**Kinetics of Insulin Release from the Perfused Rat Pancreas Caused by Glucose, Glucosamine, and Galactose**

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**ABSTRACT** Under appropriate conditions, not only glucose but also glucosamine and galactose can serve as potent stimulants for insulin release from the isolated, perfused rat pancreas. Since galactose and, probably, glucosamine are not metabolized in the islets, and since these three compounds have in all likelihood common sites of action, it is postulated that a glucoreceptor of broad specificity is involved in the mechanism of insulin release, and that metabolism of glucose is not an essential part of the releasing action of this sugar.

An important but deceptive question about the islets of Langerhans is how glucose stimulates insulin release. The question is important because it concerns a crucial aspect of normal function and of diabetes; it is deceptive because any specific releasing functions of glucose can be easily obscured by the more general role of glucose as a fuel. A promising approach to this difficulty is to compare the effects of glucose with its epimers (e.g., galactose), derivatives (e.g., glucosamine), and other closely related compounds, (e.g., mannoheptulose) and, in this way, to attempt to distinguish release and fuel functions. The perfused pancreas, although technically somewhat demanding (1), seemed an ideal system for such investigations.

In this paper we describe the peculiar kinetics of insulin secretion observed when the perfused pancreas of the rat was stimulated by glucose, galactose, or glucosamine alone, by combinations of subthreshold levels of glucose with galactose or glucosamine, and also by combinations of stimulatory amounts of any of the three sugars with theophylline.

**MATERIALS AND METHODS**

All reagents were analytical grade. Dextran (clinical grade) and α-galactose were purchased from Mann Research Laboratories. α-glucosamine was obtained from Sigma Chemical Co. α-glucose was purchased from Mallinckrodt Chemicals and theophylline from Matheson, Coleman and Bell. Dextran was dialyzed against deionized water to remove traces of contaminant glucose. Galactose was free of glucose as judged by enzymatic fluorometric assay (2), but glucosamine contained up to 1% glucose.

Male Sprague-Dawley rats, 300–500 g, fed ad libitum with Purina chow and water, served as pancreas donors.

The pancreas was isolated and perfused using the procedure described by Grodsky et al. (1) with minor modifications. The animals were fasted overnight, treated with atropine (0.1 mg/kg) intraperitoneal, and anesthetized with pentobarbital (45 mg/kg, intraperitoneal). The pancreas was removed together with the spleen, the stomach, and the proximal part of the duodenum. The celiac axis and the portal vein were cannulated and the organ complex was placed in an incubator at constant temperature (35°C). Perfusate was not recycled.

The perfusion fluid had the following composition: NaCl, 120 mM; KCl, 4.7 mM; MgSO4, 0.8 mM; CaCl2, 2.5 mM; KH2PO4, 1.2 mM; and NaHCO3, 25 mM. Dextran (8%) was added to provide a colloid osmotic pressure similar to that of serum. 1 hr prior to perfusion, the medium was warmed to 35°C and equilibrated with O2-CO2 95:5. The resulting pH was 7.4. The pH changed less than 0.1 unit during one passage through the organ complex. The perfusion pressure varied between 40 and 80 mm of Hg. The average flow rate was 7.0 ml/min and was constant throughout the experiment, which usually lasted 1 hr.

After the pancreas was perfused for 10 min with the same medium as used during the control periods of each experiment, the effluent was sampled. Usually three samples were taken as controls. At intervals, indicated in the figures, the perfusate was quickly changed by switching from one circulation medium to another and sampling was continued. The dead space of the combined arterial and venous side of the connecting tubes and cannulas was about 400 μl. This dead space, together with the extracellular water space of the whole preparation, determined the resolving time for stimulation and sampling. Transition between conditions upon medium change took 45–60 sec as judged by glucose levels in the perfusate.

Immediately after sampling, the tubes containing aliquots (about 0.5 ml) of perfusate were placed on dry ice. They were stored at −80°C until used. Insulin concentration in the perfusate was measured in duplicate by the immunoassay of Hales and Randle (3), using pork insulin as standard.

**RESULTS**

The effects of glucose (Figs. 1 and 2)

Insulin release induced by 20 mM glucose was multiphasic. The initial phase began after a short delay (45 sec) and reached its maximum within 2.5–3 min. After a transient decline at a low point at 7 min, a second, gradually rising, response occurred; this response disappeared upon cessation of the glucose stimulus. Prestimulatory insulin levels were reached within 4 min after cessation of glucose perfusion. This biphasic response is well known from other studies with perfused rat pancreas (4) and from in vivo studies with dog (5) and man (6).

The responsiveness of islets of Langerhans to stimulation with glucose was preserved (Fig. 1, control) when the pancreas was perfused for an additional 40 min with substrate-free solution. (We assume that pancreatic islets are unable to utilize the high molecular weight dextran.) The glucose-provoked response at this late stage was somewhat delayed, with insulin first appearing in the perfusate 2–3 min after addition of the sugar. Nevertheless, the typical biphasic pattern was preserved.

When substimulatory concentrations of glucose (3 mM) were present in the perfusate during the period before perfusion with 20 mM glucose, a more rapid response to high glucose was observed (Fig. 2). When, after 40 min of stimulation, the glucose concentration was decreased again to 3 mM,
Fig. 1. Release of insulin due to glucose, theophylline, and combinations of these two agents. During the control periods (−3 to 0 and 40 to 65 min) the perfusate was substrate free, except for the control experiment in which the perfusate was substrate free for 40 min, at which time 20 mM glucose was introduced. The concentrations of glucose and theophylline were 20 and 10 mM, respectively. Each releasing-profile represents one of three experiments performed under the same conditions. All gave comparable data. This approach was followed throughout the study, except in the case of the single experiment presented in Fig. 3.

Fig. 2. Release of insulin due to glucose. The perfusate contained 3 mM glucose during the control periods (−3 to 0 and 40 to 54 min in Expt. A, and −3 to 40 min in Expt. B). High glucose (20 mM) was present from 0 to 40 min and from 40 to 54 min in experiments A and B, respectively. Each curve represents the results of a typical experiment, which was repeated 3 times with comparable outcome.

The effects of glucosamine (Figs. 3 and 4)

The most notable effect occurred when theophylline was infused prior to and during the exposure to glucosamine (Fig. 3). A brief initial, and a delayed secondary, phase of secretion were produced, similar to the profile after glucose perfusion (compare Figs. 1 and 3). A pronounced "off-response" was observed when glucosamine was removed without discontinuing theophylline infusion (Fig. 3). This "off-effect" was even more marked when both theophylline and the sugar were discontinued simultaneously (Fig. 4). When glucosamine was infused alone, or in combination with 3 mM glucose, the only consistent result was a small "off-response" occurring after removal of glucosamine from the perfusate (Fig. 4).

Coore and Randle, using pieces of rabbit pancreas, demonstrated that glucosamine exhibits stimulatory or inhibitory
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**FIG. 3.** Glucosamine as a stimulus for insulin release after priming with theophylline. The concentration of glucosamine (infused from 17 to 42 min) was 20 mM. Theophylline (10 mM) was present from 0 to 52 min. Theophylline treatment was preceded and followed by perfusion with substrate-free medium. The data are the results of a single experiment of this kind.

![Graph](image)

**FIG. 4.** Release of insulin due to glucosamine and combinations of the amino sugar with glucose or theophylline. In the experiment designated "glucosamine + glucose", 3 mM glucose was present from the beginning (−3 min) to the end of the perfusion. In the other two experiments, the perfusate had no additions before Δt. The concentrations of glucosamine and theophylline were 20 and 10 mM, respectively. At 40 min, perfusion was started with medium free of sugar or theophylline. The results of typical individual experiments are recorded (see also legend to Fig. 1).

action depending on whether low or high concentrations of glucose are added to the incubation fluid (10). Lambert et al. observed that glucosamine is a potent stimulator of embryonic pancreas explants in the presence of caffeine (9). The sudden burst in insulin output seen in the present study after removal of high concentrations of glucosamine from the perfusate might be regarded as a sign of release from inhibition. Detailed dose-response studies with this sugar promise to provide more insight into the mechanism(s) involved.

**The effects of galactose (Fig. 5)**

Galactose caused insulin secretion under all conditions tested. The kinetics of the endocrine responses differed characteristically, depending upon the experimental situation. When 20 mM galactose was infused alone, the onset of the small initial phase was delayed. There followed a transient shutdown of secretion, then a second secretory phase which became manifest about 15 min later. Finally, when galactose was removed from the perfusate, insulin release increased progressively throughout the remaining period of 25 min, reaching very high concentrations. This increased insulin output seems to be due to active secretion rather than leaking of the hormone from β-cells, since Diazoixide and cyanide were able to block this phase of release (not shown). When subthreshold concentrations of glucose were present from the beginning to the end of the experiment, the initial response to galactose was equally great and occurred without lag, yet the second phase and the prolonged secretion persisting after removal of the sugar from the perfusate, seen with galactose alone, were missing.

When theophylline was present, galactose induced a pattern of hormone secretion that resembled the pattern produced by glucose: a pronounced early peak followed by a slow second phase of release, and finally, an exponential decay of insulin output after removal of the sugar.

The relative potency for insulin release of galactose, compared to glucose, ranged from 10–70% depending upon the conditions.

The duration of the "off-effects" varied greatly, depending on the conditions. The effect lasted 1 min after theophylline plus glucose, 5 min after theophylline plus glucosamine, and more than 25 min after galactose. One plausible explanation for this phenomenon might be that all three stimulants are inhibitory at high concentrations and that the duration of the off-effect is inversely proportional to the rate of efflux of the...
FIG. 5. Release of insulin due to galactose alone and in the presence of theophylline or glucose. In the experiment labeled “galactose + glucose”, 3 mM glucose was present from the beginning to the end of the perfusion. In the other two experiments, the perfusion medium had no additions before and after 40 min. The concentration of galactose was 20 mM and that of theophylline 10 mM. As in Figs. 1, 2, and 4, each curve represents the results of a typical experiment, which was repeated at least 3 times with comparable outcome.

FIG. 6. Outline of glucoreceptor theory. For explanation, see text of the Discussion section.

respective sugar from the islet cells. It is known that glucose moves extremely quickly across the cell membrane (11). Whether, as this hypothesis demands, glucosamine and galactose enter and leave the cell relatively slowly remains to be tested.

In most other in vitro studies (1, 2, 10, 12, 13) galactose has consistently failed to release insulin. However, Lambert et al. showed stimulation of insulin release by galactose with explants of fetal pancreas (9) and noted that the addition of caffeine was an absolute requirement for this effect. It is possible that the effect of galactose has been obscured previously because of the use of short periods of stimulation or the use of subthreshold concentrations of glucose which, as shown in these experiments, blocks all but the initial brief response. It is also possible that the availability of excess fuel from the high concentration of free fatty acids present in serum albumin, used by most investigators as a colloid osmotic agent, might change the responsiveness of the β-cells to galactose.

DISCUSSION

The data presented here make it difficult to accept in an unmodified form the hypothesis that attributes glucose-stimulated insulin release to metabolism of the sugar (1, 12, 14). If we accept the amply supported finding that islets are unable to metabolize galactose (15, 16), and if we assume that galactose and glucose have common site(s) of action in the β-cell, we must postulate a mechanism that involves direct stimulation of the β-cells by the intact sugars. This does not imply that metabolism of the stimulating sugar is without influence on the response. In fact, differences in the character and magnitude of the responses may—at least in part—turn
out to be due to differences in the metabolism of the stimulants by the islet cells.

One recent finding published by Renold and his coworkers deserves consideration in this context (17). These investigators observed a paradoxical enhancement of tolbutamide action by 2-deoxy-D-glucose and D-mannopentulose, two potent inhibitors of glucose-stimulated insulin release. This might be explained by postulating a mechanism that involves a tolbutamide receptor in close proximity to a sugar receptor of broad specificity. If this were the case, one might expect that glucose would also potentiate tolbutamide action, and in fact there are reports in the literature which may be interpreted as supporting this idea (12, 18). It has also been observed (A. Lambert, personal communication) that 3-O-methylglucose potentiates the insulin release due to caffeine, a result best explained if a site independent of metabolism was involved in the release mechanism.

Another line of evidence, which speaks against the primary role of glucose metabolites as triggers of release, comes from recent biochemical studies of the islets of Langerhans of the rat (19, 20). The concentrations of most of the early metabolites and of cofactors of glucose metabolism were unchanged during the first 5 min of glucose infusion, in spite of abundant insulin release, but the metabolite pattern of the islets changed markedly after prolonged glucose infusion. Accordingly, intermediates such as glucose-6-phosphate (11, 14) and 6-phosphogluconate (21), which have been implicated as signals, hardly qualify for such a role.

Therefore, we have adopted an alternative working hypothesis (Fig. 6) that could explain the major actions of glucose in β-cells of the rat (19, 20).

Four facets of glucose action are represented in the sketch: I, the glucose receptor complex; II, glucose activation; III, intermediary metabolism; and IV, the secretory complex.

According to the hypothesis, glucose itself acts as a stimulus for insulin release at the receptor (I), presumably located in the cell membrane and tentatively called glucose receptor. This site is either part of a carrier system or is an independent site. It is accessible from the outside or from the inside of the cell. The primary event, which is set off by glucose per se, is an increase of ion conductivity (Ca++, and perhaps Na+ and K+) with insulin secretion as the consequence. The essential role of Ca++ for excitation–secretion coupling in general, and for insulin secretion in particular, has found ample experimental support (22–26).

The microtubular system and the process of exocytosis as described by Lacy (27) (IV) or a form of microvesicular secretion as proposed by Orci (28) (not included in the scheme) are involved in the Ca++-dependent hormone transport and release. The exocytotic response can be elicited and can almost certainly be modified by the adenyl cyclase system, as indicated by numerous reports (7–9, 12, 17, 29–31). 3':5'-Cyclic AMP formed in the process is released from the β-cells (in analogy to events found in the liver (32)) or can increase the intracellular pool of the nucleotide.

Facilitated diffusion of glucose into the cell is extremely fast, and phosphorylation is rate limiting (11, 20) for further metabolism (II). Metabolism (i.e., alterations of the intermediate and/or cofactor pattern) influences all or selected components of the complex system by positive or negative feedback processes. Glucose itself, or factors related to its metabolism, regulate de novo synthesis of insulin (not included in the scheme) (33, 34).

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