Previous Exposure to Glucose Enhances Somatostatin Secretion from the Isolated Perfused Rat Pancreas

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Summary. Previous exposure to glucose enhances insulin and depresses glucagon secretion by the pancreas. We have investigated whether secretion of somatostatin is also influenced by a glucose priming effect. In perfused rat pancreas from 36 h fasted rats a 5 min pulse of arginine (8 mmol/l) rapidly elicited a peak of somatostatin release. A similar somatostatin response was evoked by a second, identical, pulse of arginine after perfusion with “basal” glucose (3.9 mmol/l) for 45 min. On the other hand when 27.7 mmol/l D-glucose, was administered for 20 min between arginine pulses, there was significant stimulation of somatostatin secretion. When arginine was re-introduced 15 min after the cessation of the pulse of elevated glucose the magnitude of the arginine-induced peak (min 0–2 of stimulation) was increased from 16.2 ± 4.1 to 33.1 ± 4.7 pg/2 min, $p < 0.01$, relative to the first stimulation with arginine. None of these effects of glucose could be reproduced by D-galactose. The somatostatin response to arginine was higher in pancreata from fed than from 36 h fasted animals as was also basal release (22.8 ± 5.0 vs 9.0 ± 2.0 pg/min). In the fed state the response to the second pulse of arginine was however reduced by 50% after perfusion with “basal” glucose. This decrease in responsiveness was counteracted by perfusion with 27.7 mmol/l glucose for 20 min between the arginine pulses. It is concluded that previous exposure to an elevated concentration of glucose enhances D-cell responsiveness to arginine in the fasted as well as the fed state.

Key words: Somatostatin release, D-cell, insulin release, glucagon release, glucose priming, fasting, glucose metabolism, starvation, glucose homeostasis, perfused rat pancreas.

Glucose promptly stimulates insulin secretion and amplifies the insulinotropic effect of other agents whereas glucagon secretion is affected in a reciprocal

fashion [1]. The magnitude of these acute effects of glucose on the B- and A-cells is however strikingly reinforced by a short previous exposure to the sugar [2–5]. Such exposure increases the responsiveness of the B- [2–4] and decreases that of the A-cells [5] thus demonstrating priming or “memory” effects of glucose.

Somatostatin has been identified as the hormone present in the D-cells of islets of Langerhans [6, 7]. With the advent of sensitive radioimmunoassays, secretion of somatostatin has been found to be stimulated promptly by high concentrations of glucose [8–10]. The present study was undertaken in order to test whether also a priming effect of glucose could be demonstrated in the D-cells concomitant with the effects already documented on insulin and glucagon secretion. The isolated perfused pancreas of the rat was used since this preparation retains a high sensitivity of A, B- and D-cells to stimulation. Arginine is a potent stimulator of insulin and glucagon [1], and somatostatin [10, 11] secretion; this amino acid was thus considered as a suitable agent with which to test simultaneously the effects of priming with glucose on the release of all three hormones. To test whether a priming effect of glucose was restricted or not to the fasted state, the experiments were carried out in pancreata from 36 h fasted as well as from fed animals.

Material and Methods

Animals, Experimental Procedure

Male Sprague-Dawley rats weighing 180–220 g were obtained from Anticimex, Stockholm. They were either fasted for 36 hours or fed ad libitum. The pancreata were isolated free from all surrounding tissue by the technique of Loubatières [12]. The perfusion medium was a Krebs-Henseleit-bicarbonate medium [13] supplemented with 20 g/l of bovine albumin and 3.9 mmol/l of glucose. The medium was introduced through the abdominal aorta and pumped through the pancreas without recycling at a flow rate of approximately 2.0 ml/min.

The study protocols are illustrated in Figure 1. The design of the experiments was guided by the need to obviate interexperimental variation between different preparations. Such variation was considerable in previous studies [5, 14] as well as in the present one (Table 1). Possible causes include seasonal influences on the rats used, the use of different batches of albumin [15] as well as the interassay variation between the radioimmunological determinations. The basic experimental protocol thus consisted of the administration of two identical 5 min pulses of 8 mmol/l of arginine. First and second pulse responses could then be compared within the same experiment. During an intervening period of 45 min, the glucose concentration was either kept at 3.9 mmol/l (protocol 1) or intermittently raised for 20 min to 27.7 mmol/l (protocol 2). The pulse of elevated glucose was terminated 15 min

Fig. 1. Experimental protocol for perfusion studies. A 15 min period of pre-perfusion (not shown in figures) was allowed before the start of the experimental protocols. The latter time-point was designated as min zero. ARG = arginine, G = D-glucose, GAL = galactose

before the second pulse of arginine. In one experimental series the glucose pulse was substituted by galactose (protocol 3). Experiments using protocols 1 and 2 in fed rats and protocol 3 in fasted rats were carried out 9 months later than the rest of experiments.

Samples were obtained at 60 s intervals during stimulation with arginine. The eluate from the entire pulse of glucose or galactose was pooled and a single aliquot was retained. 0.5 ml of samples were collected into pre-chilled tubes containing 0.1 ml of Trasylol (10000 IU/ml), then frozen and stored at -20°C .

Radioimmunoassay

Insulin was assayed as previously described [16]. Rat insulin which served as standard was a gift from Dr J. Schlichtkrull, Novo Industries, Bagsvaerd, Denmark. The antibodies used were raised against porcine insulin. Glucagon was assayed as described [17] using 30 K antibody (several batches), raised against porcine glucagon and obtained from Dr. Unger, Dallas, Texas. Porcine glucagon served as standard. Somatostatin was assayed using antibodies raised in the rabbit against cyclic somatostatin as described [10, 18]. The validity of the somatostatin assay was ascertained by parallel serial dilution curves of standards and somatostatin-reactive material. Neither 8 mmol/l arginine nor 27.7 mmol/l glucose influenced the binding of somatostatin to the antibody. The limit of sensitivity was 0.3 pg/assay tube or 3 pg/ml of perfusate. The inter- and intrassay coefficients of variation were 26% and 18% respectively.

Presentation of Results

These are expressed as mean $\pm$ SEM. Levels of significance were calculated by Student's t-test (two-tail) using paired differences when applicable.

Table 1. Effects of priming with glucose on arginine-induced insulin, glucagon and somatostatin secretion from fed and 36 h fasted animals. Except for experiments with galactose ($n = 7$), results are derived from data presented in Figures 2 and 3. Mean $\pm$ SEM of the integrated release above baseline during the stimulation with arginine

Conditions	Insulin $\mu\text{U}/5$ min		Glucagon ng/5 min		Somatostatin pg/5 min	
	Arginine 1st pulse	Arginine 2nd pulse	Arginine 1st pulse	Arginine 2nd pulse	Arginine 1st pulse	Arginine 2nd pulse
<i>Fasted 36 h</i>						
Control, protocol 1 (glucose 3.9 mmol/l min 0–55)	771 $\pm$ 120	463 $\pm$ 109 ^b	8.6 $\pm$ 1.1	8.8 $\pm$ 1.6	45.0 $\pm$ 8.9	42.0 $\pm$ 8.9
Priming, protocol 2 (glucose 27.7 mmol/l min 15–35)	776 $\pm$ 198	1801 $\pm$ 181 ^c	8.3 $\pm$ 0.8	4.5 $\pm$ 0.4 ^c	36.7 $\pm$ 8.3	47.7 $\pm$ 10.7 ^a
Galactose, protocol 3 (23.8 mmol/l galactose min 15–35)	127 $\pm$ 45	82 $\pm$ 36 ^b	4.3 $\pm$ 1.1	4.6 $\pm$ 1.4	30.3 $\pm$ 15.9	29.3 $\pm$ 11.3
<i>Fed</i>						
Control, protocol 1 (glucose 3.9 mmol/l min 0–55)	2824 $\pm$ 493	1522 $\pm$ 264 ^b	6.4 $\pm$ 1.1	6.2 $\pm$ 1.0	73.5 $\pm$ 23.2	32.0 $\pm$ 12.8 ^c
Priming, protocol 2 (glucose 27.7 mmol/l min 15–35)	4542 $\pm$ 439	9636 $\pm$ 1529 ^c	5.8 $\pm$ 1.2	3.7 $\pm$ 0.6 ^c	98.8 $\pm$ 24.5	104.4 $\pm$ 13.0

^a $p < 0.05$, ^b $p < 0.01$, ^c $p < 0.001$ significance of differences between 1st and 2nd pulses of arginine (Student's t-test, paired differences)

Fig. 2. Effects of priming with glucose after 36 h of fasting on arginine-induced secretion of immunoreactive insulin, glucagon and somatostatin. Protocols 1 and 2 were used. Two successive pulses of 8 mmol/l of arginine were administered 45 min apart and the glucose concentration was either kept at 3.9 mmol/l throughout the experiment (upper panel, control) or intermittently raised to 27.7 mmol/l for 20 min between pulses (lower panel, glucose priming). Results are expressed as the mean $\pm$ SEM of control ($n = 5$) or glucose priming ($n = 8$) experiments. $\square$ = period of arginine administration, $- \circ -$ = 1st pulse, $- \bullet -$ = 2nd pulse of amino acid

Results

Fasted Rats (36 hours)

A 5 minute pulse of 8 mmol/l of arginine elicited a peak and decline pattern of release of insulin, glucagon and somatostatin (Fig. 2 and Table 1). Further perfusion with 3.9 mmol/l glucose for 45 minutes did not significantly alter the basal release of glucagon or somatostatin nor the response to a second pulse of arginine. Basal insulin release was however significantly diminished (from 20 ± 2 at 0 minutes to 11 ± 4 μ U/min at 50 minutes, $p < 0.01$) as was also the stimulated secretion due to re-introduction of arginine (Table 1).

When the concentration of glucose was intermittently raised from 3.9 to 27.7 mmol/l for 20 minutes between the two pulses of arginine, the secretion of insulin and somatostatin was stimulated and that of glucagon inhibited during the presence of high glucose (Table 2). When a second pulse of arginine was presented 15 min after the cessation of the glucose pulse, the insulin response was two-fold increased at all time points while glucagon release was inhibited by 45% ($p < 0.01$, Fig. 2 and Table 1). Simultaneously, glucose priming significantly increased somatostatin release over the 5 min period of stimu-

lation with arginine ($p < 0.05$, Table 1). The effect of priming was to enhance the magnitude of the arginine induced peak (min 0–2) which was increased from 16.2 ± 4.1 to 33.1 ± 4.7 pg/2 min, $p < 0.01$.

The specificity of the priming effects of glucose were tested in experiments where 27.7 mmol/l of D-glucose was substituted for by 23.8 mmol/l of D-galactose in the continued presence of 3.9 mmol/l of glucose (protocol 3). Galactose together with "basal" glucose, did not induce insulin, glucagon or somatostatin release, (results not shown), nor did this isomer of glucose induce any of the priming effects observed after glucose (Table 1).

Fed Rats

Differences were noted in basal as well as stimulated secretion rates in pancreata from fed vs 36 h fasted animals. When calculated from all experiments (fasted $n = 20$, fed $n = 13$) the basal secretion of insulin was increased by feeding from 14 ± 4 to 37 ± 3 μ U/min, $p < 0.001$ and somatostatin secretion from 9.2 ± 2.0 to 22.8 ± 5.0 pg/min, $p < 0.01$, while basal release of glucagon was decreased from 0.45 ± 0.08 to 0.11 ± 0.04 ng/min. The effects of feeding on the insulin and somatostatin response to arginine were to increase the insulin release from 572 ± 112 to 4044

Table 2. Effects of glucose on A, B and D-cell secretion in the fed and 36 h fasted state. Results are obtained from the same experiments depicted in Figures 2 and 3 and demonstrate the effects of 27.7 mmol/l of glucose on insulin, glucagon and somatostatin release during its presence for 20 min. The glucose stimulus was administered between two pulses of arginine (protocol 2, Fig. 1). Data are presented as the mean $\pm$ SEM of the glucose effects above basal release

Conditions	Number of experiments	Insulin, μ U/min	Glucagon, ng/min	Somatostatin, pg/min
Fasted 36 h	8	144 $\pm$ 17	- 0.3 $\pm$ 0.2	9.5 $\pm$ 2.7
Fed	7	918 $\pm$ 223 ^c	$\pm$ 0.0 $\pm$ 0.0	25.6 $\pm$ 6.1 ^a

^a $p < 0.05$, ^c $p < 0.001$, significance of difference between fed and fasted state (Student's *t*-test, non-paired differences)

$\pm 467 \mu\text{U}/5 \text{ min}$, $p < 0.001$ and the somatostatin release from 36.5 ± 6.6 to $86.6 \pm 17.4 \text{ pg}/5 \text{ min}$, $p < 0.005$. Also in the experiments where 27.7 mmol/l of glucose was administered, the insulin release was 6-fold ($p < 0.001$) and somatostatin 2-fold ($p < 0.05$) higher in the fed than in the fasted state (Table 2).

In contrast to the findings in 36 h fasted animals, the basal secretion of somatostatin diminished during the two-pulse protocol in the fed state (from 23.0 ± 11.5 at zero minutes to $4.7 \pm 3.9 \text{ pg}/\text{min}$ at 50 minutes). Also the response to a second pulse of arginine was markedly reduced (by 50%, Fig. 3, Table 1). This deterioration of the arginine response was completely prevented when 27.7 mmol/l of glucose was administered between the arginine pulses. Relative to the first arginine pulse within the same experiment, the somatostatin response was however unaltered when arginine was re-introduced after glucose in the fed state.

Discussion

The present study demonstrates priming effects of glucose on insulin, glucagon and somatostatin secretion from the pancreas of 36 h fasted as well as from fed rats when measured 15 min after the cessation of a glucose stimulus. In the case of somatostatin the response was markedly enhanced in fasted and restored in fed rats. The effects of glucose could not be reproduced by an equimolar concentration of D-galactose, an isomer of glucose which is not metabolised in islets [19]. Thus it could be inferred that the priming effects of glucose were not secondary to a non-specific change of osmolality in the perfusion medium.

In control experiments, the administration of arginine depressed subsequent insulin release in the fasted and fed state and somatostatin release in the fed state. An inhibitory effect of arginine on subsequent insulin release was noted also in man [20] where the second of two consecutive arginine pulses elicited a smaller insulin release than the first one. The cellular mechanisms behind the negative effects of arginine are not clear. With regard to insulin secre-

tion, the inhibition subsides with time [20]. This may explain why no inhibition was noted in a previous series [5] where arginine was re-introduced after a longer time-period than in the present study.

The fact that priming can be demonstrated concomitantly in A, B and D-cells is compatible with the notion that similar mechanisms are operative. The common feature of enhancement of the acute effects of glucose (i. e. increased stimulation of insulin and somatostatin secretion, increased inhibition of glucagon secretion) may be taken as additional, though circumstantial, evidence along the same line. Although the effect of other secretagogues than glucose on D-cell secretion were not tested, previous studies indicate that the metabolism of glucose is essential for the induction of priming at least in A [5] and B-cells [4], since glucose cannot be substituted for by secretagogues which are non-metabolisable such as arginine [21]. It seems possible that the generation of high-energy phosphate intermediates may be of significance since ATP levels are augmented after priming (Grill and Ågren, unpublished observations). Alternatively, priming may be mediated through increased synthesis of proteins participating in the process of exocytosis. In this context it should be noted that the time-scale of induction of a priming effect is probably too short to allow altered biosynthesis of the respective hormones to be of importance [22, 23].

Paracrine effects have been suggested to supplement the systemic effects of islet hormones [24]. Experimental evidence does support the notion of intercellular communications between islet cells [25]. However, the present results concerning priming cannot be explained by postulating local interactions between the A, B or D-cells. For example, while data from 36 h fasted animals, taken alone, may suggest that heightened secretion of somatostatin could mediate the inhibitory effect by glucose priming on glucagon secretion, this does not fit with the data from fed animals where priming with glucose inhibited arginine-induced glucagon secretion without any change in the somatostatin response within the same experiment.

Fig. 3. Effects of priming with glucose in the fed state on arginine-induced secretion of immunoreactive insulin, glucagon and somatostatin. Protocols 1 (control) and 2 (glucose priming) were used. Results are expressed as the mean $\pm$ SEM of $n = 6$ and $n = 7$ experiments performed according to respective protocols. Symbols as in Figure 2

The physiological role of the priming effects of glucose are unknown. It may be speculated that they form part of adaptive mechanisms for the preservation of glucose homeostasis which act by reducing the impact of a carbohydrate challenge. Increased insulin release lowers blood glucose by increasing hepatic and peripheral uptake of glucose while a lesser glucagon secretion enhances the insulin effect on the liver. Portally delivered somatostatin could further increase glucose disposal by increasing the clearance rate of insulin over the splanchnic area [26] or by inhibiting the output of glucose from the liver (although the latter effect on the liver has so far only been shown using pharmacological concentrations of the hormone [27]). Furthermore, splanchnic somatostatin could decrease the rate of entry of carbohydrates into the organism by prolonging the transit time of nutrients in the gut [28].

In vivo the secretion of insulin is higher [29] and that of glucagon lower [30, 31] in the fed than in the fasted state. Feeding also increases the secretion of somatostatin into the portal vein [32]. Although these changes are qualitatively similar to those observed after priming, the mechanisms behind them are probably not identical because of the different time-scale used for demonstrating feeding and priming effects. In this context, it is also interesting to note that not only insulin but also the secretion of somatostatin was enhanced in the fed state in our perfused pancreas preparation. The latter observation is in contrast to results obtained in isolated islets [33] but in accordance with other data obtained from the perfused rat pancreas [34]. However, we do not wish to over-emphasise these and other quantitative differences between the response from fed or fasted pancreatic preparations since our protocols were not designed primarily to test for such differences.

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