The Immediate Insulin-secretory Response of the Rat Pancreas to Glucose Compared with Tolbutamide and Other Secretagogues

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SUMMARY

Insulin secretagogues induced characteristic secretory responses of the pancreas. While tolbutamide provoked an immediate insulin secretory response of the perfused rat pancreas, after a short delay insulin secretion appeared in response to glyceraldehyde, glucose, and mannose after different characteristic latent periods. Glucose and tolbutamide competed for insulin from the same rapidly available hormone pool during the immediate insulin-secretory response of the perfused pancreas. The mechanism of their secretory action differed.

There was a latent period between arrival of glucose at the pancreatic β-cells and the insulin-secretory response when the perfusate was switched from a medium without glucose to a medium with 16.7 mM glucose. However, when the preperfusion medium contained a substimulatory glucose concentration, this latent period was diminished or nearly abolished. The increment in glucose concentration necessary for insulin release was reduced concomitantly. The ability of glucose to sensitize pancreatic β-cells was concentration-dependent and time-dependent. Only mannose was able to substitute for glucose in its capacity to virtually abolish the latent period. Glyceraldehyde, dihydroxyacetone, and leucine only reduced the latent period. L-glucose, 3-O-methylglucose, fructose, and pyruvate were ineffective. Tolbutamide desensitized the β-cell to glucose stimulation and prolonged the latent period.

It is proposed that both glucose and tolbutamide initiate insulin secretion by interaction with a common receptor site. They differ, however, in that glucose but not tolbutamide sensitizes pancreatic β-cells. Tolbutamide cannot fully replace glucose. DIABETES 27:27-34, January, 1978.

Sudden exposure of the pancreas to secretagogues provokes an immediate (acute, early, or first) insulin secretory-response of the pancreatic β-cells.[1-9] The immediate secretory response is followed by a more or less sustained late (or second) phase of insulin secretion from the pancreas.[1,2,4,8] Glucose as well as glyceraldehyde induces a distinct late phase of insulin secretion, resulting in a biphasic secretory pattern of hormone release from the pancreas.[1,4,8,10] A transient monophasic insulin-secretory pattern is characteristic of tolbutamide and mannose.[1,2,8,11] The duration of the immediate insulin-secretory response is short (no longer than two to three minutes[7,8]), and the contribution of insulin to the total amount of hormone released during a longer stimulation period is small.[12] The duration of the latent period between arrival of the stimulus at the pancreatic β-cells and appearance of the peak immediate insulin-secretory response varies, however.[1-9] The immediate insulin-secretory response to tolbutamide appears after a short delay, whereas there are different latent periods of a few minutes in response to glucose, glyceraldehyde, and mannose.[1,3,5-9] Reduction of the sampling intervals of the perfusion medium to 10 seconds increased further the resolving power of the perfused pancreas[7,8] and made it possible to demonstrate, on simultaneous exposure of the pancreas to glucose and tolbutamide, a delay of 98 ± 2 seconds for glucose-induced immediate insulin secretion compared with tolbutamide.[7]

It is the purpose of the present communication to give a full account on the characteristics of the immediate insulin-secretory response of the perfused rat pancreas to glucose compared with tolbutamide and other insulin secretagogues. Some of the results have been presented in abstract form before.[13-15]

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MATERIALS AND METHODS

Chemicals. Pure rat insulin was kindly supplied by Novo GmbH, Mainz, and tolbutamide by Farbwerke Hoechst AG, Frankfurt. ^{125}I-insulin was obtained from Behringwerke AG, Frankfurt, bovine albumin (fraction V) from Serva, Heidelberg. L-glucose, 3-O-methyl-D-glucose, D-glyceraldehyde, dihydroxyacetone, and D-mannoheptulose were from Sigma Chemical Company, St. Louis, Mo. D-glucose, D-fructose, D-mannose, pyruvate, L-leucine, and all other chemicals of analytic grade were obtained from Merck AG, Darmstadt.

Experimental design. Male Wistar rats (200-250 gm.) were used. The pancreas and the adjacent part of the duodenum, the spleen, and the stomach were removed from 24-hour-fasted rats according to the method of Grodsky et al.,[14] with slight modifications as described in detail before.[7] After an initial equilibration period of 30 minutes (10 minutes in figures 1-4), designated zero time, the perfusion was rapidly switched to a second perfusate reservoir containing a medium with the respective stimulating agent. The effluent was collected at various timed intervals (10, 30, or 60 seconds), and immunoreactive insulin was determined by the method of Zaharko and Beck.[15] Glucose in the perfusion medium was estimated by a GOD-Perid method (Boehringer Test combination).

The dead space of the perfusion system was about 1.3 ml. The dead space consisted of the arterial tubing between the valve of the perfusion apparatus and the pancreas preparation, which accounted for 16 seconds, the pancreas preparation as such, and the venous cannula, which accounted for two seconds. The dead space of the pancreas preparation itself cannot be determined by simple measurement. Glucose in the perfusion medium was used as a plasma marker, and concomitant determination of glucose in the effluent (figure 5) showed that the total dead space accounted for about 20 seconds. In reality, it took a little longer than 20 seconds for glucose in the effluent (figure 5) to reach the final concentration of 16.7 mM. This is apparently due to the fact that it took some time for the glucose-containing perfusion medium to replace all medium without glucose in the pancreas preparation. From the kinetics of glucose in the perfusion medium (figure 5), it can be concluded that stimulatory levels of the test substances were reached after 20 seconds. Therefore, the calculations in the present communication are based on the presence of a dead space of the perfusion system of 20 seconds.

Calculations. Results were tested for statistical significance with Student's *t*-test, the Wilcoxon test, and one-way analysis of variance (figures 6-8).

RESULTS

Insulin secretagogues induced characteristic insulin-secretory responses of the endocrine pancreas. Tolbutamide (20 mg./100 ml.) induced an insulin secretory response of the pancreas virtually as soon as this pharmacologic agent reached the pancreatic β-cells, with a delay of no more than 10 seconds (figure 1 and table 1, line 7). Other insulin secretagogues provoked secretory responses after a latent period. The latent period for the initial insulin secretory response to glyceraldehyde (10 mM) was about 70

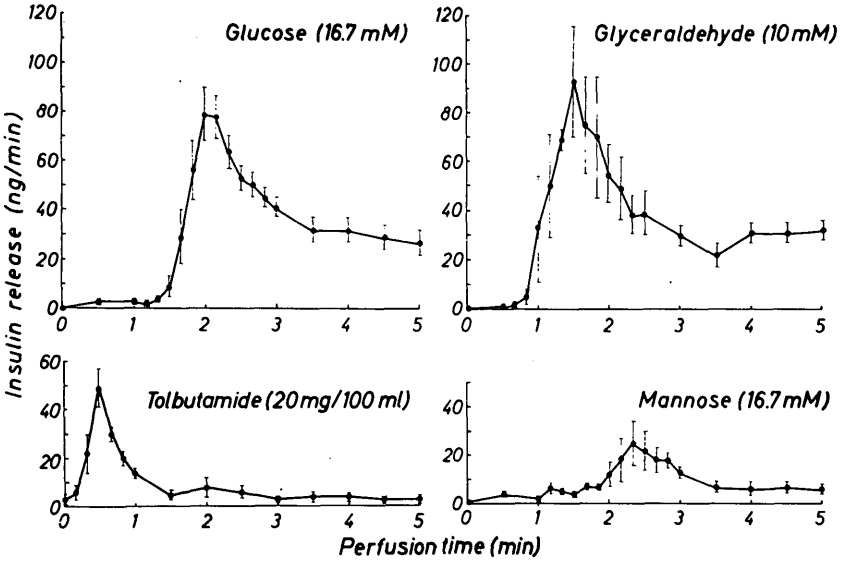

FIGURE 1

The immediate insulin secretory response of the perfused rat pancreas to glucose (16.7 mM) (n = 12), glyceraldehyde (10.0 mM) (n = 5), tolbutamide (20 mg./100 ml.) (n = 7), and mannose (16.7 mM) (n = 4). Mean ± S.E.M.

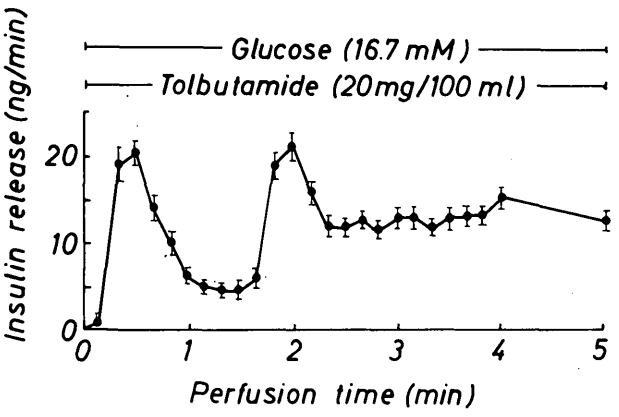

FIG. 2. The immediate insulin secretory response of the perfused rat pancreas on simultaneous exposure to glucose (16.7 mM) and tolbutamide (20 mg./100 ml.) for one to five minutes. Mean ± S.E.M. Number of experiments = 10.

seconds (figure 1 and table 1, line 5), to glucose (16.7 mM) about 100 seconds (figure 1 and table 1, line 1), and to mannose (16.7 mM) about 130 seconds (figure 1).

When tolbutamide (20 mg./100 ml.) and glucose (16.7 mM) were administered together, two sequential insulin peaks were observed (figure 2). The first peak, most likely representing tolbutamide-induced insulin release, appeared 98 ± 2 seconds earlier ($P < 0.01$) than the second peak, which probably represents glucose-induced insulin release (figure 2). The peaks were equally high (figure 2), but the total amount of insulin released was not higher than in response to glucose alone (62 ± 7 versus 74 ± 18 ng.).

When tolbutamide alone was administered to the pancreas and glucose added to the perfusion medium following the tolbutamide-induced secretory response (after one minute) (figure 3), insulin secretion was significantly higher ($p < 0.05$) during the first minute than was found when tolbutamide and glucose were perfused together (figure 2). When glucose alone was administered to the pancreas followed by tolbutamide after the glucose-induced response (after three minutes) (figure 4), the total amount of insulin released from the pancreas during the first five minutes was significantly increased ($p < 0.05$) compared with figure 2. Experiments presented in figures 3 and 4 indicate that glucose and tolbutamide competed in their insulin secretory action for a common rapidly available hormone pool, possibly via the same secretory pathway, but they did not provide an explanation for the delay of 90-100 seconds for glucose-induced insulin release compared with tolbutamide-induced release.

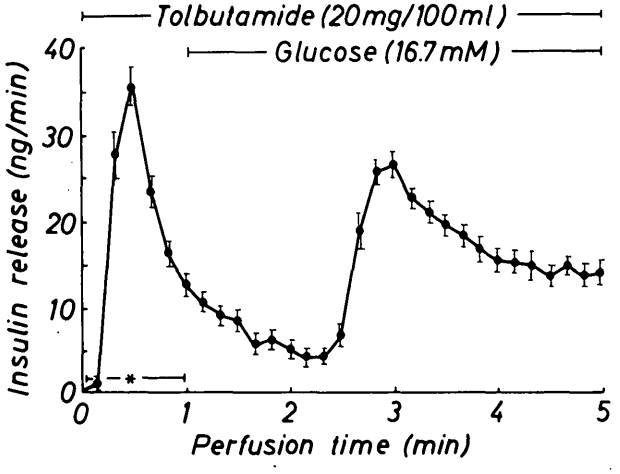

FIG. 3. The immediate insulin secretory response of the perfused rat pancreas to tolbutamide (20 mg./100 ml.) (one to five minutes) and consecutive additional exposure to glucose (16.7 mM) for two to five minutes. Mean ± S.E.M. Number of experiments = 10. *P<0.05 compared with figure 2.

The following experiments will show that it was possible to reduce the latent period for glucose-induced insulin secretion. When the perfusate was switched from a medium without glucose to a medium with 16.7 mM glucose, the peak of the immediate insulin-secretory response appeared after 121 ± 3 seconds (figure 5 and table 1, line 1). At that time the glucose concentration in the perfusion medium had reached 16 mM (figure 5). When the perfusate was switched from a medium with 5.5 mM glucose to a medium with 16.7 mM glucose, the peak of the immediate insulin secretory response appeared

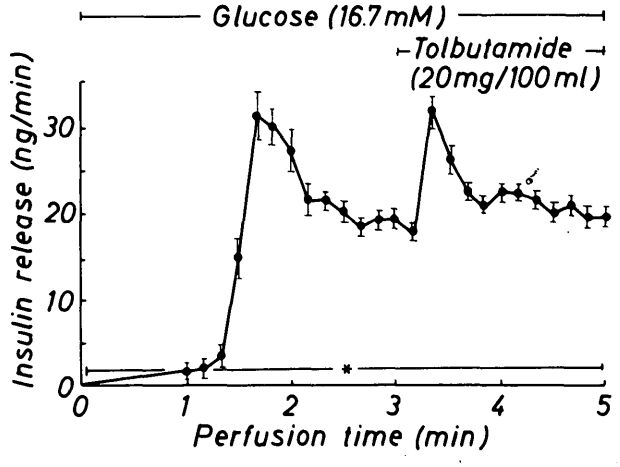

FIG. 4. The immediate insulin-secretory response of the perfused rat pancreas to glucose (16.7 mM) (one to five minutes) and consecutive additional exposure to tolbutamide (20 mg./100 ml.) for four to five minutes. Mean ± S.E.M. Number of experiments = 10. *P<0.05 compared with figure 2.

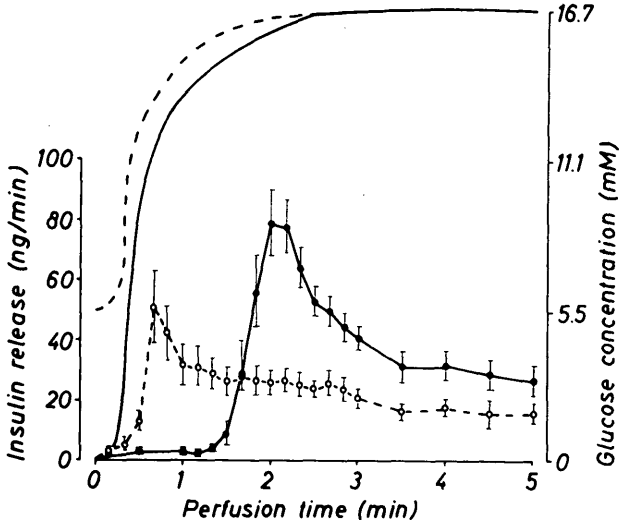

FIG. 5. The reduction of the latent period (seconds) between arrival of a stimulatory glucose concentration (upper curves) at the pancreatic β-cells and appearance of the immediate insulin-secretory response of the perfused rat pancreas after preperfusion of the pancreas with a substimulatory glucose concentration (5.5 mM) (o--o--o) compared with controls (0 mM) (●—●—●). Mean ± S.E.M. Number of experiments = 8 (o--o--o) and 12 (●—●—●).

after 48 ± 5 seconds (figure 5 and table 1, line 2), but the glucose concentration in the perfusion medium had reached only 14 mM at that time (figure 5). The pancreatic β-cells were sensitized after previous exposure to a glucose concentration of 5.5 mM, which is substimulatory in this preparation, for 30 minutes. The glucose concentration in the perfusion medium was increased by merely 8.5 mM as against a level change of 16 mM, and the peak of the insulin-secretory response to glucose appeared 73 seconds earlier. The total amount of insulin released during the first five minutes of perfusion was not significantly affected by preperfusion with glucose (figure 5) (151 ± 14 ng. versus 115 ± 17 ng.).

The ability of substimulatory glucose concentrations in the preperfusion medium to reduce the latent period between arrival of a stimulatory glucose concentration (16.7 mM) at the pancreatic β-cells and appearance of the peak immediate insulin-secretory response of the pancreas was concentration-dependent (figure 6) ($p < 0.001$) and time-dependent (figure 7) ($p < 0.001$). Increase of glucose concentration in the preperfusion medium from 0 mM to 8.3 mM reduced the latent period by 88 seconds (table 1, lines 1 and 3).

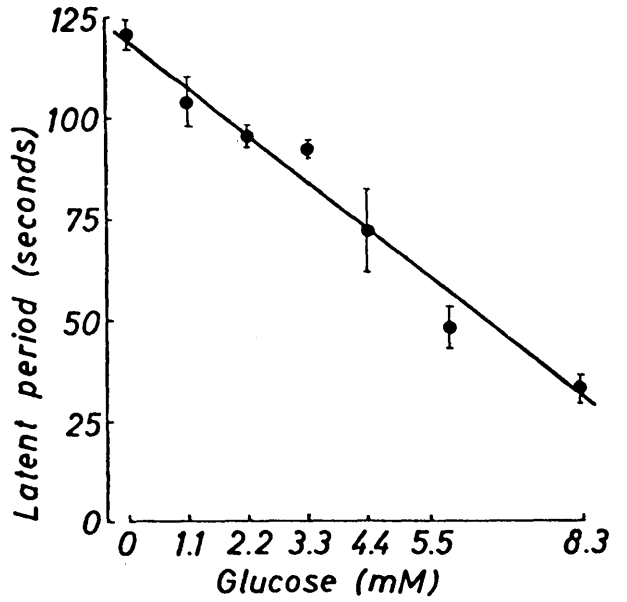

FIG. 6. Concentration dependency of the ability of glucose in the preperfusion medium to reduce the latent period between arrival of a stimulatory glucose concentration (16.7 mM) at the pancreatic β-cells and appearance of the immediate insulin secretory response of the perfused rat pancreas. Mean ± S.E.M. Number of experiments in each group = four to 12. The dead space of the perfusion system (20 seconds) must be substracted from each point to obtain the actual latent period.

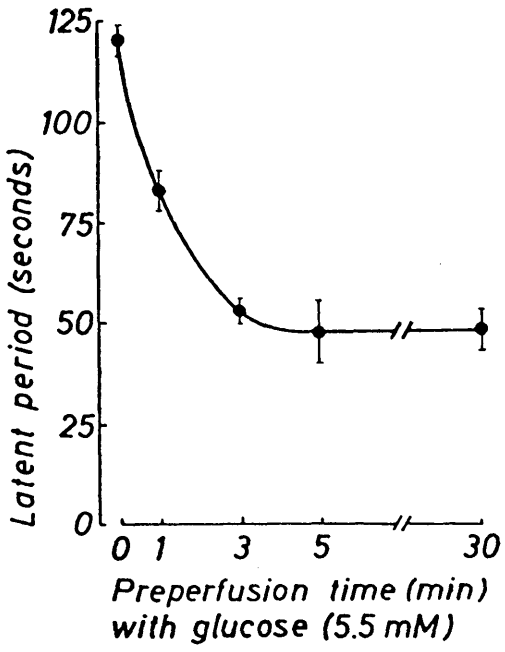

FIG. 7. Time dependency (minutes) of the ability of glucose (5.5 mM) in the preperfusion medium to reduce the latent period between arrival of a stimulatory glucose concentration (16.7 mM) at the pancreatic β-cells and appearance of the immediate insulin secretory response of the perfused rat pancreas. Mean ± S.E.M. Number of experiments in each group = three to 12. The dead space of the perfusion system (20 seconds) must be substracted from each point to obtain the actual latent period.

A preperfusion of the pancreas with 5.5 mM glucose for 30 minutes rather than five minutes did not reduce the latent period (figure 7).

Glucose in the preperfusion medium also reduced the delay of insulin release due to glyceraldehyde (10 mM) and, less pronounced, the delay for tolbutamide (20 mg./100 ml.) (table 1, lines 6 and 9), indicating a general sensitizing effect of glucose on insulin secretion in response to insulin secretagogues.

Tolbutamide could not replace glucose. In a concentration range between 1 mg./100 ml. and 20 mg./100 ml., tolbutamide could not reduce the latent period for glucose-induced (16.7 mM) insulin release. At higher concentrations the latency was even more prolonged (figure 8 and table 1, lines 25, 26) ($p < 0.001$).

We sought to determine whether the ability of the glucose present to reduce the latent period was specific for glucose. Special attention was given to whether the fuel function of glucose or some interference with a receptor site could explain the ability of this hexose to reduce the latent period between arrival of glucose at the pancreatic β-cells and appearance of the peak immediate insulin-secretory response of the pancreas. For this purpose, several other agents were tested for their ability to replace glucose in the preperfusion medium (table 1, lines 10-24). Mannose, which is poorly metabolized[18] compared with glucose, was effective only when present at a high concentration (16.7 mM) (table 1, line 11). Mannose (5.5 mM) (table 1, line 10) reduced the latent period only slightly. Glyceraldehyde (2.5 mM and 10 mM) and dihydroxyacetone (2.5 mM and 10 mM), two other substrates of glycolysis, and leucine (20 mM, but not 5 mM), a metabolizable amino acid, reduced the latent period (table 1, lines 12-17). They could not replace glucose, however. All other agents tested were ineffective (table 1, lines 18-24): fructose (5.5 mM and 16.7 mM), a poorly metabolized hexose[18] comparable with mannose in this regard, L-glucose (5.5 mM) and 3-O-methylglucose (5.5 mM and 16.7 mM), two non-metabolizable hexoses, and pyruvate (5 mM and 15 mM). The concomitant presence of mannoheptulose (1 mM) in the preperfusion medium prevented the sensitizing effect of glucose on the pancreatic β-cells (5.5 mM) (table 1, line 4).

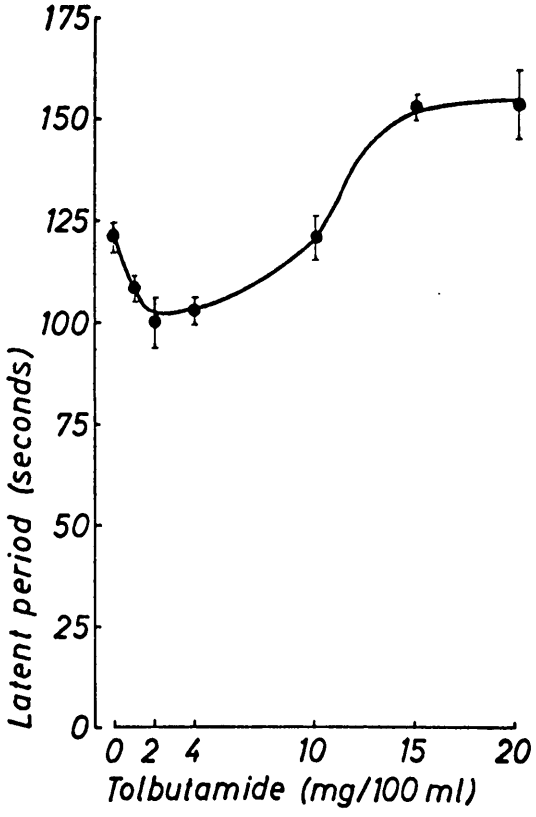

FIG. 8. Concentration dependency of the ability of tolbutamide in the preperfusion medium to increase the latent period between arrival of a stimulatory glucose concentration (16.7 mM) at the pancreatic β-cells and appearance of the immediate insulin secretory response of the perfused rat pancreas. Mean ± S.E.M. Number of experiments in each group = three to 12. The dead space of the perfusion system (20 seconds) must be substracted from each point to obtain the actual latent period.

DISCUSSION

Evidence is available for the view that tolbutamide induces insulin release by some direct interaction with a receptor in the β-cell plasma membrane.[19-23] It is not established, however, whether glucose has to be metabolized before some glucose metabolite triggers insulin release or glucose induces insulin release by some direct interaction with a receptor.[24,25] It has been speculated that the delay of about 100 seconds in glucose-induced insulin release may be caused by the necessity for glucose to be metabolized before it can induce insulin release.[3] Only glucose and mannose virtually abolished the latent period between appearance of glucose at the β-cells and initiation of the first phase of the insulin-secretory response. This explains why shorter latent periods were registered when substimulatory glucose concentrations were in the preperfusion medium.[4] Compared with glucose, mannose is a weak insulin secretagogue,[8,11] and its ability to reduce the latent period was also weaker (table 1). But even though islets metabolize mannose and fructose to a comparable degree,[18] only mannose reduced the la-

TABLE 1

The effect of different substances in the preperfusion medium (30 min.) on the latent period (seconds) between arrival of a stimulatory concentration of glucose (16.7 mM), tolbutamide (20 mg./100 ml.), or glyceraldehyde (10 mM) at the pancreatic β-cells and appearance of the immediate insulin secretory response of the rat pancreas during the perfusion.

Line	Preperfusion	Perfusion	N	Latent period (seconds)
1	Glucose (0 mM)	Glucose (16.7 mM)	12	121 ± 3
2	Glucose (5.5 mM)	Glucose (16.7 mM)	8	48 ± 5‡
3	Glucose (8.3 mM)	Glucose (16.7 mM)	4	33 ± 3‡
4	Glucose (5.5 mM) + Mannoheptulose (1 mM)	Glucose (16.7 mM)	6	100 ± 4*
5	Glucose (0 mM)	Glyceraldehyde (10 mM)	4	88 ± 5
6	Glucose (5.5 mM)	Glyceraldehyde (10 mM)	4	43 ± 3‡
7	Glucose (0 mM)	Tolbutamide (20 mg./100 ml.)	7	30 ± 0
8	Glucose (5.5 mM)	Tolbutamide (20 mg./100 ml.)	3	30 ± 0
9	Glucose (8.3 mM)	Tolbutamide (20 mg./100 ml.)	3	20 ± 0‡
10	Mannose (5.5 mM)	Glucose (16.7 mM)	8	93 ± 2‡
11	Mannose (16.7 mM)	Glucose (16.7 mM)	4	35 ± 5‡
12	Glyceraldehyde (2.5 mM)	Glucose (16.7 mM)	4	78 ± 3‡
13	Glyceraldehyde (10 mM)	Glucose (16.7 mM)	4	65 ± 9‡
14	Dihydroxyacetone (2.5 mM)	Glucose (16.7 mM)	8	77 ± 5‡
15	Dihydroxyacetone (10 mM)	Glucose (16.7 mM)	4	65 ± 5‡
16	Leucine (5 mM)	Glucose (16.7 mM)	4	98 ± 5*
17	Leucine (20 mM)	Glucose (16.7 mM)	4	60 ± 4‡
18	L-Glucose (5.5 mM)	Glucose (16.7 mM)	5	108 ± 4
19	3-O-Methylglucose (5.5 mM)	Glucose (16.7 mM)	5	105 ± 2
20	3-O-Methylglucose (16.7 mM)	Glucose (16.7 mM)	3	120 ± 4
21	Fructose (5.5 mM)	Glucose (16.7 mM)	4	125 ± 6
22	Fructose (16.7 mM)	Glucose (16.7 mM)	5	110 ± 4
23	Pyruvate (5 mM)	Glucose (16.7 mM)	4	118 ± 5
24	Pyruvate (15 mM)	Glucose (16.7 mM)	3	110 ± 0
25	Tolbutamide (1 mg./100 ml.)	Glucose (16.7 mM)	4	108 ± 3
26	Tolbutamide (15 mg./100 ml.)	Glucose (16.7 mM)	3	153 ± 3†

N = Number of experiments. Mean ± S.E.M.

*$p < 0.01$, †$p < 0.001$, ‡$p < 0.0001$ compared with the effect of controls without any supplementation of the preperfusion medium (line 1 for glucose, line 5 for glyceraldehyde, line 7 for tolbutamide in the perfusion medium). The dead space of the perfusion system accounted for 20 seconds. To obtain the actual latent period (seconds) for each experimental situation, subtract 20 seconds from each value in this table.

tent period (table 1). Three other substances—glyceraldehyde, dihydroxyacetone, and leucine—reduced the latency, but only to such an extent that the immediate insulin-secretory response to glucose appeared where the secretory response to stimulatory concentrations of these agents can be expected to appear.[8,9,24] Dihydroxyacetone, though it does not induce insulin release itself,[8,10] behaves similar to glyceraldehyde and induces insulin release in the presence of xanthines.[26] Leucine did not induce insulin secretion from the perfused pancreas in the complete absence of glucose in our hands. Matschinsky et al. reported that leucine induced insulin secretion only after a longer preperfusion period of the pancreas;[24] I can confirm this observation. All other agents except tolbutamide, which prolonged the latency, had no effect on the latent period.

The ability of mannoheptulose to prevent glucose from reducing the latent period demonstrates that it is possible to prevent the sensitizing effect of glucose in pancreatic β-cells. Mannoheptulose, however, does not help to answer the question whether glucose metabolism is a factor in the sensitizing effect of glucose, because the controversy whether mannoheptulose inhibits insulin secretion by inhibiting glucose phosphorylation[27] or by binding to glucose receptors located at the β-cell membrane[28] has not been resolved.

The results summarized in table 1 allow some comments on the mode of the insulin-secretory action of glucose that favor the idea that glucose may initiate the immediate insulin secretory response of the pancreas by some interaction with a receptor site at the β-cell membrane without a major contribution of glucose metabolism.

(1) Glucose does not simply serve as a fuel, because fructose, pyruvate, and leucine, which provide energy,[25] could not replace glucose in its ability to

diminish the latent period (table 1).

(2) Glucose does not seem to provide glycolytic intermediates, which consequently trigger the immediate insulin-secretory response of the pancreas, because fructose, glyceraldehyde, dihydroxyacetone, and pyruvate could not replace glucose in its ability to diminish the latent period (table 1).

(3) The linear relationship between glucose concentration in the preperfusion medium and the ability of glucose to reduce the latent period (figure 6) and the sigmoidal relationship between glucose concentration in the medium and glucose oxidation[27] by pancreatic islets are basically different. While 5.5 mM glucose had a close to maximal diminishing effect on the latent period (figure 6), glucose oxidation starts to increase only at a concentration of 5.5 mM glucose.[27]

(4) Mannose, only poorly metabolized[18,27] compared with glucose, reduced the latent period, even though it was less potent than glucose (table 1).

(5) Both mannose and fructose are metabolized to a comparable degree by pancreatic islets,[18,27] but only mannose reduced the latent period (table 1).

The insulin secretory actions of glucose, a physiologic agent, and tolbutamide, a pharmacologic agent, can be distinguished from each other:

1. Tolbutamide induced an immediate insulin secretory response of the pancreas, whereas glucose provoked a secretory response only after a long delay (figure 1).

2. When glucose and tolbutamide were administered together to the pancreas they induced separate secretory responses, as demonstrated by a double peak of the immediate insulin secretory response (figure 2).

3. The secretory response to glucose appeared about 100 seconds later than the response to tolbutamide (figure 2).

4. Glucose, at substimulatory concentrations in the preperfusion medium, was able to reduce the latent period that passes before the immediate insulin-secretory response to a stimulatory glucose concentration appears (figures 5-7).

5. Tolbutamide had an adverse effect; at higher concentrations, it prolonged the latent period (figure 8).

Glucose and tolbutamide, apparently, differ also in the mode of their insulin-secretory action:

1. Glucose and tolbutamide differ in the kinetic characteristics of their secretory action (figures 1-8).

2. Glucose and tolbutamide compete in their insulin-secretory action for a common, rapidly available insulin pool, possibly by the same secretory pathway (figures 2-4).

3. Both glucose and tolbutamide induce an immediate insulin secretory response, probably by interaction with a common receptor site at the β-cell membrane. It seems, however, that they elicit diverse effects at such a site even if they share a common receptor.

Glucose and tolbutamide are both initiators of insulin release, but they differ in that glucose sensitizes pancreatic β-cells whereas tolbutamide seems to have an adverse effect. Glucose sensitizes the β-cells that may finally be responsible for generation of the late phase of insulin release, probably entailing metabolism of glucose. Tolbutamide may bind to such sites and occupy them, but it seems not to interact with these sites. Tolbutamide cannot fully replace glucose.

In conclusion, the results provide evidence that the immediate insulin-secretory response of the pancreas to glucose, as well as to tolbutamide and other secretagogues, is dependent on the interaction of these stimulators with a receptor site. However, these results do not exclude a participation of glucose metabolism in glucose-induced insulin secretion, especially in maintaining the characteristic, potent, late phase of insulin secretion in response to stimulation of insulin secretion with glucose.

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REFERENCES

[1]Curry, D. L., Bennett, L. L., and Grodsky, G. M.: Dynamics of insulin secretion by the perfused rat pancreas. Endocrinology *83*:572-84, 1968.

[2]Loubatières, A., Mariani, M. M., and Chapal, J.: Insulinosécrétion etudiée sur le pancréas isolé et perfusé du rat. I. Synergie entre glucose et sulfamides hypoglycémiants. Diabetologia *6*:457-66, 1970.

[3]Curry, D. L.: Is there a common beta cell insulin compartment stimulated by glucose and tolbutamide? Am. J. Physiol. *220*:319-23, 1971.

[4]Matschinsky, F. M., Landgraf, R., Ellerman, J. E., and Kotler-Brajtburg, J.: Effects of glucose on insulin release and the metabolite pattern of islets of Langerhans of the perfused rat pancreas. Diabetes *20*:327-28, 1971.

[5]Gabbay, K. H., and Tze, W. J.: Inhibition of glucose-induced release of insulin by aldose reductase inhibitors. Proc. Natl. Acad. Sci. U.S.A. *69*:1435-39, 1972.

[6]Bennett, L. L., Curry, D. L., and Curry, C.: Differences in insulin release in response to glucose and tolbutamide stimulation. Proc. Soc. Exp. Biol. Med. *144*:436-39, 1973.

[7]Lenzen, S.: The immediate insulin secretory response of the

isolated perfused rat pancreas to tolbutamide and glucose. FEBS Lett. *49*:407-08, 1975.

[8]Lenzen, S., Joost, H. G., and Hasselblatt, A.: The inhibition of insulin secretion from the perfused rat pancreas after thyroxine treatment. Diabetologia *12*:495-500, 1976.

[9]Vague, P., Ramahandridona, G., Di Campo-Rougerie, C., and Mahmoud, F.: Kinetics of the early insulin response of the isolated perfused rat pancreas to various metabolites and tolbutamide. Diabete et Metabolisme *1*:185-89, 1975.

[10]Malaisse, W. J., Herchuelz, A., Levy, J., Sener, A., Pipeleers, D. G., Devis, G., Somers, G., and van Obberghen, E.: The stimulus-secretion coupling of glucose-induced insulin release. XIX. The insulinotropic effect of glyceraldehyde. Mol. Cell. Endocrinol. *4*:1-12, 1976.

[11]Curry, D. L.: Fructose potentiation of mannose-induced insulin secretion. Am. J. Physiol. *226*:1073-76, 1974.

[12]Grodsky, G. M.: A threshold distribution hypothesis for packet storage of insulin and its mathematical modeling. J. Clin. Invest. *51*:2047-59, 1972.

[13]Lenzen, S.: Studies on the inhibitory effect of thyroxine on insulin secretion from the perfused rat pancreas. Naunyn-Schmiedebergs Arch. Pharmakol. *287*:R 58, 1975.

[14]Lenzen, S.: Die Insulin-sekretorische Antwort des perfundierten Pankreas auf Glukose, Tolbutamid, Glycerinaldehyd und Mannose. 10. Kongress Deutsche Diabetes-Gesellschaft, R 45, Ulm, 1975.

[15]Lenzen, S.: The immediate insulin secretory response of the pancreas as a model for studies on the mechanism of action of glucose and tolbutamide. Naunyn-Schmiedebergs Arch. Pharmakol. *297*:R 40, 1977.

[16]Grodsky, G. M., Batts, A. A., Bennett, L. L., Vcella, C., McWilliams, N. B., and Smith, P. F.: Effects of carbohydrates on secretion of insulin from isolated rat pancreas. Am. J. Physiol. *205*:638-44, 1963.

[17]Zaharko, D. S., and Beck, L. V.: Studies of a simplified plasma insulin immunoassay using cellulose powder. Diabetes *17*:444-57, 1968.

[18]Jarrett, J., and Kean, H.: Oxidation of sugars, other than glucose, by isolated mammalian islets of Langerhans. Metabolism *17*:155-57, 1968.

[19]Krzanowski, J. J., Fertel, R., and Matschinsky, F. M.: Energy metabolism in pancreatic islets of rats. Studies with tolbutamide and hypoxia. Diabetes *20*:598-606, 1971.

[20]Sehlin, J.: Evidence for specific binding of tolbutamide to the plasma membrane of the pancreatic β-cells. Acta Diabetol. Lat. *10*:152-60, 1973.

[21]Bowen, V., and Lazarus, N. R.: Insulin release from the perfused rat pancreas. Mode of action of tolbutamide. Biochem. J. *142*:385-89, 1974.

[22]Davis, B., and Lazarus, N. R.: An in vitro system for studying insulin release caused by secretory granules-plasma membrane interaction: definition of the system. J. Physiol. *256*:709-29, 1976.

[23]Joost, H. G., and Hasselblatt, A.: Effects of polymer-linked sulfonylurea derivatives on insulin release. Naunyn-Schmiedebergs Arch. Pharmakol. *297*:81-84, 1977.

[24]Matschinsky, F. M., Fertel, R., Kotler-Brajtburg, J., Stillings, S., Ellerman, J., Raybaud, F., and Holowach-Thurston, J.: Factors governing the action of small calorigenic molecules on the islets of Langerhans. *In* 8th Midwest Conference on Endocrinology and Metabolism. Columbia, Missouri, October 1972, pp. 63-87.

[25]Ashcroft, S. J. H., Weerasinghe, L. C. C., and Randle, P. J.: Interrelationship of islet metabolism, adenosine triphosphate content and insulin release. Biochem. J. *132*:223-31, 1973.

[26]Hellman, B., Idahl, L.-Å., Lernmark, Å., Sehlin, J., and Täljedal, L.-B.: The pancreatic β-cell recognition of insulin secretagogues: comparisons of glucose with glyceraldehyde isomers and dihydroxyacetone. Arch. Biochem. Biophys. *162*:448-57, 1974.

[27]Ashcroft, S. J. H., Hedeskov, C. J., and Randle, P. J.: Glucose metabolism in mouse pancreatic islets. Biochem. J. *118*:143-54, 1970.

[28]Matschinsky, F. M., Ellerman, J. E., Landgraf, R., Krzanowski, J., Kotler-Brajtburg, J., and Fertel, R.: Quantitative histochemistry of glucose metabolism in the islets of Langerhans. *In* Recent Advances in Quantitative Histo- and Cytochemistry. Bern, Hans Huber Verlag, 1970, pp. 143-79.