artery. This gradient then allows insulin to stimulate net hepatic glucose uptake [7], which contributes substantially to the disappearance of glucose from the blood and thus to the avoidance of deleterious high blood glucose concentrations.

In patients with IDDM and NIDDM as a part of late complications an autonomous diabetic neuropathy might impair the sensing of the portal-arterial glucose concentration gradient, so that net hepatic glucose uptake would become insulin-insensitive. Therefore, the amount of glucose removed from the circulation by the liver after a meal would be decreased which would contribute to the hyperglycemia in patients with IDDM in spite of therapy with insulin and

NIDDM in spite of treatment with oral antidiabetic drugs or insulin. Thus, the impairment of the intrahepatic parasympathetic control could deprive the chronically diabetic patient of an adequate response to postprandial hyperglycemia.

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