Glucose Memory of Pancreatic B and A₂ Cells

EVIDENCE FOR COMMON TIME-DEPENDENT ACTIONS OF GLUCOSE ON INSULIN AND GLUCAGON SECRETION IN THE PERFUSED RAT PANCREAS

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ABSTRACT The influence of previous exposure to glucose on the subsequent B- and A₂-cell secretory responses to arginine was investigated in the perfused pancreas of the rat. Arginine (8 mM) was administered in two brief (9 min) pulses separated by a period of 66 min. In pancreata from 18-h-fasted animals the two pulses of arginine elicited biphasic glucagon secretory responses, while stimulation of insulin release was barely detectable. When 27.7 mM glucose was administered for 30 min during the intervening period up to 20 min before the second pulse of arginine, the glucagon response to arginine was diminished by 55% while the insulin release was markedly increased in comparison with the first pulse. 8.3 mM glucose, when administered before the second pulse of arginine, exerted effects that were smaller but otherwise similar to those of 27.7 mM glucose.

The inclusion of 3.9 mM glucose during the stimulation periods with arginine decreased the glucagon and greatly increased the insulin secretory response. Under these conditions, previous exposure to 27.7 mM glucose inhibited the glucagon and enhanced the insulin response to the second stimulatory pulse of arginine to the same relative degree as when arginine was administered alone.

Diazoxide (2 mM), when administered together with 27.7 mM glucose, almost completely inhibited insulin release induced by the presence of glucose, yet did not influence the modulation exerted by glucose on the subsequent insulin and glucagon secretory response to arginine. Conversely, these effects of the glucose pulse could not be reproduced by 1 μ g/ml of porcine

insulin. Previous exposure to glyceraldehyde (10 mM) mimicked the glucose effects.

Also, in pancreata from fed rats, previous exposure to 27.7 mM glucose markedly inhibited subsequent arginine-induced glucagon secretion while the concomitant insulin response was enhanced.

It is concluded that: (a) both A₂- and B-cell responsiveness is modulated by a previous exposure to glucose which produces opposite effects in the two cell types, (b) this action of glucose does not depend on its insulin-releasing capacity, and (c) instead, a "memory" of glucose is induced as a consequence of the metabolism of the sugar in the A₂ and B cells.

INTRODUCTION

Glucose stimulates insulin secretion from the pancreas within 60 s (1). This effect on the B cell is, however, modulated by a previous exposure to elevated concentrations of glucose. Such exposure augments the insulin secretory response to a second stimulation with glucose (2-4) and also to stimulation with other insulintropic agents (5, unpublished observations). This enhancing effect on B-cell responsiveness can be termed the time-dependent, priming or "memory" effect of glucose.

Glucagon secretion is stimulated when glucose is withdrawn and inhibited when the concentration of the sugar is increased. These changes brought about by glucose are much more striking in the presence of some amino acids, such as arginine which stimulates both insulin and glucagon secretion. Thus, in the absence of glucose, arginine induces a marked glucagon and only a small insulin response, while in the presence of glucose a reverse pattern of bihormonal secretion

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is observed (6, 7). The immediate effects of glucose on the A₂ cell can thus be described as "mirror" images to those exerted on the B cell. Based on these observations, the question was raised whether or not glucose exerts a priming effect on the A₂ cell reciprocal to that already documented in the B cell.

The present study was designed with the following objectives: (a) to determine whether previous exposure to glucose influences subsequent glucagon secretion from the A₂ cell, and (b) if so, to investigate whether the conditions for obtaining priming by glucose on glucagon secretion are similar to those which induce priming of insulin secretion. To this end, the release of insulin and glucagon in response to stimulation with arginine was measured before and after the pancreas had been exposed to different concentrations of glucose as well as to other agents.

METHODS

Perfused pancreas. Male Sprague-Dawley rats, weighing 160–200 g, were either fed *ad libitum* or fasted for 16 h before the experiments. They were anesthetized by i.p. injection of 100 mg/kg of pentobarbital. The pancreas was isolated free from surrounding tissue by a slight modification of the technique of Loubatières et al. (8). The gland was perfused with a Krebs-Henseleit-bicarbonate solution which contained 20 g/liter of albumin. The final solution was continuously gassed with a mixture of 95% oxygen and 5% carbon dioxide. The buffer solution was administered into the aorta and pumped through the pancreas without recycling at a flow rate of ~2 ml/min. A 15- to 20-min period of perfusion with Krebs-Henseleit-bicarbonate solution containing a nonstimulating concentration of 3.9 mM glucose was allowed before the start of the actual experiment (not shown on the figures). The total time of the preperfusion and the actual experiment was kept constant (105–110 min), including the equilibration period. The general design of all experiments was to stimulate the pancreas with two brief pulses of 8 mM arginine and to vary the experimental conditions during the time interval between these pulses. When such variation entailed exposure to 27.7 mM glucose, control measurements of glucose in the eluate were carried out which showed that the elevation of the glucose concentration subsided completely after the termination of the glucose pulse. All fractions of the eluate were collected in small prechilled plastic tubes containing 0.1 ml of Trasylol (FBA Pharmaceuticals Inc., New York). These were then rapidly frozen and stored at –20°C until later assays.

Assays. Glucagon was measured by using a charcoal separation method (9), with 30K antibodies provided by Dr. R. H. Unger, Dallas, Tex. ¹²⁵I-labeled pork glucagon was obtained from Novo Industries (Bagsvaerd, Denmark). Insulin was measured using a charcoal separation method of radioimmunoassay (10). ¹²⁵I-labeled pork insulin (obtained from the Radiochemical Centre, Amersham, United Kingdom) was used. Purified rat insulin (kindly supplied by Dr. J. Schlichtkrull, Novo Research Institute, Copenhagen, Denmark) served as standard. The glucose concentrations in the eluate were measured by a commercial glucose oxidase method (AB Kabi, Stockholm, Sweden).

Calculation of results. The integrated areas under the curves above the pre-stimulatory values were determined by planimetry. Statistical comparisons were performed using Student's *t* test when applicable for paired differences within the same experiments.

RESULTS

Stability of the perfused pancreas preparations. The stability of the pancreatic preparations during the experimental period was assessed by comparing glucagon and insulin secretion immediately before each of the two stimulatory pulses of arginine. As shown in Table I, the release of glucagon and insulin was low whether measured before the first or the second pulse of arginine.

It should be noted that in most experiments the glucose concentration was lowered, 5 min before the administration of arginine, from 3.9 to 0 to ensure that no glucose remained in the incubation medium at the commencement of the arginine pulse. No direct investigation was carried out to determine the extent of the stimulation by glucagon secretion exerted by this change in glucose concentration.

Effects of previous exposure to 3.9, 8.3, or 27.7 mM glucose on arginine-induced glucagon and insulin secretion. 8 mM arginine stimulated glucagon release markedly but induced only a slight insulin release (Fig. 1). When the brief (9 min) stimulation period with arginine was followed by perfusion with 3.9 mM glucose for 66 min, a second, identical pulse of arginine stimulated glucagon and insulin release to a degree that was not significantly different from that of the first pulse (Fig. 1A). The mean area of the glucagon response was, however, 40% larger than that elicited by the first pulse (Table II). When 27.7 mM glucose was administered for 30 min between the two pulses of arginine, however, the glucagon response to the second pulse of arginine was reduced by 55% in comparison with the first pulse when tested 20 min after cessation of the glucose stimulus (Fig. 1C; Table II). Concurrently, arginine-induced insulin release was augmented approximately fourfold in comparison to the first pulse. Reducing the concen-

TABLE I
Insulin and Glucagon Release before Stimulation
with Arginine

	Before first arginine pulse	Before second arginine pulse
IRI, μ U/min	6.9 $\pm$ 2.7	3.5 $\pm$ 1.1
IRG, pg/min	213 $\pm$ 56	283 $\pm$ 42

Insulin (IRI)* and glucagon (IRG)† were measured in the fractions of perfusate immediately preceding each of two successive pulses with arginine. Results are presented as mean $\pm$ SE of 10 experiments performed in pancreata from 18-h-fasted animals. The glucose concentration between the two pulses of arginine was kept at 3.9 mM glucose. No significant difference between the different time points of measurements was observed.

* IRI, immunoreactive insulin.

† IRG, immunoreactive glucagon.

FIGURE 1 Effects of previous exposure to different concentrations of glucose on the subsequent arginine-induced glucagon (immunoreactive glucagon [IRG]) and insulin (immunoreactive insulin [IRI]) responses. Pancreata from 18-h-fasted

tration of the glucose pulse from 27.7 to 8.3 mM resulted in changes in glucagon and insulin release in response to the second vs. the first arginine pulse that were less pronounced (Fig. 1B; Table II).

In addition to the priming effects on arginine secretion, 27.7 mM glucose, during its presence, stimulated insulin secretion while 8.3 mM had almost no such effect.

Effects of a previous exposure to 27.7 mM glucose on the secretory responses induced by arginine in the presence of 3.9 mM glucose. As expected from previous findings (6, 7), arginine, when administered together with glucose, elicited a glucagon response that was somewhat less pronounced and an insulin response that was much greater than when arginine was tested in the absence of glucose (Fig. 2; Table II). Although the immediate action of 3.9 mM glucose thus profoundly altered the arginine-induced bihormonal response, the time-dependent effect of 27.7 mM glucose on glucagon or on insulin release did not seem to be modified. In fact, when the latter glucose concentration was administered between the two pulses of arginine, the glucagon response to the second arginine pulse was inhibited by 57% in comparison to the first pulse, while the insulin response was stimulated fourfold. These percent changes of arginine-induced insulin and glucagon responses caused by the "memory" of glucose were similar to those seen when arginine was tested in the absence of glucose (compare A and B in Table II).

Effects of insulin. The possibility that the priming effects of glucose were coupled to the process of insulin release induced by the sugar was tested in two ways: first, by suppressing insulin released during the presence of 27.7 mM glucose with diazoxide and, second, by substituting exogenous insulin for glucose as a priming agent. As shown in Fig. 3, insulin release during the presence of 27.7 mM glucose was almost completely inhibited by diazoxide. However, the priming effects of glucose on arginine-induced glucagon and insulin secretion were not modified by the inclusion of the drug in the perfusion medium (Fig. 3B); the glucagon response was inhibited by 64% (23.8 ± 1.9 vs. 8.7 ± 0.6 ng/9 min, $P < 0.025$) and the insulin response was stimulated ($P < 0.05$). Diazoxide per se did not have a significant effect on subsequent arginine-induced responses (Fig. 3A).

When 1 μ g/ml of porcine insulin was administered between the arginine pulses, no effect was evident on

rats were used. Between two pulses of arginine, the pancreata from fasted rats were exposed for 30 min to 8.3 (B, $n = 5$) or 27.7 (C, $n = 4$) mM glucose. In control experiments, 3.9 mM glucose was used between the pulses (A, $n = 5$). The bar between 30 and 60 min gives the mean secretion rate of IRI and IRG, measured in the pooled perfusate. Results are given as mean $\pm$ SE.

TABLE II
*Effects of Previous Exposure to Different Concentrations of Glucose
on the Subsequent Arginine-Induced Glucagon (IRG)*
and Insulin (IRI)† Release*

Intervening glucose	n	IRG		IRI	
		First pulse	Second pulse	First pulse	Second pulse
mM		ng/9 min‡		μU/9 min‡	
A					
3.9	5	11.6±3.9	16.8±3.0	75±41	110±87
8.3	4	15.1±3.0	11.3±2.4	108±57	277±80
27.7	6	14.4±2.3	6.4±1.4	95±44	394±117
B					
3.9	4	7.2±1.1	9.4±1.3	968±281	1,000±375
27.7	5	5.3±0.9	2.3±0.6	1,238±420	4,346±1,104

Arginine, 8 mM, was administered in two pulses between min 0 and min 9, and between min 80 and min 89, respectively, either in the absence (A) or the presence (B) of 3.9 mM glucose. Before the second pulse of arginine the glucose concentration was intermittently changed from min 30 to min 60 (=intervening glucose). Pancreata from 18-h-fasted rats were used.

* IRG, immunoreactive glucagon.

† IRI, immunoreactive insulin.

‡ The values represent the mean±SE of the integrated secretion rates of insulin and glucagon release above base line obtained by planimetry.

^{||} Significantly different ($P < 0.05$ or less) from the first pulse response within the same set of experiments.

the subsequent arginine-induced glucagon response (Fig. 4); the integrated response before and after insulin was 15.9 ± 1.2 and 13.8 ± 2.0 ng/9 min, respectively. The insulin response could not be evaluated under these circumstances because basal insulin release was high (~ 100 μU/min) at the beginning of the second arginine pulse, thus suggesting residual contamination of the pancreas with exogenous insulin. Insulin, during its presence, did not significantly reduce glucagon secretion in the presence of 3.9 mM glucose.

Effects of previous exposure to glyceraldehyde. To determine whether a priming effect of glucose could be reproduced by a metabolite of the sugar, D-glyceraldehyde was tested. 10 mM of the triose was administered between the two pulses of arginine. This concentration of glyceraldehyde is known to profoundly influence islet metabolism (11). Under these conditions glyceraldehyde inhibited the glucagon secretion and stimulated the concomitant insulin secretion to a second pulse of arginine (Fig. 5). These dual effects of glyceraldehyde seemed as pronounced as those exerted by previous exposure to 27.7 mM glucose. Arginine-induced glucagon release was thus diminished by 70% (from 16.6 ± 2.8 to 5.0 ± 1.1 ng/9 min, $P < 0.025$) and insulin release tended to be increased (from 59 ± 37 to 702 ± 348 μU/9 min, $P < 0.1$). In addition to these time-dependent effects, during its presence in the perfusion medium glyceraldehyde had an immediate

stimulatory effect on insulin release which was comparable to that of 27.7 mM glucose.

Effects of nutrition. To determine whether the demonstration of priming effects of glucose on arginine-induced secretory responses was exclusively limited to the fasting state, pancreata from fed rats were tested in four experiments, using the same protocol as for fasted animals in Fig. 1. Several differences were noted between the secretory responses of the pancreata from fed and fasted rats. Basal insulin release was significantly higher in the pancreata from fed animals (22.7 ± 0.5 as compared to 6.9 ± 2.7 μU/min in the fasted state, $P < 0.001$). Furthermore, the first pulse of arginine elicited a much more marked insulin response in the fed than in the fasted state. (886 ± 163 as compared to 96 ± 44 μU/9 min). In contrast to these differences of responsiveness of the pancreatic A₂ and B cells, the priming effects of 27.7 mM glucose were similar in the fed and fasted state, in the sense that glucagon responses to arginine were markedly inhibited and insulin responses stimulated. In comparison to the first pulse of arginine, glucagon release was thus inhibited from 12.6 ± 2.1 to 3.3 ± 0.9 ng/9 min (74%, $P < 0.05$) and the insulin release stimulated from 1,238 to 4,346 μU/9 min (3.5-fold, $P < 0.02$) by the previous exposure to glucose.

In three other experiments with pancreata from fed animals in which glucose was kept at 3.9 mM between the arginine pulses, the glucagon response to the first

FIGURE 2 Effects of previous exposure to 27.7 mM glucose on the secretory response induced by arginine simultaneously with glucose. Two successive pulses of arginine were administered in the presence of 3.9 mM glucose. Between pulses, the glucose concentration was either kept at 3.9 mM (A, $n = 4$) or intermittently raised to 27.7 mM during a 30-min period (B, $n = 5$). Pancreata from 18-h-fasted rats were used. Symbols as in Fig. 1.

arginine pulse was 16.3 ± 2.9 and to the second 20.8 ± 2.8 ng/9 min, a difference which is not significant. In the same experiments, however, insulin release to a second arginine stimulus was almost completely suppressed (from $1,738 \pm 87$ to 86 ± 48 μ U/9 min).

DISCUSSION

Our experiments were designed to determine if a priming effect of glucose on the A_2 cell could be observed and characterized in vitro. To ensure maximal detectability of the glucose effects, inter-experimental variation was excluded by investigating how the response to two successive and identical pulses of arginine on the same pancreas could be modulated by changes in the glucose concentration during the rest period;

FIGURE 3 Effects of previous exposure to glucose in the presence of diazoxide. Diazoxide (2mM) was administered together with either 3.9 (A) or 27.7 (B) mM glucose. Pancreata from 18-h-fasted rats were used. Two experimental series, each consisting of four experiments. Symbols as in Fig. 1.

the first pulse served as a control for the second one. The appropriateness of this protocol was indicated by the finding that the insulin release in response to the two arginine pulses was similar under control conditions at least in the fasted state.¹ Also, the glucagon release

¹ In pancreata from fed rats, however, insulin release in response to the second pulse of arginine was profoundly suppressed in comparison to first-pulse response. We have no

FIGURE 4 Effects of previous exposure to exogenous insulin on IRG release. 1 μ g/ml porcine insulin was administered for 30 min between two pulses of arginine. Pancreata from 18-h-fasted rats were used, $n = 6$. Symbols as in Fig. 1.

induced by the second pulse of arginine was in no series significantly different from that elicited by the first pulse, although a tendency to higher second-pulse glucagon release was observed. Because the effect of raising the glucose concentration between the administrations of arginine was to change in the opposite direction (i.e., inhibit) the glucagon response to a second pulse of arginine, we conclude that the time-dependent effects of glucose would in no case be overrated and would possibly be underestimated by the present method of analysis.

We have been unable to find prior clear documentation of an inhibitory effect on A_2 -cell secretion caused by previous exposure to glucose. In our experiments, the "memory" of glucose lasted for at least 29 min as measured from the cessation of the glucose pulse to the end of the stimulation by arginine. Weir et al. noted inhibition of first phase isoproterenol-induced glucagon secretion 5 min after exposure to 16.7 mM glucose (12). In their study, no lasting suppression by glucose was evident 10 min after cessation of the glucose stimulus. We have no obvious explanation for this partial discrepancy.

In our previous work (4) we were able to define some of the conditions for inducing a time-dependent effect of glucose on insulin secretion. It could be shown that some factors that are thought to regulate the immediate insulin release process are not essential for induction of the glucose "memory." Thus the latter was not dependent on a normal concentration of Ca^{++} in the

obvious explanation for this difference between fasted and fed rats. However, a similar time-dependent inhibition of the second insulin response to arginine has been observed in overnight-fasted man. Cerasi, E. Unpublished observations.

FIGURE 5 Effects of previous exposure to glyceraldehyde on IRG and IRI response. 10 mM D-glyceraldehyde was administered for 30 min between two pulses of arginine. Pancreata from 18-h-fasted rats were used, $n = 4$. Symbols as in Fig. 1.

extra-cellular medium, on an intact insulin-releasing effect of glucose, nor on stimulation of islet cyclic AMP. On the other hand, induction of the "memory" was abolished by a blocker of glucose metabolism, mannoheptulose, and the glucose metabolite D-glyceraldehyde could substitute for glucose as a priming agent. On the basis of these findings we suggested that the time-dependent effect of glucose on insulin release is coupled directly or indirectly to the metabolic degradation of the sugar in the islet. These observations and conclusions can now be extended to the A_2 cell. Thus diazoxide, which inhibits the islet accumulation of cyclic AMP (13), uptake of Ca^{++} (14), as well as insulin release (15), did not inhibit the "memory" effect of glucose on the arginine-induced secretory responses, thus suggesting that the factors inhibited by diazoxide are not of crucial importance for the glucose effect on either the A_2 or the B cell. Furthermore, the fact that glyceraldehyde could reproduce the priming effects of glucose on the A_2 cell suggests that, also in this cell, the induction of priming requires the metabolic degradation of glucose.

Also from another viewpoint, the time-dependent effects of glucose on glucagon and insulin secretion seemed similar. Thus no obvious differences were observed as to the dependency of the glucose concentration; 8.3 mM glucose as a priming agent exerted small effects on the subsequent arginine-induced bi-hormonal responses. However, it has to be emphasized that although our findings are compatible with a common mechanism related to the metabolism of glucose for the induction of a "memory" both in the B and A_2

cells, the results do not permit the conclusion that identical mechanisms are operative in both types of cells.

The exact mechanisms underlying the priming effects of glucose remain obscure. The present study has shown that the time-dependent effect can be expressed in the absence of extracellular glucose, and indeed in the absence of any metabolizable substrate, because arginine is not metabolized in islet cells (16). In fact, inclusion of glucose with the arginine pulse, while changing dramatically the insulin to glucagon ratio of the response, did not change appreciably the relative effects of priming with glucose. Thus, while induction of priming by glucose probably requires the metabolism of the sugar, the expression of priming may not be dependent on metabolism of the secretagogue. It may be speculated, without being in conflict with the present data, that the "memory" of glucose may be related to increased intracellular levels of high-energy nucleotides or altered synthesis and accumulation of proteins involved in the process of exocytosis.

Hyperglucagonemia in insulinopenic diabetics is corrected by insulin administration only after some latency (17), thus suggesting that the insulinopenic state exerts a lasting effect on the A₂ cell. If so, it could be inferred that exposure to insulin exerts a delayed effect on glucagon secretion. Thus, although the experiments with diazoxide show that induction of the priming effect by glucose does not depend on the insulin-releasing action of the sugar, the possibility was considered that, in addition to the glucose action, insulin per se could exert a time-dependent effect on glucagon secretion in the perfused pancreas. However, in our experiments no significant time-dependent effect of exogenous insulin could be observed (Fig. 3). While a small effect might have been overlooked in the present experimental protocol, it is also possible that a "memory" of insulin can be registered only after previous insulinopenia as seen in diabetes.

What relation to physiological events can be ascribed to the "memory" for glucose documented here? It is well established that fasting as well as a diet poor in carbohydrates lowers the ratio of insulin to glucagon release in the rat (18) and in man (19) under basal conditions and during stimulation, while the reverse situation is observed after a carbohydrate-rich diet. Our present results with the perfused pancreas from fed rats as compared with fasted rats confirm that ratios of insulin to glucagon secretion increase with feeding, and also show that feeding the animals a balanced diet does not establish an endpoint in this respect because ratios could be further increased by priming. It seems possible that the mechanisms which alter the long-term secretory responsiveness in vivo on the scale fasting-fed-excessively carbohydrate-fed state are partly similar to those which regulate the time-dependent effects of glucose

presently observed. However, the "memory" of glucose in the present experiments was induced after a time period much shorter than the one previously used for studying the effect of antecedent diet on insulin and glucagon secretion (18, 19). It should be realized that glucose also alters insulin and glucagon secretion by mechanisms which require prolonged time to express themselves. For instance, while glucose initiates insulin biosynthesis instantaneously, newly synthesized insulin is secreted in appreciable amounts only 2 h after the introduction of the glucose stimulus (20, 21). Thus nutrient regulation of A₂ and B cell responsiveness must be a more complex process than the "memory" effects of glucose presently observed. Future study of the similarities and dissimilarities between the present effects and diet-induced enhancement of secretory responsiveness might help to distinguish the different mechanisms which participate in the long-term regulation of insulin and glucagon secretion.

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