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Glucose-Induced Time-Dependent Potentiation of Insulin Release, but not Islet Blood Perfusion, in Anesthetized Rats

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Abstract

Background: Repeated administration of glucose *in vivo* leads to a time-dependent potentiation of insulin release. Glucose is also known to stimulate pancreatic islet blood flow, but whether this is associated with a time-dependent potentiation is unknown. We therefore repeatedly administered glucose to anesthetized rats and evaluated effects on insulin release and islet blood flow.

Methods: Male Wistar-Furth rats, anesthetized with thiobutabarbital, were injected intravenously with 1 ml of saline or glucose at times 0, 30 and 60 min. The combinations used were saline + saline + saline (SSS), glucose + saline + saline (GSS), saline + saline + glucose (SSG) and glucose + glucose + glucose (GGG). Regional organ blood flow values were measured 3 min after the final injection with a microsphere technique, and at this time also serum insulin concentrations were determined with ELISA.

Results: Serum insulin concentrations as well as total pancreatic, pancreatic islet and duodenal blood flow were higher in SSG and GGG-treated rats when compared to those given SSS and GSS. However, only insulin concentrations, not blood flow values, were higher in GGG rats when compared to SSG animals.

Conclusions: Glucose-induced time-dependent potentiation of insulin release occurs *in vivo* in thiobutabarbital-anesthetized rats, but is not associated with a further increase in islet blood flow.

Introduction

Time-dependent potentiation of insulin release, also known as "priming", refers to the fact that previous exposure within 60 min to a stimulatory glucose concentration augments the insulin secretory response to a subsequent stimulation by glucose or other secretagogues (1–4). Thus, the priming does not necessitate the continuous presence of glucose. The underlying mechanisms are still poorly understood. Glucose metabolism seems to be required for priming to occur (2, 5), whereas the dependence on Ca^{2+} is controversial (2, 5–7). In a more recent publication it was shown that only minute extracellular Ca^{2+}-concentrations are needed, and it seems to be mainly intracellular pH which is of importance for priming to occur (8). The priming is likely mediated by Ca^{2+}- and pH-senstive mitochondrial enzymes (8), and in line with this is the notion that mitochondrial ATP production is linked to the phenomenon (9).

Most of the studies referred to above have been performed in vitro, but there is evidence that priming is seen also in vivo and in humans (3, 10). An intriguing find-

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ing in this context is that the impaired insulin response to glucose can be corrected by inducing priming in some patients with type 2 diabetes (3, 10), and this is also seen in an animal model for type 2 diabetes (11).

Glucose is known to induce an acute increase in pancreatic islet blood flow, mediated through complex interactions between nervous and metabolic mediators (12), which is returned to normal within approximately 30 min (13). Furthermore, animals with impaired glucose tolerance or type 2 diabetes have a permanently increased islet blood perfusion (14–17). The mechanisms behind this chronic islet blood flow increase are likely to be similar to those mediating the acute increase, even though this is not completely clarified (16, 17). In view of the fact that insulin release can be primed by repeated exposure to glucose, we deemed it of interest to evaluate if also islet blood perfusion can be similarly affected, in view of possible contributions to the islet blood flow increase seen in type 2 diabetes.

Materials and methods

Animals

Male Wistar-Furth rats weighing 290–310 g purchased from B & M (Ry, Denmark) were used in all experiments. The animals had free access to food (Type R3; Scanbur, Sollentuna, Sweden) and water before the experiments. All experiments were approved by the local animal ethic committee at Uppsala University (Uppsala, Sweden).

Surgical preparation for blood flow measurements

The rats were anaesthetized with an intraperitoneal injection of thiobutabarbital sodium (120 mg/kg body weight; Inactin®; Research Biochemicals International, Natick, MA, USA) and placed on a heated operating table to maintain body temperature at 37.5ºC. Polyethylene catheters were inserted into the ascending aorta, via the right carotid artery, and into the left femoral artery and vein. The former arterial catheter was connected to a pressure transducer (PDCR 75/1; Druck Ltd., Groby, UK), whereas the latter was used to infuse Ringer solution (5 ml/kg body weight/h) to substitute for fluid losses.

When the blood pressure had remained stable for at least 20 min each animal were given a total of 3 intravenous injections of 1 ml of saline or 30% (w/v) D-glucose at time points 0, 30 and 60 min. The following experimental groups were used:

1) Animals receiving 1 ml of saline at 0, 30 and 60 min (SSS)
2) Animals receiving 1 ml glucose at 0 min and then 1 ml of saline at 30 and 60 min (GSS)
3) Animals receiving 1 ml of saline at 0 and 30 min and 1 ml of glucose at 60 min (SSG)
4) Animals receiving 1 ml of glucose at 0, 30 and 60 min (GGG)

Blood flow measurements

Blood flow measurements were performed with a microsphere technique as previously described (18, 19) 3 min after the last injection of saline or glucose. Briefly, a total of 2.5 x 10^5 black non-radioactive microspheres (EZ-Trac™; Triton Microspheres, San Diego, CA, USA), with a diameter of either 10 μm were injected during 10 sec via the catheter with its tip in the ascending aorta. Starting 5 sec before the microsphere injection, and continuing for a total of 60 sec, an arterial blood sample was collected by free flow from the catheter in the femoral artery at a rate of approximately 0.6 ml/min. The exact withdrawal rate was confirmed in each experiment by weighing the sample. Arterial blood was collected from the carotid catheter for determination of hematocrit and blood glucose and serum insulin concentrations as given below.

The animals were then killed and the pancreas and adrenal glands were removed *in toto*, blotted and weighed. Samples (approximately 100 mg) from the mid regions of the duodenum colon and left kidney were also removed, blotted and weighed. The number of microspheres in the samples referred to above, including the pancreatic islets, was counted in a microscope equipped with both bright and dark field illumination after treating the organs with a freeze-thawing technique (20). The number of microspheres in the arterial reference sample was determined by sonicating the blood, and then transferring samples to glass microfibre filters (pore size <0.2 μm), and then counting them under a microscope.

The organ blood flow values were calculated according to the formula $Q_{org} = Q_{ref} \times N_{org}/N_{ref}$ where Q_{org} is organ blood flow (ml/min), Q_{ref} is withdrawal rate of the reference sample, N_{org} is number of microspheres present in the organ and N_{ref} is number of microspheres in the reference sample.

Blood flow values based on the microsphere contents of the adrenal glands were used to confirm that the microspheres were adequately mixed in the circulation. A difference <10% in the blood flow values was taken to indicate sufficient mixing, and this occurred in all animals in the present study (data not shown).

Blood glucose and insulin concentration determinations

The blood glucose concentration was analyzed with reagent strips based on the glucose oxidase method (Medisense, Solna, Sweden), and serum insulin concentrations were measured with an ELISA kit (Rat Insulin ELISA; Mercodia, Uppsala, Sweden).

Statistical calculations

All values are given as means ± SEM. Probabilities (P) of chance differences were calculated with one-way repeated measurement ANOVA with Tukey's correction (SigmaStat®; SSPD, Erfart, Germany). For calculation of statistical differences between fractions non-parametric ANOVA with Tukey's correction was used. A value of $P<0.05$ was considered to be statistically significant.

Table 1. Anesthetized, male Wistar-Furth rats were injected intravenously with 1 ml of saline (S) or 30% D-glucose (G) at times 0, 30 and 60 min. Measurements were made at 63 min.

Treatment	S+S+S	G+S+S	S+S+G	G+G+G
No of animals	8	8	10	9
Mean arterial blood pressure (mm Hg)	116 ± 7	118 ± 6	120 ± 7	119 ± 6
Hematocrit (%)	43.1 ± 0.9	40.5 ± 3.7	43.6 ± 0.7	42.0 ± 0.6
Fractional islet blood flow (% of pancreatic blood flow)	9.8 ± 0.7	9.5 ± 0.4	17.7 ± 1.4*	16.5 ± 1.7*
Colonic blood flow (ml/minxg)	1.08 ± 0.18	0.75 ± 0.16	1.70 ± 0.32	1.11 ± 0.10
Renal blood flow (ml/minxg)	3.57 ± 0.30	3.03 ± 0.24	4.16 ± 0.32	2.97 ± 0.21
Adrenal blood flow (ml/minxg)	5.11 ± 0.50	4.34 ± 0.33	8.86 ± 1.39	6.43 ± 0.61

Values are means ± SEM. * denotes $P<0.05$ when compared to animals given S+S+S or G+S+S (ANOVA).

Results

All animals tolerated the surgical procedures well, and the hematocrit values and mean arterial blood pressure were similar in all groups (Table 1). Blood glucose concentrations were as expected unaffected in saline-injected rats, SSS rats, and in animals injected with glucose at time 0, and thereafter with saline, GSS rats (Table 2). In animals given 2 initial saline-injections and a final glucose injection, SSG rats, the blood glucose was higher at the last measurement (Table 2). Animals given 3 repetaed glucose injections, GGG rats, demonstrated similar peak glucose values 3 min after each injection (Table 2). No differences between glucose concentrations obtained 3 min after a gluose injection were seen when the different groups were compared.

Serum insulin concentrations were similar in SSS- and GSS-treated rats, but higher in SSG and GGG rats (Figure 1). The insulin concentrations were higher in GGG rats when compared to SSG rats (Figure 1).

Total pancreatic (Figure 2), islet (Figure 3) and duodenal blood flow (Figure 4) were higher in SSG and GGG rats when compared to the other two groups. In none of the cases was there any difference in flow values between SSG and GGG rats.

There were no differences in colonic, renal or adrenal blood flow between the investigated groups (Table 1), even though there was a tendency of an increased renal blood flow in SSG rats ($P=0.062$; ANOVA) when compared to SSS rats.

Table 2. Blood glucose concentrations (mmol/l) in anesthetized, male Wistar-Furth rats injected intravenously with 1 ml of saline (S) or 30% D-glucose (G) at times 0, 30 and 60 min. Measurements were made at different times after injection.

Treatment	S+S+S	G+S+S	S+S+G	G+G+G
No of animals	8	8	10	9
Before any injection	4.8 ± 0.1	4.9 ± 0.1	4.9 ± 0.1	5.0 ± 0.2
3 min	4.9 ± 0.2	16.1 ± 1.4**	5.0 ± 0.1	16.0 ± 1.4**
30 min	5.1 ± 0.2	8.5 ± 0.4*	4.5 ± 0.1	8.7 ± 0.5*
33 min	5.0 ± 0.2	7.4 ± 0.5*	4.5 ± 0.2	17.7 ± 0.7**
60 min	4.8 ± 0.2	5.6 ± 0.2	4.2 ± 0.1*	8.9 ± 0.3*
63 min	4.9 ± 0.2	5.6 ± 0.3	13.5 ± 1.4**	17.4 ± 0.8**

Values are means ± SEM. * denotes P<0.05 and **P<0.01 when compared to the value before administration (ANOVA).

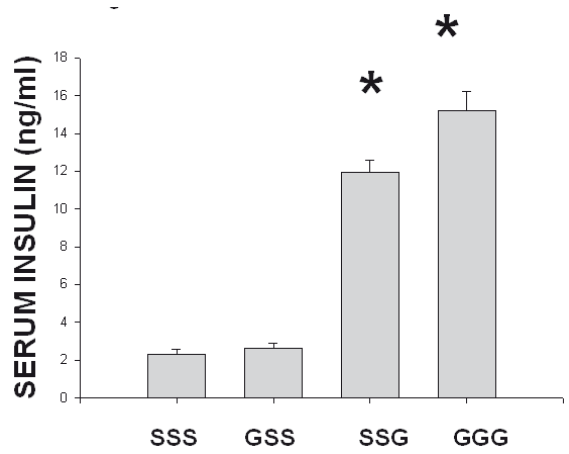

Figure 1: Serum insulin concentrations after three intravenous injections of 1 ml of saline or 30% glucose at times 0, 30 and 60 min. The combinations used were saline + saline + saline (SSS), glucose + saline + saline (GSS), saline + saline + glucose (SSG) and glucose + glucose + glucose (GGG). Values are means ± SEM for 8-10 experiments. * denotes P<0.05 when compared to SSS and GSS (ANOVA).

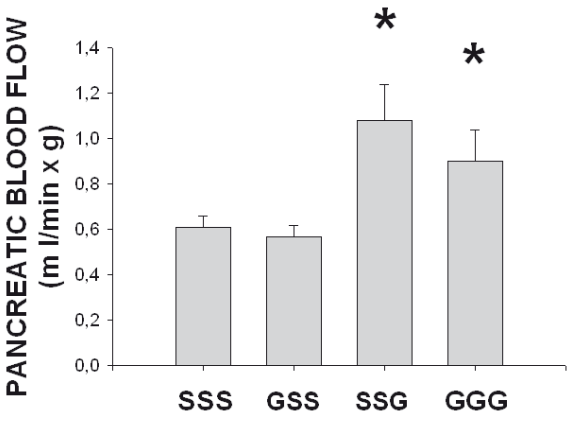

Figure 2: Total pancreatic blood flow after three intravenous injections of 1 ml of saline or 30% glucose at times 0, 30 and 60 min. The combinations used were saline + saline + saline (SSS), glucose + saline + saline (GSS), saline + saline + glucose (SSG) and glucose + glucose + glucose (GGG). Values are means ± SEM for 8-10 experiments. * denotes P<0.05 when compared to SSS and GSS (ANOVA).

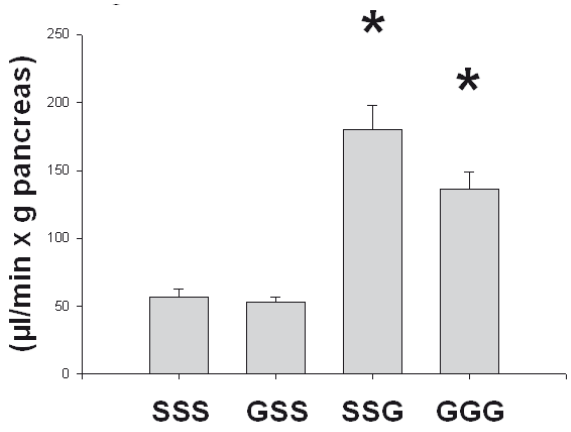

Figure 3: Pancreatic islet blood flow after three intravenous injections of 1 ml of saline or 30% glucose at times 0, 30 and 60 min. The combinations used were saline + saline + saline (SSS), glucose + saline + saline (GSS), saline + saline + glucose (SSG) and glucose + glucose + glucose (GGG). Values are means ± SEM for 8-10 experiments. * denotes P<0.05 when compared to SSS and GGG (ANOVA).

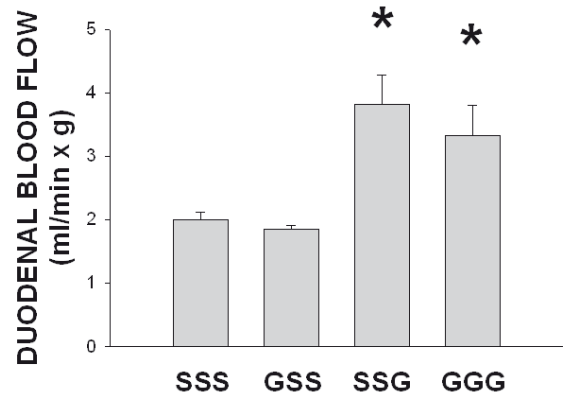

Figure 4: Duodenal blood flow after three intravenous injections of 1 ml of saline or 30% glucose at times 0, 30 and 60 min. The combinations used were saline + saline + saline (SSS), glucose + saline + saline (GSS), saline + saline + glucose (SSG) and glucose + glucose + glucose (GGG). Values are means ± SEM for 8-10 experiments. * denotes P<0.05 when compared to SSS and GSS (ANOVA).

Discussion

In confirmation of previous results we observed that glucose administered 3 min before blood flow measurements preferentially increased islet blood flow (18, 21). This effect is mediated by interactions between the parasympathetic nervous system and locally produced metabolites, mainly adenosine (12). We also verified that this glucose-induced acute islet blood flow increase had returned to normal 1 h later (22). Also total pancreatic and duodenal blood flow were increased during this condition, suggesting an general increase in the blood flow through the superior mesenteric artery (23, 24). However, the increase in islet blood perfusion was more pronounced as seen previously (18), and in the present study. This is not due to osmotic effects (24), since it is not seen after administration of the non-metabolizable glucose derivative 3-O-methyl glucose (25). However, the exact mechanisms for the non-specific glucose-induced increase in total pancreatic and duodenal blood flow are still unknown.

When glucose was administered three times during 1 h, it induced similar increases in islet, total pancreatic and duodenal blood flow as those seen acutely after

only one glucose injection. Thus, no priming as seen in insulin secretion was observed with regard to blood perfusion. That priming would occur by direct effects on vascular smooth muscle and/or endothelial cells seems unlikely, since priming in the β-cell is dependent on glucose metabolism and presumably an activation of mitochondrial enzymes (8). It could, however, be speculated that increased endocrine cell release from the islets could directly affect the islet afferent islet arterioles to increase blood perfusion. We have previously noted that insulin in itself does not affect islet blood flow (26), whereas high doses of islet amyloid polypeptide (IAPP) (27), somatostatin (28) and pancreatic polypeptide (29) may do so. However, the present findings suggest that neither insulin, IAPP or any other product co-secreted with insulin, e.g. chromogranins (30), can potentiate the glucose-induced islet blood flow increase. It can also be speculated that increased release of metabolites from the islets, e.g. adenosine, associated with repeated glucose stimulation cannot further increase islet blood flow when compared to one acute glucose challenge.

As expected repeated glucose administration caused a more pronounced insulin secretion, thereby verifying previous observations (1, 2). This priming effect has been suggested to remain in some patients with type 2 diabetes (3, 10). We therefore speculated above that it could be that islet blood perfusion can be similarly affected, since islet blood flow is increased in type 2 diabetes (16, 17). Our present findings that no priming effect was seen on islet blood flow, suggests that this is not the case, but that other mechanisms are involved in islet blood hyperperfusion during impaired glucose tolerance. The mechanisms for the latter are likely to involve both the nervous system and metabolic by-products from the islets.

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