Evidence that a ‘memory’ for glucose metabolism desensitizes A-cell responsiveness in the perfused pancreas of the rat

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Abstract. The effects of prior exposure to glucose or an inhibitor of glycolysis (iodoacetate) on A-cell sensitivity to glucose in the perfused pancreas of the rat was investigated. Inhibition of glucagon secretion by a high glucose concentration (22 mM) was attenuated and delayed when tested 20 min after a previous infusion with the same glucose concentration. Previously elevated glucose also delayed for 2 min a glucagon response to glucose omission whereas the total response was not significantly affected. During a 20 min perfusion with 1 mM iodoacetate, glucagon secretion increased and rates of secretion were further augmented after withdrawal of iodoacetate. When introduced 10 min after cessation of the iodoacetate pulse, 22 mM glucose failed to affect insulin or somatostatin release but, conversely, induced a profound decrease in glucagon secretion which was more marked than during control conditions.

Conclusions: A-cell sensitivity to glucose is diminished and enhanced by prior fuel abundance and deprivation, respectively. Such effects could be due to persisting changes in A-cell energy availability rather than to perturbations in insulin or somatostatin secretion.

Glucose profoundly influences all aspects of glucagon secretion; elevation of ambient glucose inhibits basal as well as stimulated release while opposite changes are induced by a decrease in glucose concentration (review Gerich et al. 1976). The mechanisms whereby glucose inhibits glucagon secretion have not been completely elucidated. Evidence has been presented for an intrinsic glucose effect, attributed either to an effect of the intact glucose molecule or to the metabolic fate of glucose (review Gerich et al. 1976). It has however, also been proposed that the glucose effect is secondary to stimulation of insulin release (Unger & Orci 1981) either by a systemic or by an intra-islet (paracrine) effect. A paracrine effect of somatostatin can also be envisaged as modulating a glucose effect on A-cell secretion (Efendić et al. 1980).

We have previously demonstrated that a ‘memory’ of previous exposure to glucose influences stimulation of glucagon secretion by arginine (Grill et al. 1979). A 30 min pulse of 27 mM glucose presented 20 min before a 10 min pulse of arginine thus markedly attenuated the stimulatory effect of arginine. We have also presented evidence that this priming effect of glucose is secondary to metabolic effects of the sugar since it could be reproduced by the glycolytic intermediate D-glyceraldehyde, but not by exogenous insulin.

The aim of the present work was to test whether not only the response to amino acids but also other aspects of regulation of glucagon secretion were desensitized by previous glucose. As a corollary to the importance of glucose metabolism in priming, we furthermore tested whether inhibition of glycolysis by previous exposure to iodoacetate could—reciprocally to the effects of glucose—sensitize the regulation of A-cell secretion.

Material and Methods

Animals and perfusion system

Male Sprague-Dawley rats (Anticimex, Solna, Sweden), weighing 200–250 g, were used. They were fed ad libitum with a commercial pelleted diet (Anticimex). The animals were anaesthetized by an ip injection of pentobarbital, 100 mg/kg body weight. The pancreas was isolated free from all adjacent organs (Loubatières et al. 1981).
1969) and perfused with a Krebs-Henseleit bicarbonate buffer with the following millimolar composition of electrolytes: Na$^+$ 143.5, K$^+$ 5.9, Mg$^{2+}$ 1.2, Ca$^{2+}$ 2.6, Cl$^- 128.4$, HCO$_3^-$ 25.0, H$_2$PO$_4^-$ 1.2, SO$_4^-$ 1.2. The medium was supplemented with 20 g/l of bovine albumin (Sigma, St. Louis, Missouri, USA) and $-3.9$ mM of D-glucose. The perfusate was delivered by way of a peristaltic pump from a buffer reservoir. Elevations in glucose as well as the administration of iodoacetate were achieved by the aid of a side-arm connected syringe which was driven by an infusion pump (Braun Melsungen, W. Germany). Using constant pressure, flow rates through the pancreas were between 2.7 and 3.2 ml/min. A pre-perfusion period of 20 min was allowed before starting the experimental protocol; the latter time-point was designated as min zero. No visible oedema of the pancreas developed during perfusion. Samples of perfusate were obtained at times indicated in the figures and collected in pre-chilled plastic tubes containing 500 Kallikrein Inhibitor Units per ml of aprotinin (Trasylol, Bayer, Leverkusen, W. Germany), then frozen and stored at $-20^\circ$C.

**Assays**

Glucagon was assayed as described (Faloona & Unger 1974) using 30 K antibodies obtained from Dr. Unger, Dallas, Texas. The sensitivity was about 10 pg/ml and the inter-assay coefficient of variation $\pm 7\%$.

Insulin was assayed radioimmunologically using charcoal addition to separate free and bound antibody (Herbert et al. 1965). The sensitivity of this assay was 8 mU/ml and the inter-assay coefficient of variation $\pm 10\%$. Somatostatin was assayed as described (Efendic et al. 1979) using $[^{125}\text{I}]\text{Tyr}^1$-somatostatin-14 and our own antibodies (R141E). The sensitivity of the somatostatin assay was about 2 pg/ml and the inter-assay coefficient of variation $\pm 15\%$.

**Presentation of results**

All results were expressed as mean $\pm$ se. Levels of significance were calculated using Student's $t$-test (two-tail) for paired or unpaired differences as evident or indicated in the text. Incremental or decremental integrated effects were calculated as the area above or under the mean of measurements preceding the change in glucose concentration.

**Results**

*Effects of previous glucose on suppression of glucagon secretion by elevated glucose*

As expected, glucagon secretion was depressed when the glucose concentration in the perfusion medium was changed from 3.9 to 22 mM glucose (Fig. 1). Depression was maximal after about 4 min and persisted throughout the 20 min period of administration of elevated glucose. This period was followed by a 20 min perfusion with 3.9 mM glucose, at the end of which glucagon secretion rates were similar to rates observed at the start of the experiment. When a second pulse of 22 mM glucose was administered glucagon secretion was again inhibited. However, the total inhibition was now only 50% of that recorded during the first pulse of elevated glucose (mean $-3220 \pm 500$ during the first, $-1640 \pm 660$ pg/20 min during the second pulse, $P < 0.05$). The attenuated inhibition by glucose priming occurred concomitant with an expected 2-fold enhancement of the insulin response during the second vs the first pulse of elevated glucose. As to glucose-induced somatosta-
Glucagon, insulin and somatostatin secretion. The perfusion schema was identical to that of Fig. 1, however, samples were obtained only from 2 min before and during the first 3 min of each perfusion period with 22 mM glucose. Mean ± SEM of 5 experiments.

Effects of previous glucose on stimulation of glucagon secretion by glucose omission
Glucagon secretion was not significantly changed during a 60 min perfusion period with the 'basal' glucose concentration (Fig. 3). During a 2 min period following a change from 3.9 to zero mM glucose, glucagon secretion rose from 518 ± 81 to 620 ± 84 pg/min (min 58–60 vs min 60–62 in Fig. 3; P < 0.02). The rise in glucagon levels in response to glucose omission was delayed in pancreases which had been pre-perfused with 22 mM glucose for 30 min, followed by 3.9 mM glucose for another 30 min. No rise was thus seen during the first 2 min of glucose omission (451 ± 72 and 437 ± 87 pg/min for min 58–60 vs min 60–62 in Fig. 3). This lack of response was significantly different from the response during the same time period in control experiments (P < 0.05). Total stimulation did, however, not differ significantly during control and test experiments (mean increment during the 20 min period of glucose omission 2460 ± 660 vs 1660 ± 700 pg/min. 

Secretory rates of insulin and somatostatin were low before and during the period of glucose omission in control experiments. In test experiments insulin secretion was slightly elevated immediately before glucose omission and declined during this
Effects of previous glucose on hormonal response to glucose withdrawal. Test experiments (●) included perfusion with 22 mM glucose during min 0–30, then 3.9 mM during min 30–60. Control experiments (○) included perfusion with 3.9 mM glucose min 0–60. Mean ± SEM of 6 experiments in each series.

Effects of previous iodoacetate on suppression of glucagon secretion by elevated glucose

Glucagon secretion was enhanced during the 20 min presence of the iodoacetate (Fig. 4). Ten min after withdrawal of iodoacetate secretion rates were further elevated. Transition from 3.9 to 22 mM glucose, however, markedly and persistently inhibited glucagon secretion. This inhibition by glucose was more marked in pancreases pre-treated with iodoacetate than in control pancreases. The mean inhibition during the 15 min period of elevated glucose was thus −11130 ± 3630 vs −2010 ± 690 pg/15 min, P < 0.01. However, secretion rates reached during the continued presence of 22 mM glucose were still elevated from iodoacetate – pre-treated compared with control pancreases.

In marked contrast to the suppressive effect on glucagon secretion, elevated glucose completely failed to elicit an insulin or a somatostatin response after pre-treatment with iodoacetate.

Discussion

Glucose exerts not only acute but also priming or 'memory' effects on the endocrine pancreas. A
priming effect on B-cell secretion is well documented and characterized (Cerasi 1975; Grill et al. 1978). A priming effect on D-cell secretion is less general and/or profound. We observed a small enhancing effect on arginine-induced somatostatin secretion (Grill et al. 1981). However, when glucose-primed pancreases were again stimulated with the hexose, a priming effect was observed in one study (Kanatsuka et al. 1981) but not in another (Gerber et al. 1984) and not in the present study.

The present study investigated a priming effect of glucose on A-cell secretion. A 'memory' of glucose has been shown to inhibit subsequent arginine-induced glucagon secretion in vitro (Grill et al. 1979). We now show that the regulation of glucagon secretion by glucose per se is also influenced by previous exposure to glucose. Thus the stimulatory effect on glucagon secretion of decreasing, and the suppressive effect of raising, extracellular glucose are both delayed by previous exposure to the sugar. Hence, it would appear that all aspects of glucose regulation of glucagon are indeed desensitized by glucose priming. In this context it should be pointed out that priming effects in Figs. 1 and 2, were compared within experiments. An influence of perfusion time per se was not ruled out in these experiments; however, such an influence seems unlikely since the basal glucagon secretion did not change significantly during 60 min of perfusion with 3.9 mM glucose (Fig. 3) and since the effects of 22 mM glucose on glucagon secretion in control experiments of Fig. 4 were comparable to that of the first glucose pulse of Fig. 1, despite differences in the duration of preceding perfusion with 3.9 mM glucose.

Indications that glucose exerts a priming effect on glucagon secretion can be obtained from two other studies. Weir et al. (1974) showed in the perfused rat pancreas that a sudden change in glucose concentration from 300 to 25 mg/100 ml elicited a more gradual increase in glucagon secretion than transition from 100 to 25 mg/100 ml; however, the presence of a 'memory' for previous glucose was not investigated. Suppression of basal glucagon secretion rates after withdrawal of elevated glucose has been demonstrated (Lecerq-Meyer et al. 1983); however, the relationship of the suppression to a desensitizing effect on regulation by glucose was not tested directly as in the present study.

It is not likely that the priming effect is mediated by changes in insulin secretion, since previous exposure to exogenous insulin did not mimic a priming effect of glucose on arginine-induced glucagon secretion (Grill et al. 1979). Concerning somatostatin, the present experiments do not support a role for this hormone in mediating a priming effect on glucagon secretion. Thus no or very small changes were seen in somatostatin secretion due to previous glucose during conditions where priming markedly altered glucagon secretion.

Observations showing augmented ATP levels 30 min after a pulse of elevated glucose (Grill & Ågren 1980) indicate that lasting changes in energy availability in islets are associated with priming. This notion is also compatible with our finding that iodoacetate sensitized A-cell secretion to the inhibitory influence of glucose. This effect of iodoacetate is most likely due to energy depletion since the concentration of iodoacetate used fully inhibits glycolysis but does not appear to exert unspecific toxic effects (Sener et al. 1978).

In other respects our results confirm several known effects of iodoacetate, such as inhibition of glucose-induced insulin secretion (Sener et al. 1978) and enhancement of glucagon secretion during the presence of iodoacetate (Edwards & Taylor 1970) and persisting inhibition of glucose-induced insulin secretion (Zawalich et al. 1977) after withdrawal of the glycolytic inhibitor. A novel finding is that previous iodoacetate also suppressed glucose-induced somatostatin release. The latter results emphasize the importance of glucose metabolism for D-cell secretion.

What is the physiological significance of glucose priming on glucagon secretion? We have previously shown that arginine-induced glucagon release is delayed after oral glucose in man (Adamson et al. 1981). A recent report furthermore demonstrates that 2 h of preceding hyperglycaemia obtunds the glucagon response to subsequent insulin-induced hypoglycaemia (Lager et al. 1984). Hence priming effects on glucagon secretion are likely to be operative in vivo under physiological conditions. It seems possible that glucose priming is also involved in the aberrations of A-cell responsiveness found in diabetes mellitus. Although it should be realized that multiple factors influence the responsiveness of the A-cell in diabetes, a role for an A-cell 'memory' of hyperglycaemia per se should be considered in the evolution of the diminished responsiveness to hyper- or hypoglycaemia (review Gerich et al. 1976; Unger & Orchi 1981) associated with diabetes.
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References


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