

# Immediate and Time-Dependent Effects of Glucose on Insulin Release from Rat Pancreatic Tissue

## EVIDENCE FOR DIFFERENT MECHANISMS OF ACTION

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**ABSTRACT** Glucose-induced insulin secretion is enhanced by a preceding glucose stimulus. The characteristics of this action of glucose were investigated in perfused pancreas and collagenase-isolated islets of Langerhans. A 20- to 30-min pulse of 27.7 mM glucose enhanced both the first and second phase of insulin release in response to a second glucose stimulus by 76–201%. This enhancement was apparent as an augmented maximal insulin release response to glucose. The effect of priming with glucose was seen irrespective of whether the pancreatic tissue was obtained from fed or fasted rats. Separating the two pulses of hexose by a 60-min time interval of exposure to 3.3 mM glucose did not abolish the potentiation of the second pulse. Omission of  $\text{Ca}^{++}$  as well as the inclusion of somatostatin or mannoheptulose during the first pulse abolished insulin secretion during this time period; however, only the inclusion of mannoheptulose deleted the potentiation of the second pulse. D-Glyceraldehyde, but not pyruvate, D-galactose, or 3-isobutyl-1-methylxanthine, could substitute for glucose in inducing potentiation.

In islets labeled with  $[2\text{-}^3\text{H}]\text{adenine}$ , the  $[^3\text{H}]\text{cyclic AMP}$  response to glucose was increased by 35% when measured after 1 min, but was increased only marginally after 2–10 min of stimulation with a second pulse of glucose. The production of  $^3\text{H}_2\text{O}$  from glucose was not affected by glucose priming.

It is concluded that (a) the induction of the glucose-induced, time-dependent potentiation described here is dependent on glucose metabolism but not on stimulation of cyclic AMP, calcium fluxes, or insulin release per se; (b) the mechanisms that mediate the

pancreatic “memory” for glucose are unknown but do not seem to involve to a major extent an increased activity of the adenylate cyclase–cyclic AMP system of the beta-cell; (c) the evidence presented supports the hypothesis of a dual role of glucose for insulin release.

## INTRODUCTION

Insulin release induced by glucose is a process of considerable complexity. Exposing the pancreas to a high concentration of glucose gives rise to a biphasic pattern of insulin release in which a short-lived burst of insulin output is later followed by a slowly increasing secretion rate (1, 2). However, this acute insulin response is also influenced by a “memory” for glucose. It has thus been demonstrated both in vitro (3) and in vivo (4) that a first pulse of glucose is able to enhance the insulin response to a second one.

Although some characteristics of this glucose-induced, time-dependent potentiation (TP)<sup>1</sup> of insulin secretion have been described in man (4, 5), the mechanisms that govern this phenomenon are completely unknown. The aims of the present investigation were (a) to characterize the time dimensions as well as the glucose dose dependency of TP; (b) to determine whether the demonstration of TP depends on the nutritional state; (c) to establish whether the glucose stimulation which generates TP is coupled to a glucose action on fluxes of  $\text{Ca}^{++}$ , stimulation of cyclic AMP, or the metabolism of the hexose; and (d) to investigate whether TP is mediated by an increased cyclic AMP response, by increased metabolism of glucose, or by other factors. To this end the effects of glucose in two in vitro systems, the perfused pancreas

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<sup>1</sup>Abbreviations used in this paper: IBMX, 3-isobutyl-1-methylxanthine; KHB, Krebs-Henseleit-bicarbonate buffer; TP, time-dependent potentiation.

and collagenase-isolated islets of Langerhans from the rat, were studied.

## METHODS

### Perfused pancreas

Male Sprague-Dawley rats weighing 160–200 g were either fed *ad lib.* or fasted for 24 h before the experiments. They were anesthetized by i.p. injection of 100 mg/kg of pentobarbital, and the pancreas was isolated free from surrounding tissue by a slight modification of the technique of Loubatières (6). The gland was perfused with a Krebs-Henseleit-bicarbonate (KHB) solution which contained 20 g/liter of albumin and, when not otherwise indicated, 3.9 mM of glucose. 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. The flow rate was kept around 2.0 ml/min.

### Isolated islets

**Preparation of islets, labeling with [2-<sup>3</sup>H]adenine, and incubation of islets.** Male Sprague-Dawley rats weighing 100–150 g were either fed *ad lib.* or fasted for 48 h until decapitation. Pancreatic islets were isolated by the collagenase method of Lacy and Kostianovsky (7). The collagenase employed was from Worthington Biochemical Corp., Freehold, N. J. KHB buffer with 2 g/l bovine serum albumin, 10 mM Hepes, and, when not otherwise indicated, 3.3 mM glucose, was used throughout the experiments. Islets were pulse-labeled with 100  $\mu$ Ci/ml of [2-<sup>3</sup>H]adenine (25 Ci/mmol, New England Nuclear, Boston, Mass.) during a 60-min preincubation period as previously described (8). Batches of 15 islets were then transferred to "baskets." These were prepared by cutting small microcentrifugation plastic tubes with an upper inner diameter of approximately 4 mm (Beckman Instruments, Inc., Fullerton, Calif.). The hollow cylinders thus obtained were capped with nylon gauze (70 threads/cm<sup>2</sup> of gauze, 36% open space). The baskets with the islets were placed in small incubation tubes in a final volume of 0.5 ml. Incubations were carried out at 37°C together with the agents to be tested, the baskets being transferred to new incubation tubes according to the experimental protocol. In the final incubations, 0.1 mM of 3-isobutyl-1-methylxanthine (IBMX) was always present unless otherwise indicated.

**Measurement of islet [<sup>3</sup>H]cyclic AMP.** After removal of an aliquot of the incubation medium, 100  $\mu$ g of cyclic AMP was added and the samples kept in a boiling water bath for 5 min. [<sup>3</sup>H]cyclic AMP was extracted as previously described (8, 9), and the radioactivity was counted by liquid scintillation. Under all conditions, efflux of [<sup>3</sup>H]cyclic AMP into the incubation media was less than 5% of the tissue content of radioactive nucleotide; this was not corrected for.

**Estimation of glucose utilization.** The utilization of glucose was determined from the production of tritiated water formed from [5-<sup>3</sup>H]glucose during glycolysis. The technique described by Ashcroft et al. (10) was followed with minor modifications. Batches of 10 islets were incubated in 30  $\mu$ l of KHB containing 400,000–600,000 cpm of radioactive glucose (The Radiochemical Centre, Amersham, England). The reactions were stopped by the addition of 10  $\mu$ l of 0.25 N HCl. The recovery of the microdiffusion process was measured in each experiment (range from 50% to 60%) and corrected for.

### Insulin assay

Insulin was measured using a charcoal separation method of radioimmunoassay (11). <sup>125</sup>I-labeled pork insulin (obtained from The Radiochemical Centre, Amersham, England) was used. Purified rat insulin (kindly supplied by Dr. J. Schlichtkrull, Novo Research Institute, Bagsvaerd, Denmark) served as standard.

## RESULTS

### Time- and dose-dependent characteristics of glucose-induced TP

Fig. 1 demonstrates glucose-induced TP in the perfused pancreas. In these experiments, 27.7 mM glucose was present for two 30-min periods interrupted by a 30-min perfusion with 3.9 mM glucose. Whether in pancreata from 24-h-fasted animals or from fully fed animals, the insulin response to the second stimulus was significantly greater than the first response (*P*

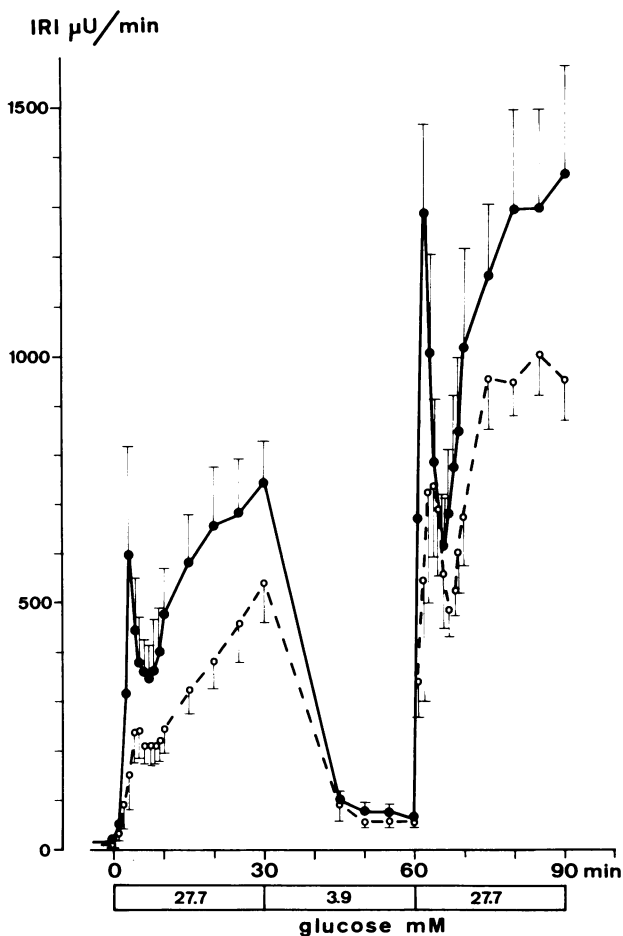


FIGURE 1 Effect of two 30-min pulses of 27.7 mM glucose on insulin secretion in perfused pancreas in fasted (○ --- ○) or fed (● — ●) animals. Mean  $\pm$  SEM of 10 and 5 experiments, respectively.

< 0.001 according to Student's *t* test, paired differences). The total insulin response was enhanced by 201±27% in the fasted and by 131±21% in the fed animals. Both first and second phases of insulin release were potentiated (148 and 218% for the first 10 min as compared to 90–112% for minutes 20–30 in the fed and fasted animals, respectively).

When the time interval between two 20-min pulses of glucose stimulation was prolonged from 10 to 60 min, no clear differences in the degree of TP were observed (Table I). Thus a 60-min period of 3.9 mM glucose was not sufficient to delete the potentiating effect induced by the first glucose (27.7 mM) pulse.

The effects of glucose priming on subsequent stimulation with a range of glucose concentrations were tested in isolated islets (Fig. 2). Islets were incubated for 30 min with 27.7 mM glucose, then for 20 min in 3.3 mM glucose, and finally for 10 min in media containing various glucose concentrations. Glucose priming augmented the insulin response to all glucose concentrations (0–27.7 mM) in the final incubation; the priming effect, however, was more marked with increasing glucose concentrations. This effect was observed both in islets from 48-hr-fasted animals and from fed animals, although the maximal insulin response was lower in the fasted animals under all circumstances. Since it is well established, also in our hands (8), that 27.7 mM is a maximally effective concentration of glucose for stimulating acute insulin release in isolated rat islets, it would appear that priming with glucose increased the maximal response to glucose in the final incubations.

The effect of varying the glucose concentration during the first pulse on the subsequent response to glucose was studied in isolated islets (Table II). 27.7 mM glucose was a maximally effective concentration for inducing an enhancing effect on the second pulse; 8.3 mM exerted a small effect.

TABLE I

Effect of Two 20-min Pulses of 27.7 mM Glucose on Insulin Secretion from the Perfused Pancreas from Fasted Animals\*

No. of experiments	Time intervals between pulses min	First pulse,	Second pulse,	Percent increment of first pulse %
		IRI μU	IRI μU	
3	10	178±5	356±59	98
4	20	307±80	641±162	115
3	30	335±15	590±33	76
3	60	390±35	710±98	81

\* Between the two pulses the glucose concentration was kept at a nonstimulatory level (3.9 mM) for 10, 20, 30, or 60 min. The secretory response is expressed as mean±SEM of the total amount of insulin released during the respective stimulatory pulse.

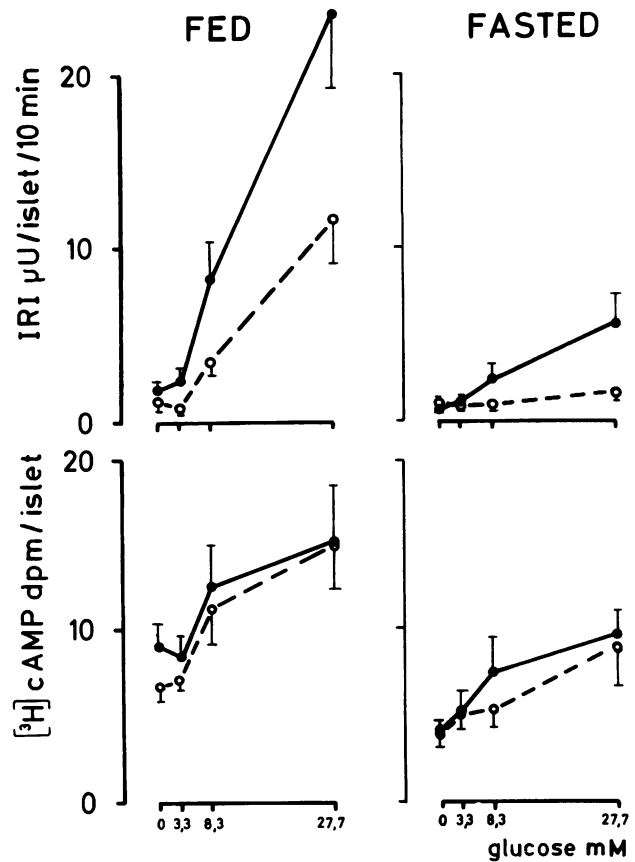


FIGURE 2 Effects of glucose priming on insulin and [<sup>3</sup>H]-cyclic AMP responses to a subsequent glucose stimulus. Islets from fed or 48-h fasted animals (*n* = 4) were preincubated with 27.7 (●—●) or 3.3 mM (○---○) of hexose for 30 min, then with 3.3 mM glucose for 20 min, and finally with different glucose concentrations for 10 min. 0.1 mM IBMX was included in the final incubations.

To determine whether a prolonged incubation of the islets with a low glucose concentration could affect the subsequent responsiveness to a stimulatory glucose concentration, islets were incubated from 0 to 90 min in 3.3 mM glucose and then transferred to a 10-min incubation with 27.7 mM glucose (Fig. 3). With prolonged time of exposure to 3.3 mM glucose, increasing deterioration of the insulin response to 27.7 mM glucose was observed (at 60 min, 32% inhibition, at 90 min, 54% inhibition).

#### Induction of TP: modification of the stimulus for TP

In the perfused pancreas, as well as in isolated islets, the insulin response to a first pulse of 27.7 mM glucose was inhibited by two-thirds or more when Ca<sup>++</sup> was omitted (Fig. 4, Tables II and III). In contrast, TP was not abolished, the insulin response of

TABLE II  
Effects of Modifying Glucose Priming in Isolated Islets from Fed Animals\*

No. of experiments	First pulse				Percent increment of the response to 27.7 mM glucose	Second pulse	
	Glucose concn.	Ca <sup>++</sup>	Additions	IRI		IRI	[ <sup>3</sup> H]cAMP
	mM	mM		μU/islet		μU/islet	dpm
5	3.3	2.56	—	3.3±1.2		10.8±1.2	12.9±0.6
5	8.3	2.56	—	6.8±2.0		15.7±2.5	14.1±0.7
5	27.7	2.56	—	37.7±3.3		23.4±3.1‡	14.9±0.8
5	55.4	2.56	—	47.5±2.4		22.7±2.1‡	16.6±1.8
5	3.3§	2.56	—	3.3±1.2		2.8±1.1	7.6±1.4
4	3.3	2.56	—	2.2±0.7		10.6±1.5	13.2±0.7
4	27.7	2.56	—	39.4±3.7		23.8±3.9	15.4±0.5
4	3.3	0	—	2.3±0.5		13.4±2.3	16.1±3.2
4	27.7	0	—	8.2±1.3	-79	21.6±3.3‡	17.3±2.3
4	3.3	2.56	Mannoheptulose, 13.8 mM	2.2±0.7		11.8±2.1	14.0±1.7
4	27.7	2.56	Mannoheptulose, 13.8 mM	1.5±0.5	-96	12.2±1.9	16.2±1.4
4	3.3§	2.56	—	2.2±0.7		3.5±1.2	8.7±1.2
5	3.3	2.56	—	3.0±1.0		9.4±1.7	12.1±1.2
5	27.7	2.56	—	38.6±3.0		22.3±3.4‡	13.9±1.5
5	0	2.56	Glyceraldehyde, 10 mM	7.2±1.2	-81	19.8±2.6‡	16.3±1.5
5	3.3§	2.56	—	3.0±1.0		3.5±1.2	7.4±1.6
4	3.3	2.56	—	3.7±1.7		11.4±1.5	12.7±0.7
4	27.7	2.56	—	35.2±1.4		21.4±0.7‡	13.2±0.7
4	3.3	2.56	Pyruvate, 10 mM	3.2±1.7		12.5±1.4	14.2±1.2
4	3.3	2.56	Galactose, 27.7 mM	4.2±2.2		11.4±1.9	12.6±0.7
4	3.3§	2.56	—	3.7±1.7		2.4±1.1	7.6±0.9
4	3.3	2.56	—	3.4±1.7		12.2±2.2	12.9±0.7
4	3.3	2.56	IBMX, 1.0 mM	5.8±0.3	-84	11.9±1.1	14.1±1.6
4	8.3	2.56	—	5.2±1.2		13.4±1.0	14.5±2.1
4	8.3	2.56	IBMX, 1.0 mM	38.9±4.4	5	13.9±1.7	11.9±0.6
4	27.7	2.56	—	36.9±2.9		23.2±1.7‡	14.0±0.7
4	3.3§	2.56	—	3.4±1.7		1.9±0.8	7.2±1.2

\* Islets were stimulated by two pulses (first and second pulse, respectively). The first pulse was 30 min and the second was 10 min of duration. Between these two pulses, islets were incubated with a nonstimulating glucose concentration (3.3 mM) for 20 min. 27.7 mM glucose was present during the second pulse as well as 0.1 mM IBMX. Mean±SEM of the insulin and [<sup>3</sup>H]cyclic AMP responses in the separate sets of experiments delineated by the horizontal lines.

‡  $P < 0.05$  or less, according to Student's  $t$  test (paired differences) from the response obtained in islets preincubated with 3.3 mM glucose and 2.56 mM Ca<sup>++</sup>.

§ 3.3 mM glucose was present throughout the first and second pulse.

the second pulse being enhanced by 74% in comparison to that of the first pulse, measured in control incubations. Similarly, somatostatin (tested in the perfused pancreas only) inhibited insulin response to the first pulse by 82% but did not inhibit the TP induced by the hexose. On the other hand, mannoheptulose, a known inhibitor of glycolysis, totally inhibited TP in addition to abolishing the insulin response to the first pulse (Fig. 4, Tables II and III).

When glucose in the first pulse was replaced by the glucose metabolite D-glyceraldehyde (Fig. 5, Tables II

and III), the insulin response to 27.7 mM glucose (second pulse) was potentiated to the same extent as when preceded by 27.7 mM glucose in the first pulse. Another glucose metabolite, pyruvate, neither generated TP nor induced insulin release during the first pulse (Tables II and III). Galactose could not substitute for glucose during the priming period, nor could the phosphodiesterase inhibitor IBMX (tested in isolated islets only). It is noteworthy that IBMX, when added to media containing 8.3 mM glucose, enhanced the insulin secretion of the first pulse to a

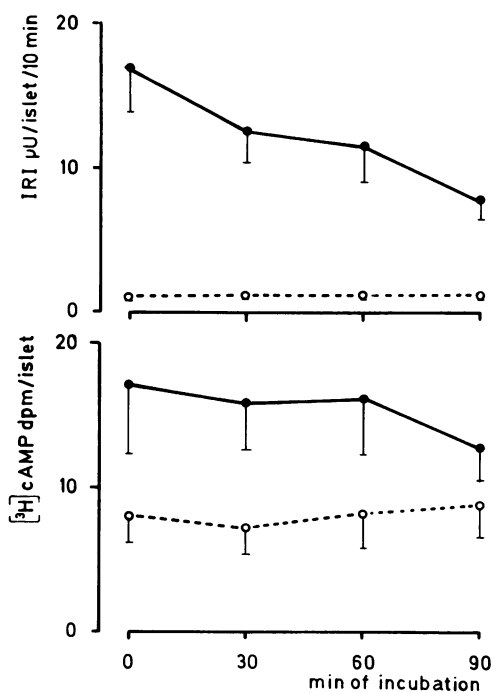


FIGURE 3 Effects of prolonged exposure to a low glucose concentration on the subsequent glucose-induced insulin and [<sup>3</sup>H]cyclic AMP responses. Islets from fed rats were incubated from 0 to 90 min together with 3.3 mM glucose and finally for 10 min in incubation media containing 3.3 or 27.7 mM of hexose. ○···○ and ●---● denote final incubations with 3.3 and 27.7 mM of glucose, respectively; 0.1 mM IBMX was included in all final incubations. Mean ± SEM of seven experiments.

rate not different from that induced by 27.7 mM of hexose (without further additions); yet the methylxanthine was completely ineffective in generating TP (Table II).

#### Mediation of glucose-induced TP

**Role of cyclic AMP.** The possibility that the adenylate cyclase-cyclic AMP system of the islet would be involved in TP was tested in several ways in islets prelabeled with [<sup>3</sup>H]adenine. First, it was observed that basal [<sup>3</sup>H]cyclic AMP levels were not significantly elevated by priming whether in the absence or presence of a high concentration of the phosphodiesterase inhibitor IBMX (Table IV). Second, when measured after 10 min of final incubation, the stimulatory effect of 27.7 mM glucose on [<sup>3</sup>H]cyclic AMP was only marginally affected by glucose priming. Indeed a small augmentation of [<sup>3</sup>H]cyclic AMP levels was observed only when results from several experimental series were compiled (from Table II and Fig. 2, 14.0 vs. 13.0 dpm;  $n = 15$ ,  $P < 0.05$  according to Student's  $t$  test, paired differences). In contrast, the insulin response measured in the same incubations

was invariably increased twofold or more by the priming procedure.

To investigate whether priming with glucose influenced preferentially an early component of the cyclic AMP response, the time course of changes in [<sup>3</sup>H]cyclic AMP during a 1- to 5-min-long second pulse with 27.7 mM glucose was studied (Fig. 6). In these experiments, glucose priming moderately augmented only the 1-min [<sup>3</sup>H]cyclic AMP level (35%,  $P < 0.01$  according to Student's  $t$  test, paired differences), whereas the insulin responses were enhanced by more than 150% after 2 and 5 min of incubation.

The markedly reduced insulin response to 27.7 mM glucose after prolonged (90 min) incubation with 3.3 mM glucose (Fig. 3) was accompanied by a reduction of the mean [<sup>3</sup>H]cyclic AMP response, which, however, was not significant ( $P < 0.1$  according to Student's  $t$  test, paired differences).

**Role of glucose metabolism.** Since the potentiated insulin response during the second pulse might be the consequence of an enhancement of the metabolism of glucose, glucose utilization was measured in islets which had been incubated in 27.7 mM glucose for 30 min and then selected for a final 10-min incubation in media containing 3.3 or 19.4 mM glucose. Production of <sup>3</sup>H<sub>2</sub>O from [5-<sup>3</sup>H]glucose and—in parallel incubations—insulin release were measured (Table V). In line with the previous results, insulin release was markedly enhanced by the glucose priming. No significant effect on glucose utilization could be registered, however.

## DISCUSSION

The present results confirm and extend the observations that Grodsky et al. made several years ago (3) on glucose-induced, time-dependent potentiation of insulin secretion, where in the perfused pancreas of fasted rats, preincubation with a high glucose concentration for 60 min greatly enhanced the insulin response to a second glucose stimulus. In conjunction with the present results, a broad time range would appear to be established for the induction of TP (from 20 to at least 60 min), for the memory of TP (at least 60 min) as well as for its expression (at least 30 min). The definite time limits for any of the above components of TP have not been determined, however. Our data showing that TP can be induced in fed as well as in fasted rats, as well as the finding that TP can be demonstrated in man (4), further indicate the role of glucose-induced TP as a basic physiological phenomenon.

Which mechanisms are involved in the induction of TP? Our results indicate that the metabolism of glucose must be important in this respect. Thus, an inhibitor of glycolysis, mannoheptulose, when given during the first pulse, completely inhibited TP. Conversely, glucose-induced TP was abolished by omission of Ca<sup>++</sup>, a

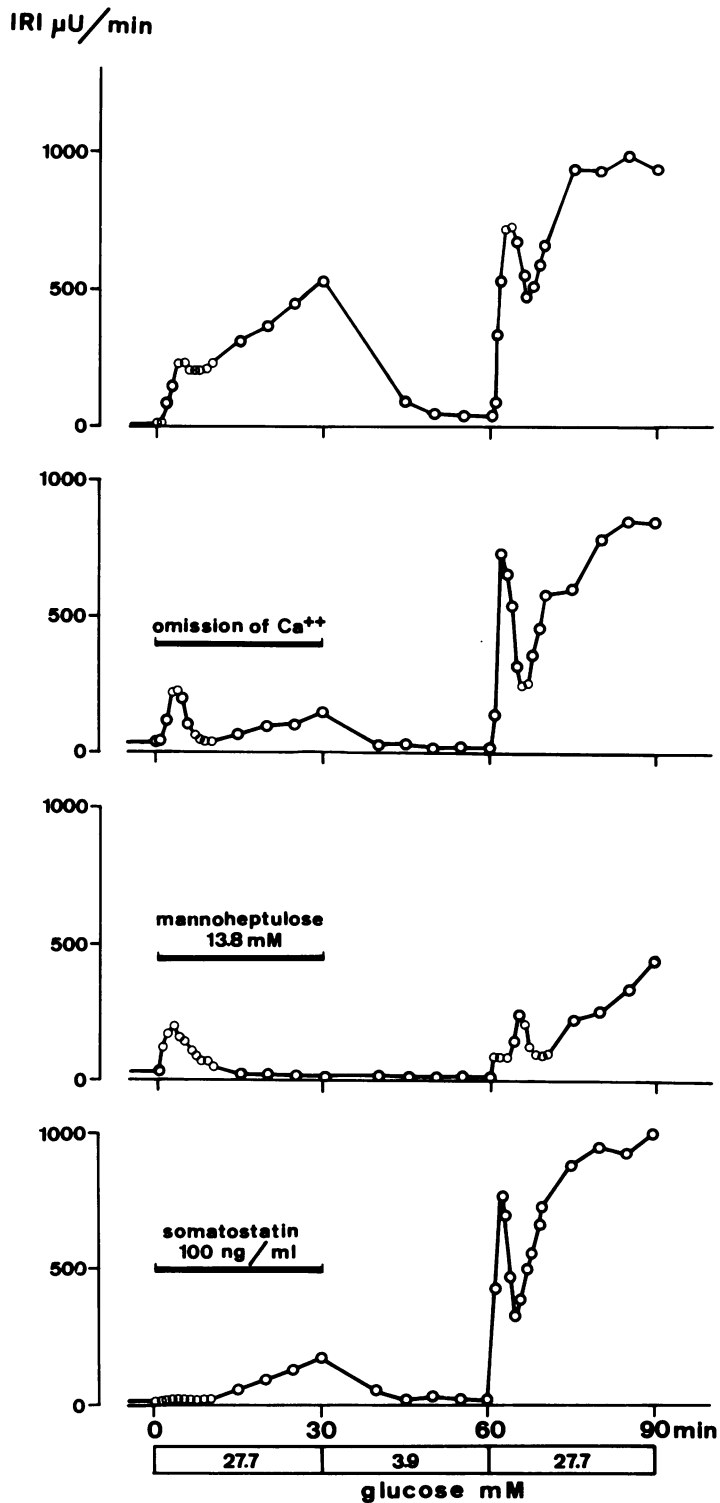


FIGURE 4 Effects of modifying a first glucose pulse by omission of  $\text{Ca}^{++}$  or by addition of mannoheptulose or somatostatin. Representative experiments from the perfused pancreas preparation (fused rats) are shown in comparison with the mean of experiments derived from Fig. 1 (upper graph). The experimental conditions are given in Table III.

TABLE III  
Effects of Modifying Glucose Priming in the Perfused Pancreas from Fasted Animals\*

No. of experiments	First pulse					Second pulse	
	Glucose concn.	Ca <sup>++</sup>	Additions	IRI	Percent increment of the response to 27.7 mM glucose	IRI	Percent increment of the response to 27.7 mM glucose and 2.56 mM Ca <sup>++</sup> during first pulse
	mM	mM		$\mu$ U	%		
10	27.7	2.56	—	287±44	—	787±101‡	201‡
4	27.7	—	—	103±32	-65	515±50‡	74‡
3	27.7	2.56	Mannoheptulose, 13.8 mM	25±13	-92	216±12	-27
4	27.7	2.56	Somatostatin, 100 ng/ml	53±9	-82	596±75‡	101‡
4	3.9	2.56	D-Glyceraldehyde, 14 mM	314±18	6	1,049±169‡	253‡
4	3.9	2.56	Pyruvate, 14 mM	14±1	-95	332±94	16

\* The pancreata were stimulated by two 30-min pulses (first pulse and second pulse, respectively). Between these two pulses, the pancreata were perfused with a nonstimulating glucose concentration (3.9 mM) for 30 min. 27.7 mM glucose was present during the second pulse. The secretory responses are expressed as mean±SEM of the total amount of insulin released during the respective stimulatory pulse.

‡  $P < 0.05$  or less according to Student's  $t$  test (unpaired differences) from the response to 27.7 mM glucose and 2.56 mM Ca<sup>++</sup> during the first pulse.

condition which inhibits glucose-induced insulin release (12) while affecting glucose metabolism only slightly (13). The finding that the glycolytic intermediate D-glyceraldehyde, but not the nonmetabolizable glucose isomer D-galactose, could substitute for glucose as inducer of TP further indicates that glucose metabolism is essential for TP. The failure of pyruvate, which is metabolized in the beta-cell (14), to induce TP may indicate that a product of glycolysis is important in this respect.

Apart from a probable influence of glucose metabolism on TP, our studies have excluded but not revealed other factors of importance in this context. It seems clear that the insulin release process per se does not induce TP. This is illustrated by the findings that omission of Ca<sup>++</sup>, as well as the inclusion of somatostatin, inhibited glucose-induced insulin release but not TP. Conversely, IBMX greatly potentiated insulin release during glucose priming, whereas TP was unaffected. Since cyclic AMP is inhibited by somatostatin (15) (which also may inhibit Ca<sup>++</sup> fluxes [16]) and by omission of Ca<sup>++</sup> (17), and is increased by IBMX, it seems clear that cyclic AMP is not the factor that induces TP. In a similar manner, the maintenance of TP in the absence of Ca<sup>++</sup> indicates that a glucose-induced accumulation of this ion, which could be part of the action by which glucose initiates insulin secretion (18), is not a crucial factor in the induction of TP.

Translocation of insulin granules from the interior of the beta-cell to its periphery could be a mechanism whereby glucose induces TP, the insulin granules close to the cell membrane presumably being more easily released than those located further away from the site of exocytosis. However, such a mechanism is unlikely to be operative in our system, because the migration of the granules are dependent on the presence of calcium (19). Glucose promotes the synthesis of tubulin (20) as well as its polymerization from pre-existing soluble subunits (21). Such actions could lead to an increased delivery of granules to the emiocytotic sites in response to a repeated glucose stimulus. However, also cyclic AMP induces synthesis (20) as well as polymerization of tubulin (22), while no effects on TP were observed in the present experiments. Thus no evidence favoring the involvement of the microtubular system in TP is apparent.

Which mechanisms mediate the potentiating effect of glucose? To study this question, isolated islets had to be employed. This system has the disadvantage of showing a time-dependent deterioration of the glucose responsiveness after prolonged incubations with low glucose. The possibility that the enhancing effect of a first pulse of 27.7 mM glucose was partly due to correction of this deterioration cannot be excluded. However, the fact that our findings concerning insulin release were qualitatively quite similar in

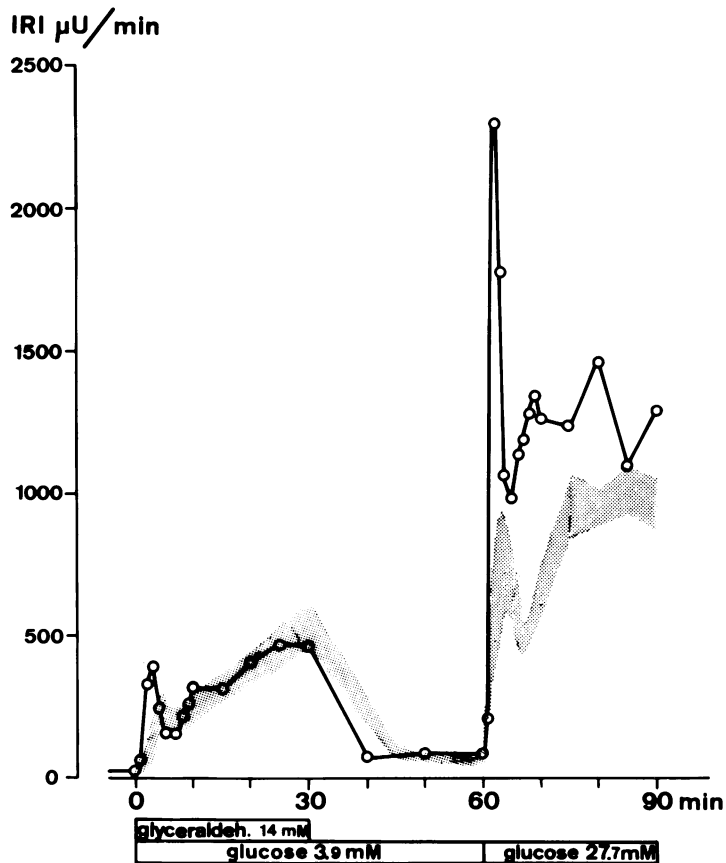


FIGURE 5 Effect of priming with D-glyceraldehyde (14 mM) on the subsequent response to glucose 27.7 mM. One representative experiment with the perfused pancreas from a fasted rat is shown. The experimental conditions are given in Table III. Shaded areas represent mean  $\pm$ SEM of experiments with two pulses of 27.7 mM glucose derived from Fig. 1.

both experimental systems used supports the validity of the results obtained with isolated islets.

With the above reservation in mind, the islet data show that priming with glucose increases the maximal insulin release in response to glucose on insulin release during a subsequent stimulation with the hexose. A concomitant change also in the sensitivity to glucose is not apparent; our results, however, are insufficient to rule out this possibility. These findings are analogous to results obtained in man (4).

One main purpose of the islet studies was to investigate the role of cyclic AMP in mediating TP. A prelabeling method for measuring changes in cyclic AMP levels was used, the validity of which has recently been evaluated (23). A moderate increase in the [ $^3$ H]cyclic AMP response due to glucose priming was found after 1 min of second pulse stimulation, while effects were absent or marginal after longer times of incubations. It seems difficult to envisage how the 1-min cyclic AMP response could directly influence the 5-min insulin as seen in Fig. 6. Although the evidence is not unambiguous, we thus favor the idea that an in-

creased cyclic AMP response to glucose—the response to other agents not being investigated here—is at least not a major factor for the mediation of TP. It should be emphasized, however, that the acute

TABLE IV  
Lack of Effect of Glucose Priming on the Basal Islet  
[ $^3$ H]Cyclic AMP Levels and Insulin Release\*

Glucose during preincubation	IBMX, 1.0 mM	[ $^3$ H]cyclic AMP	IRI
mM		dpm/islet	$\mu$ U/islet per 10 min
27.7	-	4.2 $\pm$ 0.8	1.8 $\pm$ 0.7
	+	22.9 $\pm$ 1.7	3.8 $\pm$ 1.4
3.3	-	4.6 $\pm$ 1.0	3.3 $\pm$ 1.7
	+	19.8 $\pm$ 4.2	3.5 $\pm$ 1.1

\* Islets from fed rats were preincubated for 30 min either in 27.7 or 3.3 mM glucose followed by 20 min together with 3.3 mM and finally for 10 min in the absence of hexose. Insulin release and islet [ $^3$ H]cyclic AMP were measured at the end of the final incubation.



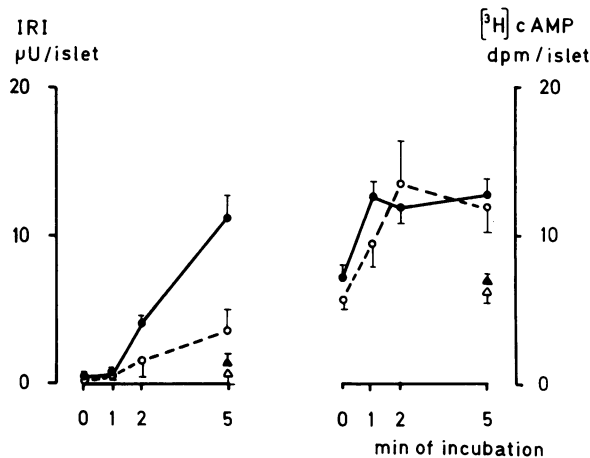


FIGURE 6 Time course of the insulin and  $[^3\text{H}]$ cyclic AMP responses after glucose priming. Islets from fed rats were incubated either in 27.7 or 3.3 mM glucose for 30 min, then with 3.3 mM hexose for 20 min. In the final incubations (where 0.1 mM IBMX was included), the response to 27.7 mM glucose was measured. ● — ●, first and finally incubated in 27.7 mM glucose; ○ --- ○, first incubated in 3.3 mM, finally in 27.7 mM glucose; ▲, first incubated in 27.7 mM, finally in 3.3 mM glucose; △, first and finally incubated in 3.3 mM glucose. Insulin release from the glucose-primed islets vs. unprimed islets was significantly enhanced according to Student's *t* test (paired differences) after 2 min ( $P < 0.005$ ) and 5 min ( $P < 0.001$ ), and the islet  $[^3\text{H}]$ cyclic AMP level increased after 1 min ( $P < 0.01$ ). Mean  $\pm$  SEM of nine experiments.

action of glucose, which is necessary during both the first and the second pulse, does involve stimulation of cyclic AMP.

Howell et al. (24) have documented that the activity of islet adenylate cyclase is increased by high glucose after a stimulation period of 2 h. Although cyclic AMP metabolism in the islet can thus be profoundly influenced by glucose over a prolonged period of time, this time scale does not seem to be equivalent to that of TP as described here. Whether the mechanisms that mediate TP are partly similar to those responsible for the alterations of the islet responsiveness due to prolonged exposure to glucose is unknown.

Our study does not give a positive answer as to which factor(s) mediate TP. A gross change in the metabolism of glucose does not seem to be associated with the potentiated insulin response, but more subtle and specific alterations in the metabolic handling of glucose have not been excluded. In this context, it should also be pointed out that our measurements of glucose utilization were carried out in much smaller volumes of incubation than those employed for the study of insulin release; the possible importance of this difference has not been investigated.

Other possible mechanisms that may mediate TP and

TABLE V  
Effects of a Preincubation with 27.7 mM Glucose on  $^3\text{H}_2\text{O}$  Production from  $[5\text{-}^3\text{H}]$ Glucose\*

Glucose in preincubation	Glucose in final incubation			
	3.3 mM		19.4 mM	
	Glucose utilized	IRI	Glucose utilized	IRI
mM	pmol islet per 10 min	$\mu\text{U}/\text{islet}$	pmol islet per 10 min	$\mu\text{U}/\text{islet}$
3.3	$3.2 \pm 0.4$	$1.7 \pm 0.9$	$10.6 \pm 2.7$	$11.8 \pm 3.8$
27.7	$2.9 \pm 0.4$	$4.3 \pm 0.7$	$12.2 \pm 2.3$	$21.0 \pm 6.0 \ddagger$

\* Islets from fed rats were exposed immediately after isolation for 30 min at 37°C to either glucose 3.3 or 27.7 mM, after which they were selected in 3.3 mM glucose for final incubations in 30  $\mu\text{l}$  of buffer containing radioactive glucose. The samples were incubated in sextuplicate for 10 min in either 3.3 mM or 19.4 mM glucose, the reactions being stopped by the addition of HCl. Separate incubations where insulin release was measured were carried out in 0.6 ml of KHB. 0.1 mM IBMX was included in all final incubations. Mean  $\pm$  SEM of five experiments.

‡  $P < 0.02$  according to Student's *t* test (paired differences) from the response obtained in islets preincubated with 3.3 mM glucose.

which remain for further study include increased energy stores in the beta-cell, changes in cell-membrane structure and function, or an increased number of protein molecules associated with the process of exocytosis. The present studies, however, demonstrate clearly that glucose exerts two distinct actions on insulin release: one which is immediate and involves  $\text{Ca}^{++}$  ions and the cyclic AMP system of the islet, and one which is delayed and seemingly independent of these factors, the expression of which requires the presence of the first, initiating action. Thus our results offer experimental support for the hypothesis of a dual role of glucose for insulin release (4, 25). It is tempting to speculate that TP represents a primitive function in the beta-cell, while the acute glucose effect, involving cyclic AMP, has evolved at a later stage for the minute-to-minute regulation of glucose homeostasis with high sensitivity.

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