Potentiation of Glucose-induced Insulin Release by Glucose in the Isolated Pancreas of Fed and Fasted Rats

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SUMMARY

Glucose, apart from its acute insulin-releasing effect, exerts a time-dependent potentiating action on subsequent stimulations of the pancreas.[1] The influence of a 24-hour starvation on these two actions of glucose was studied with the completely isolated, perfused rat pancreas preparation.

Starvation had no effect on the time kinetics of the insulin response, but the magnitudes of the early and late insulin-secretion phases were reduced to similar extents. It was demonstrated by using a wide glucose concentration range that the maximal insulin response is not significantly modified by starvation. In contrast, both the threshold of stimulation by and the K_m for glucose were higher in the pancreas from fasted rats. Thus, starvation reduces the sensitivity of the islet for the insulin-releasing action of glucose.

When the stimulatory concentrations of glucose were preceded for 40 minutes by the perfusion of 8.3 mM instead of the basal, 4.4 mM glucose, insulin secretion from the pancreas of fed animals was not modified. In contrast, raising the glucose concentration of the equilibrium period to 8.3 mM potentiated markedly the insulin response to subsequent stimulations in the pancreas from fasted rats. This potentiation expressed itself as increase in the maximal response: the K_m for glucose was not reduced. Thus a 40-minute pretreatment with 8.3 mM glucose does not correct the diminished sensitivity induced by a 24-hour starvation.

It is concluded that in starvation (1) the sensitivity for glucose of the mechanisms that initiate insulin release is diminished; (2) the sensitivity of the pancreas for the potentiation-inducing action of glucose is augmented. (3) In both respects, the insulin response of fasted rats is similar to that of mildly diabetic subjects.[2] These and other findings[3] suggest that the effect of glucose on initiation of insulin release and on generation of a state of potentiation in the islet are mediated by different mechanisms. DIABETES *25*:949-54, **October, 1976.**

The vast majority of investigations regarding glucose-induced insulin release have dealt with the effect of the hexose on the acute initiation of hormone secretion. Glucose, however, exerts a second, time-dependent effect on the pancreatic beta cell, which results in potentiation of insulin release. This was first demonstrated by Grodsky et al.,[4] who used the isolated, perfused rat pancreas. It was shown that, when stimulated consecutively by two pulses of glucose, the insulin response of the pancreas to the second stimulation could be markedly enhanced under certain circumstances. On the basis of such studies, Grodsky[5] has postulated that glucose generates a hypothetical "potentiator" in the beta cell, which augments the responsiveness of the islet to later stimulation.

In extensive studies by one of us (E.C.), the characteristics of glucose-induced potentiation of the insulin response has been described in man.[1-3,6] Evidence suggesting that the initiating and potentiating actions of glucose may utilize distinct mechanisms was

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presented.[3] In subjects with impaired initiating effect of glucose (so-called low insulin responders, and patients with mild diabetes mellitus), the ability of the hexose to generate potentiation was found to be intact. In fact, glucose had a stronger potentiating effect in subjects with decreased insulin release than in controls.[2]

In analogy with mild diabetes mellitus, impaired insulin response to glucose is found in fasted animals and man.[7-15] Therefore, the fasted rat may serve as a suitable model for studies aiming at the description of the potentiating action of glucose on the pancreatic islet. The present paper reports on some characteristics of the potentiation induced by glucose in the isolated, perfused pancreas obtained from fasted and fed rats.

METHODS

Sprague-Dawley rats, weighing 200-250 gm., fed ad libitum or fasted for 24 hours, were used for the preparation of the completely isolated, perfused pancreas. The animals were anesthetized by intraperitoneal injection of 50 mg. per kilogram of pentobarbital, and the pancreas was isolated by a slight modification of the technique of Loubatières et al.[17] The gland was perfused with a Krebs-Ringer bicarbonate solution containing 4.4 or 8.3 mM glucose and 20 gm. per liter of beef albumin (fraction V, Armour Co.). The final solution was adjusted to pH 7.4 with 0.1 N HCl, and then continuously gassed with a mixture of 95 per cent oxygen and 5 per cent carbon dioxide. The perfusate was administered into the celiac artery and run into the prepared pancreas by an open circuit, nonrecycling perfusion system. The portal effluent was collected every 60 seconds. Flow rates were kept approximately at 2.5 ml. per minute by making minor changes in arterial pressure. A 40-minute equilibration period preceded the stimulatory concentration of glucose.

Insulin was measured by a double-antibody radioimmunoassay[18] using insulin reagent kits

TABLE 1

Effect of nutritional state on basal insulin release from isolated perfused rat pancreas (in μU. min.$^{-1}$)

Glucose level in equilibrium (mM)	Nutritional state: Fasted	Fed	
4.4	23 ± 2 (n = 27)	82 ± 12 (n = 20)	P < 0.001
8.3	203 ± 24 (n = 18)	361 ± 25 (n = 18)	P < 0.001
	P < 0.001	P < 0.001	

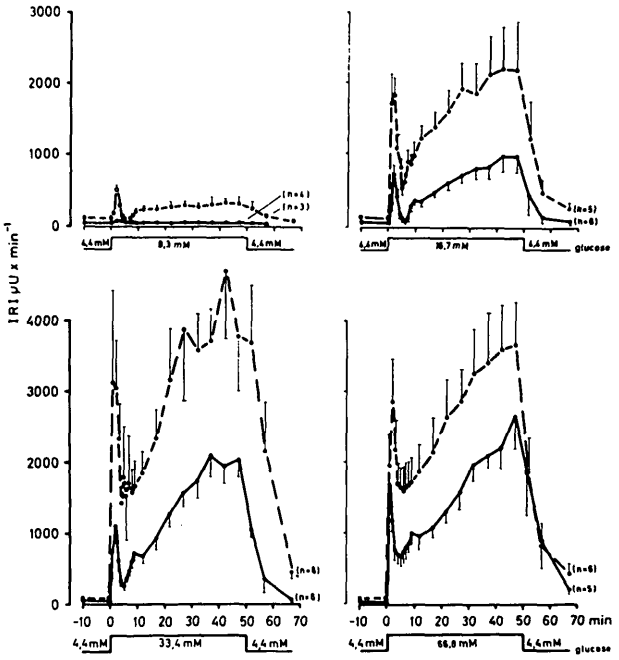

FIG. 1. Secretion rate of insulin from perfused pancreases isolated from fed (broken curves) or fasted (solid curves) rats. The pancreas were equilibrated for 40 minutes with 4.4 mM glucose prior to the application of stimulating glucose concentrations (0-50 minutes). Mean ± S.E.M. of the indicated number of experiments are given.

(Radiochemical Centre, Amersham) and a rat insulin standard, kindly provided by Dr. J. Schlichtkrull (Novo Research Institute, Copenhagen). The magnitude of insulin release was determined by comparison of the incremental areas under the insulin curves. The initial phase of insulin release corresponds to zero to seven minutes after the start of the stimulation, while the second phase comprises the period between seven and 32 minutes. Conventional statistical methods have been employed. Results are expressed as mean ± S.E.M.

RESULTS

As expected, the basal insulin secretion rate, measured at the end of a 40-minute equilibration period with 4.4 or 8.3 mM glucose, was dependent on the nutritional state of the animal, significantly lower values being observed after fasting (table 1).

When the perfusate concentration of glucose was raised from 4.4 to 16.7, 33.4, and 66.8 mM, respectively, biphasic insulin responses were obtained whether the pancreas was isolated from fed or fasted rats (figure 1). At all stimulatory levels, however, the response of the fed pancreas was significantly greater, the initial (zero to seven minutes) and the late (seven

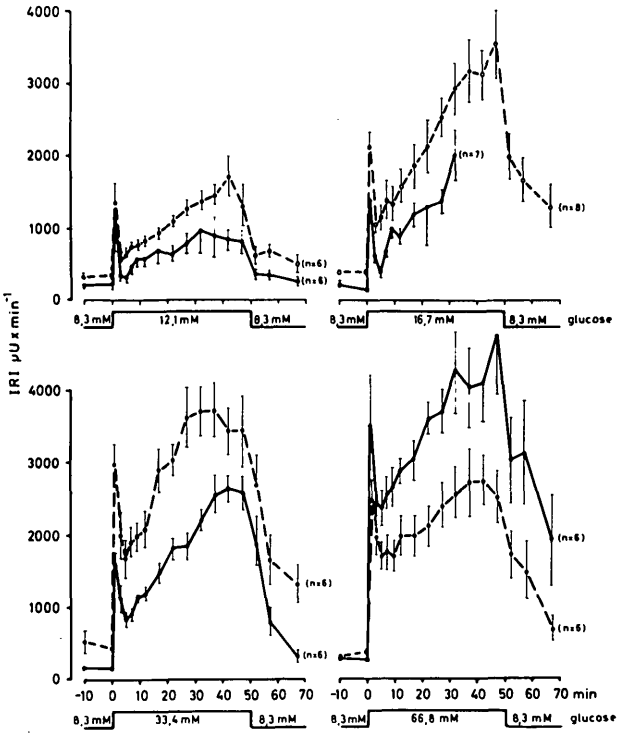

FIG. 2. Effect of a higher glucose concentration during equilibration on the insulin response of pancreas from fed (broken curves) and fasted (solid curves) rats. Legend as in figure 1, except that the pancreases were perfused with 8.3 mM glucose between −40 and zero minutes.

to 32 minutes) insulin responses being reduced by similar proportions in the fasted animal. Thus, the time kinetics of insulin release do not seem to be influenced by starvation for 24 hours.

The pancreas from fed rats responded with a small but clearly significant insulin response when stimulated by 8.3 mM glucose. In the pancreas from fasted rats, this glucose concentration was below the stimulatory threshold (figure 1).

In a second series of experiments, the pancreases of fed and fasted animals were equilibrated with 8.3 mM glucose and then stimulated with 12.1, 16.7, 33.4, and 66.8 mM, respectively. Figure 2 shows that both preparations responded to all glucose concentrations with biphasic insulin release. The response of the pancreas from fasted rats was, however, lower, except at the highest stimulatory concentration, when it even exceeded that of the control.

The quantitative analysis of the two series of results (table 2) demonstrates that increasing the glucose level of equilibration medium enhanced significantly the insulin response of the fasted animal to most glucose concentrations, while no effect was observed in the pancreas from fed rats.

The results are summarized in figure 3, where the dose-response relationship between the glucose concentration and the integrated insulin response is depicted. Two sets of information may be obtained from the figure. First, if one compares the dose-response curves of the fed and fasted animals, it may be seen that starvation increases the apparent "Km" for glucose: around 33 mM for the initial and 28 mM for the late insulin response in the fasted rat, as against about 17 mM for both phases in the fed animals. A clear-cut maximal secretion rate was observed only in the pancreas from fed rats. Second, raising the glucose level of the equilibration medium increased the "Vmax" of the response of the pancreas from fasted rats, while the apparent "Km" was not changed. In the fed rat, no significant modification of the dose-response curves was observed by increasing the glucose concentration during equilibration.

DISCUSSION

Although numerous authors have described a reduced insulin response after starvation,[7-16] few have studied simultaneously the changes that occur in the time and dose kinetics of the glucose effect.[9,12] The present work has the merit of giving a detailed description of these two important kinetic features. A 24-hour starvation was selected since we wanted to study the effect of glucose (and calorie) deprivation as such; during more prolonged fasting, such as 48-72 hours, other secondary events like protein wastage may obscure the picture.

In the present series of experiments starvation clearly reduced the sensitivity of the pancreas for glucose. This was expressed by the elevation of the threshold of stimulation and the larger apparent Km of the insulin response. This observation is in agreement with the findings of Bosboom et al.[12] on the perfused rat pancreas, and those of Hedeskov and Capito[13] and Hahn and Fiedler[15] on isolated mouse and rat islets. In our hands, the initial and late insulin responses were affected in a symmetrical manner by fasting, in contrast with the data of Fink et al.,[14] who found the initial response to be reduced to a greater extent. One possible reason for this discrepancy is that the latter authors used animals starved for 48 hours.

The mechanism for the derangement of the insulin response during starvation is not known. We have recently shown that, at least in the perfused pancreas, the reduced response is not due to an exaggerated

TABLE 2

Effect of nutritional state on the insulin response to glucose of the isolated perfused rat pancreas (in μU. min.$^{-1}$)

Nutritional state:	Fasted					
Insulin area:	1-7 min.			7-32 min.		
Glucose in equilibrium (mM):	4.4	8.3	P	4.4	8.3	P
Glucose concentration during stimulation (mM):						
8.3	103 ± 28 (4)		109 ±	77 (4)		
12.1	1,304 ± 227 (6)	1,796 ± 331 (6)	NS	6,681 ± 2,219 (6)	12,024 ± 3,508 (6)	NS
16.7	1,759 ± 257 (6)	3,427 ± 456 (7)	<0.01	12,387 ± 1,932 (6)	29,051 ± 4,461 (7)	<0.01
33.4	3,471 ± 458 (6)	6,288 ± 550 (6)	<0.005	26,366 ± 3,807 (6)	36,148 ± 2,122 (6)	<0.05
66.8	5,922 ± 921 (5)	15,535 ± 1,470 (6)	<0.001	32,146 ± 4,566 (5)	76,514 ± 7,606 (5)	<0.001

inhibitory effect of catecholamines.[16] Recent findings from our group (Rabinovitch and Cerasi, unpublished observations) as from other authors[19] indicate that the

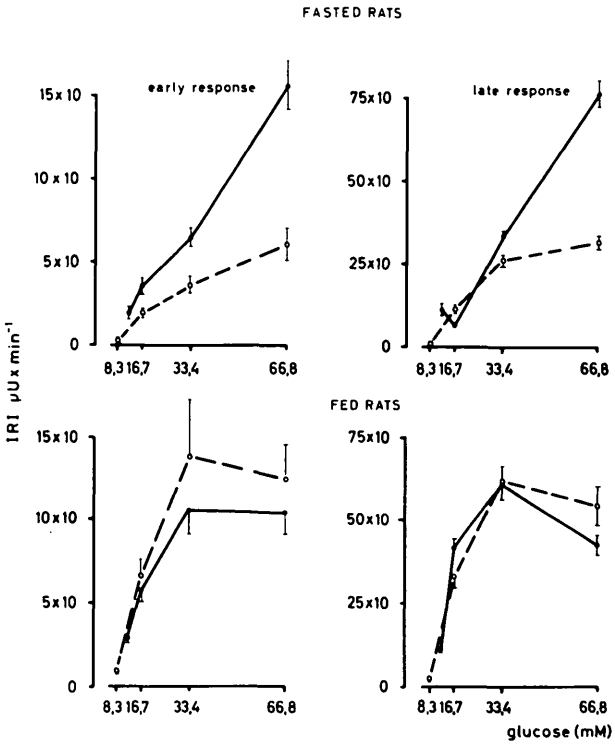

FIG. 3. The dose-response relationships of glucose-induced insulin release from the perfused pancreas. Early response corresponds to the integrated insulin values between zero and seven minutes, late response to seven to 32 minutes. The stimulatory glucose concentrations were preceded by a 40-minute perfusion with 4.4 mM (broken curves) or 8.3 mM glucose (solid curves). Mean ± S.E.M. of three to eight experiments (see table 2).

stimulation of islet cyclic AMP due to glucose is impaired during starvation. Furthermore, it has been demonstrated that caffeine may restore the impaired insulin release in starvation.[20] Thus, it is likely that changes in islet cyclic AMP metabolism are (at least partly) responsible for the diminished insulin response of fasted animals, although other metabolic changes in the beta cells may also be of importance.

It has often been stated that starvation bears many similarities to mild diabetes. Indeed, the kinetic changes of the insulin response described here were found also in patients with mild maturity-onset diabetes and subjects with decreased insulin response only.[21] Furthermore, at least in two species of rodents with spontaneous diabetes, the spiny mouse[22] and the Chinese hamster (Rabinovitch and Cerasi, unpublished observations), also the islet cyclic AMP response to glucose was deficient. This paper presents a third feature common to starvation and mild diabetes, namely increased sensitivity to the potentiating action of glucose.

Glucose induces two distinct effects on the beta cell: on the one hand it initiates an acute insulin release; on the other hand it generates a time-dependent state of potentiation in the islet, resulting in greater insulin responses to consecutive stimulations by glucose, and also by tolbutamide or glucagon.[23] In patients with mild diabetes the ability of glucose to generate potentiation was not decreased,[2] while the sensitivity of the acute insulin response for glucose was reduced.[21] Especially in subjects without diabetes but with decreased acute insulin response to glucose (low insulin responders), sensitivity to the potentia-

TABLE 2 (Cont.)

Effect of nutritional state on the insulin response to glucose of the isolated perfused rat pancreas (in μU. min.$^{-1}$)

Fed					
1-7 min.	1-7 min.		7-32 min.		
4.4	8.3		4.4	8.3	
		P			P
767 ± 162 (3)			3,595 ± 816 (3)		
	2,794 ± 225 (6)			16,985 ± 920 (6)	
6,566 ± 1,015 (5)	5,632 ± 793 (8)	NS	34,067 ± 6,343 (5)	42,553 ± 6,425 (8)	NS
13,651 ± 3,629 (6)	10,515 ± 1,514 (6)	NS	61,427 ± 9,976 (6)	60,993 ± 6,037 (6)	NS
12,384 ± 1,998 (6)	10,424 ± 1,207 (6)	NS	49,389 ± 15,165 (6)	43,037 ± 5,477 (6)	NS

ting action of the hexose was markedly increased.[2] The pancreas from fasted rats seems to follow quite closely this pattern. Indeed, in this preparation a moderate increase in the glucose concentration of the equilibration medium (from 4.4 to 8.3 mM) amplified markedly both phases of the insulin response to a subsequent glucose stimulation, while no such effect was observed in the fed rat. This is very similar to the findings in low insulin responders, where the threshold for eliciting potentiation was around 7 mM, as against 15 mM for control subjects.[2,6] Thus, the sensitivity of the islets for the potentiating action of glucose seems to be enhanced in situations where acute insulin response to glucose is diminished. Obviously, complete dose-response studies of the potentiating effect of glucose in the perfused pancreas from fed and fasted rats will be necessary in order to estimate the quantitative differences in sensitivity caused by starvation.

It has to be emphasized that, although the insulin response of the fasted pancreas after potentiation may exceed that of the fed pancreas (see figure 2), this does not mean that a 40-minute equilibration with 8.3 mM glucose corrects the defect created by 24 hours of starvation. Indeed, the basic change induced by fasting, i.e. decrease of the affinity of the acute response for glucose, is not modified by the prior exposure of the islets to the higher concentration of the hexose.

The mechanisms by which glucose induces potentiation in the islet is not known. The fact that the insulin-initiating and -potentiating actions of the hexose may be dissociated (in terms of affinities) in situations such as starvation or diabetes strongly supports the previous suggestion of one of us[3,23] that distinct mechanisms may be responsible for their transmission in the beta cell.

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REFERENCES

[1]Cerasi, E., Fick, G., and Rudemo, M.: A mathematical model for the glucose induced insulin release in man. Eur. J. Clin. Invest. *4*:267-78, 1974.

[2]Cerasi, E.: Potentiation of insulin release by glucose in man. III. Normal recognition of glucose as a potentiator in subjects with low insulin response and in mild diabetes. Acta Endocrinol. (Kbh.) 79:511-34, 1975.

[3]Cerasi, E.: Potentiation of insulin release by glucose in man. II. Role of the insulin response, and enhancement of stimuli other than glucose. Acta Endocrinol. (Kbh.) 79:502-10, 1975.

[4]Grodsky, G.M., Curry, D., Landahl, H., and Bennett, L.L.: Further studies on the dynamic aspects of insulin release in vitro with evidence for a two-compartmental storage system. Acta Diabetol. Lat. 6 (Suppl. 1):554-79, 1969.

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

[6]Cerasi, E.: Potentiation of insulin release by glucose in man. I. Quantitative analysis of the enhancement of glucose-induced insulin secretion by pretreatment with glucose in normal subjects. Acta Endocrinol. (Kbh.) 79:483-501, 1975.

[7]Cahill, G.F., Jr., Herrera, M.S., Morgan, A.P., Soeldner, J.S., Steinke, J., Levy, P.L., Reichard, G.A., and Kipnis, D.M.:

Hormone-fuel interrelationships during fasting. J. Clin. Invest. *45*:1751-69, 1966.

[8]Malaisse, W.J., Malaisse-Lagae, F., and Wright, P.H.: Effect of fasting upon insulin secretion in the rat. Am. J. Physiol. *213*:843-48, 1967.

[9]Buchanan, K.D., Vance, J.E., and Williams, R.H.: Effect of starvation on insulin and glucagon release from isolated islets of Langerhans of the rat. Metabolism *18*:155-62, 1969.

[10]Grey, N.J., Goldring, S., and Kipnis, D.M.: The effect of fasting, diet and actinomycin D on insulin secretion in the rat. J. Clin. Invest. *49*:881-89, 1970.

[11]Voyles, N., Sutman, R.A., Selawry, H., Fink, S., Penhos, J.C., and Recant, L.: Interaction of various stimulators and inhibitors on insulin secretion in vitro. Hormone Res. *4*:65-73, 1973.

[12]Bosboom, R.S., Zweens, J., and Bouman, P.R.: Effect of feeding and fasting on the insulin secretory response to glucose and sulfonylureas in intact rats and isolated perfused rat pancreas. Diabetologia *9*:243-50, 1973.

[13]Hedeskov, C.J., and Capito, K.: The effect of starvation on insulin secretion and glucose metabolism in mouse pancreatic islets. Biochem. J. *140*:423-33, 1973.

[14]Fink, G., Gutman, R.A., Cresto, J.C., Selawry, H., Lavine, R., and Recant, L.: Glucose induced insulin release patterns: Effect of starvation. Diabetologia *10*:421-25, 1974.

[15]Hahn, H.J., and Fiedler, H.: Investigations on isolated islets in vitro. IX. Influence of food deprivation and glucose refeeding in vitro on insulin secretion. Acta Diabetol. Lat. *9*:457-67, 1974.

[16]Efendić, S., Cerasi, E., and Luft, R.: Effect of phentolamine and preperfusion with glucose on insulin release from the isolated perfused pancreas from fasted and fed rats. Diabetologia *11*:407-10, 1975.

[17]Loubatières, A., Mariani, M.M., Ribes, G., and de Malbosc, H.: Etude expérimentale d'un nouveau sulfamide hypoglycémiant particulièrement actif, le HB 419 ou glibenclamide. I. Action bêtacytotrope et insulinosécrétrice. Diabetologia *5*:1-10, 1969.

[18]Hales, C.N., and Randle, P.J.: Immunoassay of insulin with insulin-antibody precipitate. Biochem. J. *88*:137-46, 1963.

[19]Bouman, P.R., Konijnendijk, W., and Wolters, G.H.J.: Effect of fasting on insulin release, cyclic AMP and glucose oxidation in isolated islets of Langerhans. Diabetologia *11*:334, 1975 (abstract).

[20]Hedeskov, C.J., and Capito, K.: The restoring effect of caffeine on the decreased sensitivity of the insulin secretory mechanism in mouse pancreatic islets during starvation. Horm. Metab. Res. *7*:1-5, 1975.

[21]Cerasi, E., Luft, R., and Efendić, S.: Decreased sensitivity of the pancreatic beta cells to glucose in prediabetic and diabetic subjects. A glucose dose-response study. Diabetes *21*:224-34, 1972.

[22]Rabinovitch, A., Gutzeit, A., Grill, V., Kikuchi, M., Renold, A.E., and Cerasi, E.: Defective insulin secretion in the spiny mouse (Acomys cahirinus). Isr. J. Med. Sci. *11*:730-37, 1975.

[23]Cerasi, E.: Insulin secretion: mechanism of the stimulation by glucose. Q. Rev. Biophys. *8*:1-41, 1975.