

Glucose Insensitivity and Amino-acid Hypersensitivity of Insulin Release in Rats with Non-insulin-dependent Diabetes

A Study with the Perfused Pancreas

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SUMMARY

Non-insulin-dependent diabetes (NIDDM) was obtained in adult rats following a neonatal streptozotocin injection. Rats with NIDDM exhibited slightly lowered plasma insulin, slightly elevated basal plasma glucose values (<200 mg/dl), and low pancreatic insulin stores (50% of the controls). Insulin secretion was studied in this model using the isolated perfused pancreas technique.

Insulin response to glucose stimulation over the range 5.5–22 mM was lacking, thus indicating complete loss of B-cell sensitivity to glucose. Even in presence of theophylline, the B-cells remained insensitive to glucose. In contrast, glyceraldehyde elicited an insulin release as important as that obtained in the control pancreata. This could possibly suggest that the B-cell dysfunction in rats with NIDDM involves a block in glucose metabolism in the early steps of glycolysis prior to the triose-phosphate. Mannose stimulated insulin secretion less in the diabetics than in the controls. The insulin secretion obtained in response to isoproterenol indicated that the ability of the adenylylase to generate cAMP in the B-cells of the diabetics was not decreased. The insulinotropic actions of acetylcholine and tolbutamide were normal and increased, respectively, as compared with the controls.

In the absence of glucose, the B-cells of the diabetics were unexpectedly hypersensitive to arginine and leucine. The α -ketoisocaproate effect in the diabetics was not significantly different from that obtained in the controls. The possibility that enhancement of insulin response to leucine in the diabetics might be related to a more active conversion of leucine to ketoisocaproate along the first steps of intraislet leucine metabolism is proposed. *DIABETES* 32:445–451, May 1983.

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We have previously developed^{1,2} an experimental model of non-insulin-dependent diabetes (NIDDM) in the rat, which is obtained by a neonatal streptozotocin injection and is later on characterized by chronic and stable low insulin response to glucose and amino acids, slight but consistent elevation of basal plasma glucose values, and slightly impaired glucose tolerance.

Because the B-cell number^{3,4} and the insulin stores² in the pancreata of these diabetic rats were low, the defective insulin response observed *in vivo* could be attributed to these quantitative abnormalities of the islets. In fact, in a preliminary report,⁵ we observed in these diabetic rats a disparity between the effect of glucose and arginine on *in vitro* insulin release, as attested by a loss of the response to glucose contrasting with the partial preservation of the response to arginine. This suggested that in the diabetic pancreas, the alteration of the B-cell responsiveness might be variable according to the nature of the stimulus. The present study was designed to characterize the secretion of insulin in this model using the isolated perfused pancreas.

MATERIAL AND METHODS

Animals. Albino Wistar rats bred in the laboratory were fed *ad libitum* with commercial pelleted chow (no. 113, UAR, Villemoisson s/Orge, France). On the day of their birth, the rats received streptozotocin (100 μ g/g) in 25 μ l of citrate buffer (0.05 mol/L), pH 4.5, through the saphenous vein made directly accessible by transcutaneous puncture as previously described.¹ The litters were limited to eight. Four days after birth, all the neonates exhibited glycosuria reaching 3+ Clinistix values (Ames Co., Division Miles Lab, Paris, France). Spontaneous evolution of this neonatal diabetes led to a non-insulin-dependent diabetic (NIDDM) state in the adult which was stable and chronic as previously described.² In control litters, newborns received only citrate buffer. All the animals were weaned 21 days after birth.

TABLE 1
Characteristics of the diabetic and control male rats used in the study

	Body wt (g)	Plasma		Pancreas	
		Glucose (mg/dl)	Insulin (μ U/ml)	Weight (g)	Insulin (U/pancreas)
Controls	346 \pm 8 (43)	141 \pm 2 (43)	67 \pm 2 (42)	2.5 \pm 0.1 (42)	2.4 \pm 0.1 (42)
Diabetics	343 \pm 12 (34)	161 \pm 6* (34)	54 \pm 2* (33)	2.7 \pm 0.1 (34)	1.2 \pm 0.1* (34)

Values are means \pm SEM. The number of observations is shown in parentheses.
*P < 0.001 as compared with values in controls.

Perfusion technique. Nonfasted 3–5-mo-old male rats were used in all the experiments. The animals were anesthetized with pentobarbital (4 mg/100 g body wt, i.p.). Isolation and perfusion of the rat pancreas were performed by a modification⁶ of the technique described by Sussman et al.⁷ The pancreas was isolated with the proximal portion of the duodenum and separated from the spleen and stomach. The pancreas + duodenum block was placed in an incubator and perfused through the coeliac and mesenteric arteries via a cannula inserted into the aorta, at a constant 37°C temperature without recycling the medium. Flow rate was regularly measured and was usually 3 ml/min. The perfusate was a Krebs-Ringer bicarbonate buffer with the following components: NaCl, 118 mM; KCl, 4 mM; CaCl₂, 2.5 mM; MgSO₄, 1.2 mM; KH₂PO₄, 1.2 mM; NaHCO₃, 25 mM; fatty free bovine serum-albumin, 1.25 g/L (Sigma Co., St. Louis, Missouri); dextran T70, 40 g/L (Pharmacia, Uppsala, Sweden). In some experiments, the perfusate was supplemented with 5.5 mM glucose or with 5.5 mM glucose + 5 mM theophylline. The perfusate was continuously gassed (1 h before and during the whole experiments) with a mixture of O₂ + CO₂ (95:5); final pH 7.4. All the pancreata were first perfused with the selected perfusate for a 20-min equilibration period which followed the end of the surgery. When needed, L-arginine (Sigma), L-leucine (Sigma), α -keto-isocaproate (Sigma), D-glucose (Merck, Darmstadt, West Germany), D-mannose (Sigma), D-glyceraldehyde (Sigma), tolbutamide (Hoechst, Frankfurt, West Germany), isoproterenol (Sigma), and acetyl- β -methylcholine bromide (Sigma) were administered through a side-arm syringe at a flow rate of 0.3 ml/min. The complete

effluent was collected from the cannula in the portal vein at 1-min intervals in chilled tubes and frozen for storage at -20°C until assay.

The functional integrity of the pancreata was assessed by: (1) the constancy of perfusion pressure over the whole experiment time (60–80 mm Hg); (2) presence of duodenal peristaltic activity; (3) presence of a rebound insulin secretion following a bolus of 19 mM arginine at the end of the experiment. Pancreata that did not meet all these criteria were discarded. In some experiments, to compare the effects of some agents with glucose or leucine, 2 or 3 stimuli were given sequentially within a given perfusion. Because of the possibility that prior exposure of the pancreas to one agent could influence the subsequent response to another challenge, the order in which the agents were given was reversed in separate experiments. No significant difference could be detected concerning the amount of insulin release when the order of the stimuli was changed.

Samples. Blood was sampled from the tail vein just before laparotomy, and immediately centrifuged at 4°C; plasma was stored at -20°C until assayed. After the perfusion experiments, the pancreas was dissected, weighed, and homogenized by ultrasonic disintegration at 4°C (Sonifier Branson B12 Heat Systems, Ultrasonics, Plainview, New York) in an acid-alcohol solution (75% ethanol, 1.5% vol/vol, 12 mol/L HCl, 23.5% distilled water). After one night at -20°C, the extracts were centrifuged and the supernatants kept at -20°C until assayed.

Assays. Plasma glucose was determined using a glucose analyzer (Beckman Inc., Palo Alto, California). Plasma in-

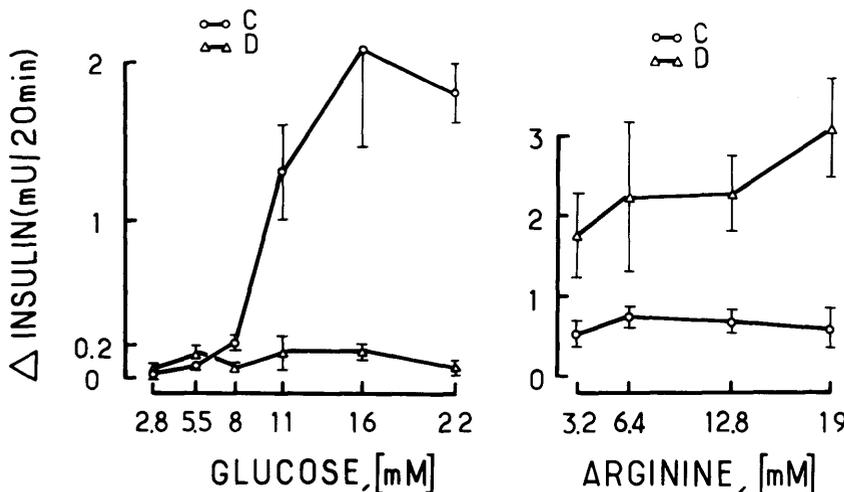


FIGURE 1. Left panel: Effect of glucose on insulin release from the perfused pancreas of control (O) and diabetic (Δ) rats. Each point represents the incremental insulin output above the prestimulation level, during the 20-min glucose infusion. These experiments were carried out in the absence of glucose during the prestimulatory period. Each point is the mean \pm SEM of 5–11 observations in each group. Right panel: Effect of arginine on insulin release from the perfused pancreas of control (O) and diabetic (Δ) rats. Each point represents the incremental insulin output above the prestimulation level, during the 20-min arginine infusion. These experiments were carried out in the absence of glucose during the prestimulatory period. Each point is the mean \pm SEM of 4–10 observations in each group.

munoreactive insulin (IRI) was estimated using purified rat insulin as a standard (R 171, Novo, Copenhagen, Denmark), antibody to a mixture of porcine + bovine insulin, and porcine monoiodinated ^{125}I -insulin.⁸ This method allows the determination of 6 $\mu\text{U/ml}$ (0.25 ng/ml) with a coefficient of variation within and between assays of 10%. Silicate was used to separate free from bound hormone.⁹ The pancreatic insulin was assayed with the same procedure.

Calculations. Insulin secretion rate per total pancreas was calculated by multiplying the insulin concentration in the samples by the flow rate and expressed as $\mu\text{U/min}$. In some experiments, insulin secretion rate was related to the pancreatic stores. In other experiments, total insulin response to glucose or arginine stimulation was obtained by planimetry of the individual perfusion profiles and expressed as the difference in hormonal secretion rate (Δ insulin: mU/20 min or $\mu\text{U/min}$) relative to the mean hormonal output recorded at the end of the prestimulation period (between min 0 and 10). All results were expressed as mean \pm SEM and statistical analysis was performed using Student's *t* test for unpaired data.

RESULTS

CHARACTERISTICS OF THE DIABETIC ANIMALS

Table 1 shows the basic characteristics of the diabetic and normal male rats used in this study. The body weight of the diabetics is not significantly different from that of the controls. Basal plasma glucose and plasma insulin measured in the fed state are slightly increased ($P < 0.001$) and slightly decreased ($P < 0.001$), respectively, in the diabetics as compared with the controls. The pancreatic insulin content of the diabetics is twice lower ($P < 0.001$) than that of the controls.

INSULIN RELEASE FROM THE ISOLATED PERFUSED PANCREAS OF DIABETIC RATS IN RESPONSE TO GLUCOSE, GLYCERALDEHYDE, AND MANNOSE

Effect of glucose. After a prestimulation period without glucose in the perfusate, exposure of the control pancreata to 2.8 mM or 5.5 mM glucose did not significantly change the

insulin release. When the control pancreata were challenged with 8 mM glucose or higher glucose concentrations, the amount of insulin released during the stimulation period was significantly increased (Figure 1) and the hormonal response displayed the well-known biphasic pattern (data not shown). The dose-response curve relating the insulin output to the glucose concentration was a typical sigmoidal curve (Figure 1).

In the diabetics, when perfusate did not contain glucose, the basal insulin release was not different from that measured in the controls, but these determinations were just at or below the detection limit of our insulin assay ($14 \pm 1 \mu\text{U/min}$, $N = 6$, and $13 \pm 3 \mu\text{U/min}$, $N = 6$, respectively). In contrast, exposure of the pancreata of the diabetics to glucose concentrations that were stimulatory in the controls, did not elicit any significant increase of the insulin output. This indicated clearly a marked defect in the diabetic rats concerning sensitivity to glucose of the B-cells.

In other experiments, the pancreata as soon as isolated, were perfused with 5.5 mM glucose. Under these conditions, the basal insulin release in the diabetics was not significantly different from that in the controls (Table 2), and during a 20-min exposure to high glucose (16 mM), the mean insulin release was not significantly modified in the diabetic group, whereas it was increased fivefold in the control group (Table 2).

In the next experiments, theophylline was used to test the possibility that prior exposure to this agent could reconstitute in the diabetics the insulin response to high glucose. In the control group, the insulin release was 15 times more elevated in response to perfusate with 5.5 mM glucose and 5 mM theophylline than in response to perfusate with 5.5 mM glucose only. In response to 16 mM glucose, the mean insulin output of the control pancreata was 2.6 times more elevated in the presence than in the absence of theophylline (Table 2 and Figure 2). In the same experimental conditions, with 5.5 mM theophylline, 16 mM glucose did not induce any significant increase of the insulin output in diabetic pancreata (Table 2 and Figure 2).

TABLE 2

Insulin secretory rates from the perfused diabetic rat pancreas. Basal release values were expressed as absolute values. Stimulated release values were calculated as the mean increase above release with 5.5 mM glucose only or with 5.5 mM glucose + 5 mM theophylline

	Addition	Insulin output ($\mu\text{U/min}$)	
		Controls	Diabetics
Basal release	5.5 mM glucose	42 ± 10 (9)	27 ± 9 (8)
Stimulated release (increase above basal release)	16 mM glucose	170 ± 40 (6)	4 ± 1 (8)*
	19 mM arginine + 5.5 mM glucose	981 ± 177 (9)	278 ± 38 (8)†
Basal release	5.5 mM glucose + 5 mM theophylline	609 ± 160 (5)	540 ± 199 (5)
Stimulated release (increase above basal release)	16 mM glucose + 5 mM theophylline	450 ± 85 (5)	22 ± 15 (5)*
	19 mM arginine + 5.5 mM glucose + 5 mM theophylline	1085 ± 153 (5)	551 ± 130 (5)‡

Values are means \pm SEM. The number of observations is shown in parentheses.

* $P < 0.001$, † $P < 0.01$, ‡ $P < 0.05$ as related to respective control groups.

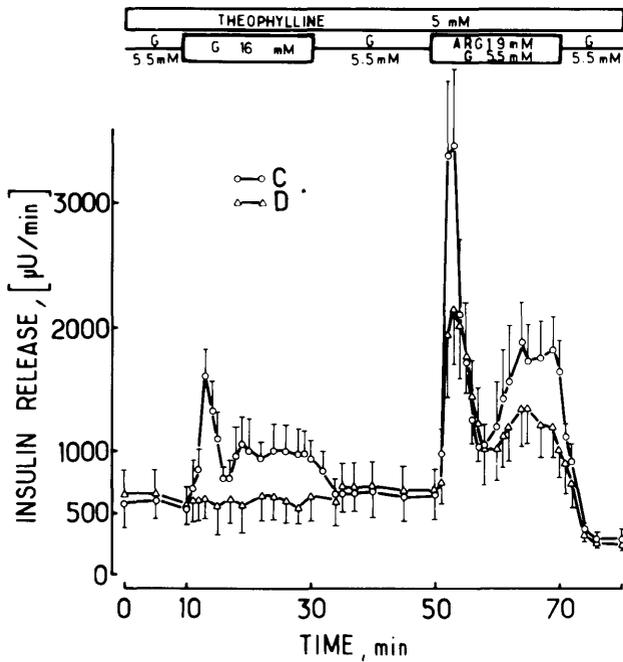


FIGURE 2. Effect of 16 mM glucose and of 19 mM arginine + 5.5 mM glucose on insulin release from the perfused pancreas of control (○) and diabetic (△) rats. These experiments were carried out in the presence of 5 mM theophylline in the perfusate. Glucose (5.5 mM) was present during the pre- and poststimulatory periods. Each point is the mean ± SEM of 5 observations in each group.

Effect of glyceraldehyde and mannose. The effect of 20-min infusion of 5 mM D-glyceraldehyde or 22 mM mannose on insulin release was studied in the absence of glucose. In the normal pancreata, the triose elicited a clearcut biphasic insulin output quantitatively similar to that elicited by a previous 20-min exposure to 22 mM glucose (Figure 3 and Table 3). In the diabetic pancreata, D-glyceraldehyde increased insulin output to an extent similar to that of the control response, this feature contrasting sharply with the lack of insulin response by the same pancreata during their exposure

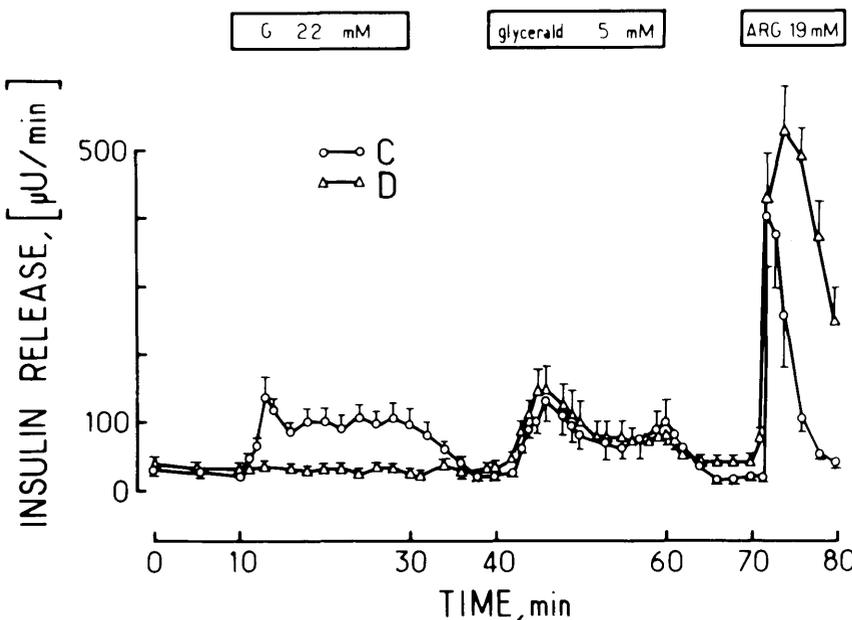


FIGURE 3. Effect of 22 mM glucose, 5 mM glyceraldehyde, and 19 mM arginine on insulin release from the perfused pancreas of control (○) and diabetic (△) rats. These experiments were carried out in the absence of glucose during the pre- and poststimulatory periods. Each point is the mean ± SEM of 4–6 observations in each group.

to 22 mM glucose (Figure 3). Mannose significantly increased the insulin secretion both in the normal and the diabetic preparations (Table 3). Nevertheless, the increase of insulin release above basal level was significantly lower ($P < 0.05$) in the diabetics than in the controls (Table 3).

INSULIN RELEASE FROM THE ISOLATED PERFUSED PANCREAS OF DIABETIC RATS IN RESPONSE TO ARGININE, LEUCINE, OR α -KETOISOCAPROATE

Effect of arginine. In the absence of glucose, 3.2 mM arginine caused a slight but significant insulin release from normal pancreata. This increased secretion over the basal release was not modified when arginine concentrations were enhanced (Figure 1). Quite unexpectedly, insulin responses in the diabetic group were always significantly higher than those obtained in the controls, whatever the arginine concentration (Figure 1), thus indicating higher sensitivity of diabetic B-cell to arginine under glucose deprivation.

The insulin response to 19 mM arginine was also tested when perfusate contained 5.5 mM glucose. The mean increase of the insulin output by the diabetic pancreata was only 28% ($P < 0.01$) of that measured in the controls (Table 2), when expressed as absolute output, and was 57% ($P < 0.01$) of that measured in the controls when the release was related to the pancreatic insulin stores which were decreased in the diabetic rats (Table 1). Moreover, it may be observed that with a background of 5.5 mM glucose, the control pancreata when stimulated with 19 mM arginine, released 33 times more insulin ($981 \pm 177 \mu\text{U}/\text{min}$, $N = 9$) than in experiments performed without glucose in the basal perfusate ($30 \pm 11 \mu\text{U}/\text{min}$, $N = 7$). On the other hand, the increase of the insulin output by the diabetic pancreata when stimulated with arginine + glucose ($278 \pm 38 \mu\text{U}/\text{min}$, $N = 8$) was only 1.8 times more elevated than in experiments performed without glucose in the basal perfusate ($152 \pm 33 \mu\text{U}/\text{min}$, $N = 6$).

With a background of 5 mM theophylline + 5.5 mM glucose, no significant increment of the mean insulin response of the control group to 19 mM arginine could be detected

TABLE 3

Insulin secretory rates from the perfused diabetic rat pancreas in response to 22 mM glucose, 22 mM mannose, or 5 mM glyceraldehyde. Basal perfusate contained no glucose. Insulin output was calculated as the mean increase above the basal release during a period of 20 min

	Increase of insulin output ($\mu\text{U}/\text{min}$)		
	22 mM Glucose	22 mM Mannose	5 mM Glyceraldehyde
Controls	89 \pm 10 (7)	86 \pm 21 (6)	70 \pm 24 (4)
Diabetics	2 \pm 1* (6)	31 \pm 6† (5)	55 \pm 18 (6)

*P < 0.001, †P < 0.05 as compared with value in controls.

as compared with experiments without theophylline (Table 2). The same experiments performed in the diabetic group showed that the mean insulin response was amplified when theophylline was present in the perfusate but the difference was not significant (Table 2). When the insulin responses were related to the respective pancreatic insulin stores, there no longer existed any significant difference between the diabetic and control groups (data not shown). It may be stressed that the insulin response of the diabetic pancreata to arginine was always typically biphasic, whatever the experimental conditions used (data not shown).

Effect of leucine and α -ketoisocaproate. In the absence of glucose, 10 mM leucine caused a very slight increase of insulin above basal level in the controls (5.3 \pm 1.7 $\mu\text{U}/\text{min}$, N = 7) (Figure 4). In contrast, in the diabetic group insulin response to the same leucine concentration was greatly enhanced with a mean insulin release above basal of 76 \pm 20 $\mu\text{U}/\text{min}$ (N = 5) (Figure 4).

The α -ketoisocaproate (10 mM), infused in the same experiments, induced a stimulation of insulin release clearly biphasic, similar in the two groups (318 \pm 88 $\mu\text{U}/\text{min}$, N = 8, in the control group, and 402 \pm 76, N = 5, in the diabetic group), and much greater than that induced by leucine 10 mM (Figure 4). In the same pancreata (Figure 4), 19 mM arginine elicited an enhanced insulin response in the diabetics as previously mentioned.

INSULIN RELEASE FROM THE ISOLATED PERFUSED PANCREAS OF DIABETIC RATS IN RESPONSE TO TOLBUTAMIDE, ISOPROTERENOL, AND ACETYLCHOLINE
A 20-min exposure to 0.4 mM tolbutamide, in the absence of glucose, determined a slight but significant monophasic insulin response in the control group (Figure 5). The response of the diabetic pancreata was biphasic and the 20-min insulin release was significantly greater than that obtained with the controls (49 \pm 14 $\mu\text{U}/\text{min}$, N = 4 as compared with 6.0 \pm 0.5 $\mu\text{U}/\text{min}$, N = 4).

Without glucose in the basal perfusate, isoproterenol 4 \times 10⁻⁵M did not stimulate insulin release of the control pancreata. When 2.8 mM glucose was added to the perfusate, a very slight insulin response to isoproterenol was obtained in the control group (3.0 \pm 0.3 $\mu\text{U}/\text{min}$, N = 5). The response of the diabetic pancreata was significantly 2.7 times more elevated (8.0 \pm 2.7 $\mu\text{U}/\text{min}$, N = 5) as compared with that of the controls (Figure 5). When the pancreata were challenged with acetylcholine 0.1 mM in the absence of glucose, the insulin response of the diabetics was higher (165 \pm 50 $\mu\text{U}/\text{min}$, N = 4) than that measured in the controls

(86 \pm 16 $\mu\text{U}/\text{min}$, N = 4), but the difference was not significant (Figure 5).

DISCUSSION

The most striking result of the study was the extreme glucose insensitivity of the B-cells in this diabetic model, contrasting sharply with normal or occasionally higher responses to other stimuli such as amino acids, isoproterenol, acetylcholine, or tolbutamide. To further elucidate whether this selective insensitivity applied to intact glucose molecule or also to glucose metabolite, glyceraldehyde, which stimulates insulin release in the normal rat pancreas,¹⁰ was therefore tested. Our results clearly indicated that the triose elicited an insulin release by the diabetic pancreata as important as that obtained in the control pancreata. Thus, the present finding could possibly suggest that the B-cell dysfunction in rats with this type of NIDDM involves an alteration of the glycolytic pathway prior to the triose phosphate. This is a new example of an altered insulin response, supporting the concept that the metabolism of glucose within the B-cell is fundamentally

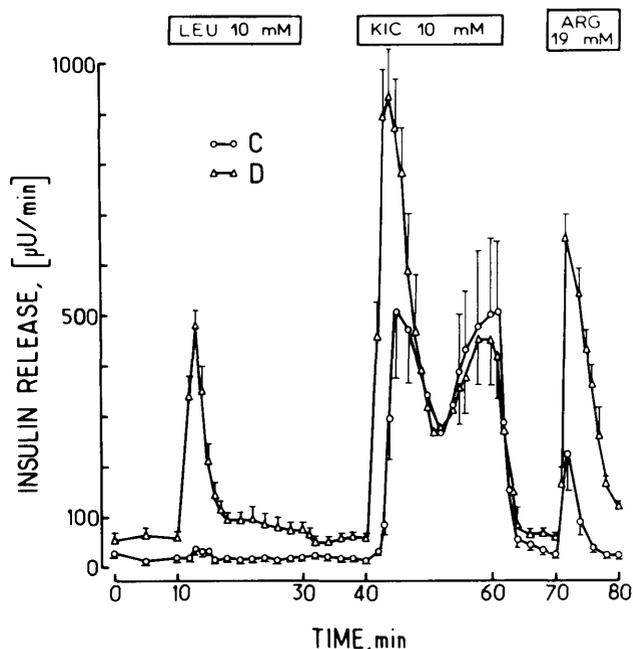


FIGURE 4. Effect of 10 mM leucine, 10 mM α -ketoisocaproate (KIC), and 19 mM arginine on insulin release from the perfused pancreas of control (○) and diabetic (△) rats. These experiments were carried out in the absence of glucose during the pre- and poststimulatory periods. Each point is the mean \pm SEM of 5–8 observations in each group.

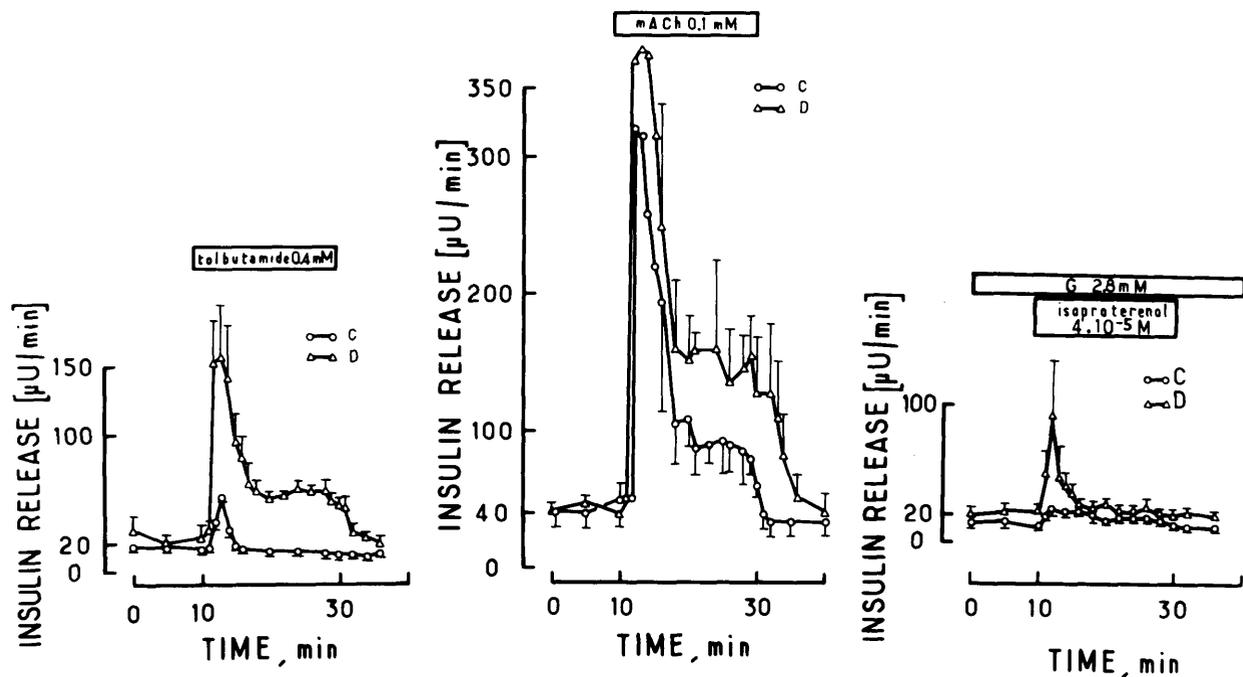


FIGURE 5. Effect of 0.4 mM tolbutamide, 0.1 mM methylacetylcholine (mACh), and 4×10^{-5} M isoproterenol on insulin release from the perfused pancreas of control (○) and diabetic (△) rats. The experiments with tolbutamide and acetylcholine were carried out in the absence of glucose, and those with isoproterenol were carried out in the presence of 2.8 mM glucose. Each point is the mean \pm SEM of 4–7 observations in each group.

involved in the normal process of glucose recognition and the hormonal response it induces.¹¹ Experiments with 22 mM mannose indicated that this hexose possesses the ability to increase significantly insulin release by the diabetic pancreata. Nevertheless, the response was significantly lower than that obtained in the control pancreata. As far as mannose has been reported to be metabolized by islet tissues and is presumably acted on by the same enzyme systems that act on glucose,¹² the lower efficiency of mannose was also compatible with a defect of the hexose metabolism in the B-cells of the diabetic rats. However, in the framework of the metabolic theory of stimulated insulin secretion, the discrepancy between the respective effects of mannose and glucose on insulin release in the diabetic rats remains at present unexplained.

Because in some diabetic rodents (*Acomys*¹³ and hamsters) infected with Venezuelan encephalitis virus,¹⁴ it has been shown that alteration in insulin response to glucose was associated with defective glucose-induced cAMP production in the B-cells, we examined insulin secretion in our diabetic model in the presence of theophylline. Our aim in this experiment was to identify a possible insulin release in response to glucose under conditions where cAMP concentrations were raised inside the B-cells. It was noticeable that even in presence of theophylline the B-cells of the diabetics remained completely insensitive to glucose. This observation did not enable us to fully eliminate the assumption that glucose is no longer able to increase cAMP level in the B-cells of the diabetic rats, but it was consistent with the existence of another B-cell defect that could contribute to the abnormality of the insulin response to glucose. Besides, our results indicated that theophylline in presence of a nonstimulating glucose concentration, elicited similar insulin response in the normal and diabetic rats. The B-cell response to theophylline

was also found to be normal in the diabetic Chinese hamster, a rodent with spontaneous diabetes.¹⁵ Moreover, the responses obtained with the β -agonist isoproterenol clearly indicated that the ability of the adenylcyclase to generate cAMP in the B-cells of the diabetics was normal, if not increased.

Results related to the response to amino acids, arginine and leucine, in the absence of glucose, indicated that in control rats, the increase of insulin release was low, as previously shown.^{16,17} Unexpectedly, in the diabetics, the B-cells were hypersensitive to arginine and probably also to leucine. Concomitantly, α -ketoisocaproate, which is the first catabolic product of leucine, induced in these diabetic B-cells a striking stimulation of insulin release. This effect of α -ketoisocaproate was not significantly different from that obtained in the controls. Thus, in the diabetic rats, because insulin response to ketoisocaproate was normal while response to leucine was greater as compared with that of controls, it would be conceivable that leucine was more actively converted to ketoisocaproate along the first steps of the intraslet pathway of leucine metabolism. Nevertheless, data concerning the insulin response to arginine in presence of glucose indicated that glucose has lost its ability to potentiate B-cell response to arginine in the diabetic rats.

Since Weir et al.¹⁸ recently studied the secretion of insulin in an experimental NIDDM¹⁹ induced by neonatal streptozotocin injection like our own model,¹ it was interesting to compare the data. In the Weir experiments, a very low increase of insulin secretion by the diabetic pancreata was only obtained in response to glucose concentration very far (7.5 g/L) from the physiologic range and in the presence of 5 mM theophylline. Insulin response to tolbutamide was also absent. The discrepancy between this model and ours concerning the response to tolbutamide was probably related

to the severity of the diabetes: the animals used in the Weir experiments exhibited a frank basal hyperglycemia (from 2 to 3.5 g/L) and their pancreatic insulin stores were three times lower than in the controls,¹⁹ this indicating a more severe diabetes than in our model.

In summary, the present in vitro data were in agreement with our previous experiments indicating a low insulin response to glucose in vivo.^{1,2} This impaired insulin release that we had first detected in vivo could not be entirely attributed to decreased B-cell number, and the in vitro experiments indicated that it was also related to altered B-cell responsiveness varying according to the nature of the stimulus. At first, a selective B-cell insensitivity to glucose has been demonstrated in this experimental model. Such a defect was similar to that known to be present in human non-insulin-dependent diabetes and in various animal models of diabetes. In the case of our own model, we suggest that this insensitivity is associated with a block in B-cell glucose metabolism prior to the triose-phosphate level. The second striking observation was the paradoxical hypersensitivity of the B-cells to arginine or leucine in the absence of glucose. Though the significance of this hypersensitivity to amino acids might be interpreted from a teleologic point of view as a means of increasing insulin secretion in the diabetic pancreata, the mechanism of this unexpected data remains unknown.

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