Abnormalities of Insulin Responses After Ambient and Previous Exposure to Glucose in Streptozocin-diabetic and Dexamethasone-treated Rats

Role of Hyperglycemia and Increased B-Cell Demands

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SUMMARY

In NIDDM, B-cells are insensitive to glucose. We studied the specificity and evolution of this abnormality in 6–10-wk-old neonatally streptozocin-diabetic (STZ) and in dexamethasone-treated (DMT) rats. Not only the effect of ambient but also that of previous glucose (priming effect) was characterized in the perfused pancreas. In fed STZ, blood glucose was elevated to 9.2 ± 0.8 versus 5.3 ± 0.2 mM in control (C) rats. Ambient glucose (27 mM) in the perfusate induced a significant but reduced total response (11% of C) that was predominantly monophasic. Secretion was promptly induced (in <20 s) both in STZ and C. Other nutrients, i.e., glyceraldehyde (10 mM) and α-ketoisocaproic acid (KIC) (5 mM) also induced reduced and monophasic responses, whereas, in contrast, 3-isobutyl-1-methylxanthine (IBMX) induced an enhanced response that was 3.8-fold larger than in C. In DMT, blood glucose was normal (5.4 ± 0.3 mM). Ambient glucose (27 mM) in the perfusate induced a normal first phase and a moderately reduced second phase (52% of untreated rats). DMT rats were hyperresponsive to IBMX, this agent inducing 2.5-fold higher release than in untreated rats.

Previous perfusion with 27 mM glucose enhanced twofold the effect of a second stimulation period with glucose in C. This induction of priming by glucose could not be demonstrated in fed STZ or in DMT. However, when STZ were fasted or insulin treated for 36 h, induction of priming reappeared, i.e., the second pulse of glucose evoked 2–3-fold more insulin release than the first pulse.

We conclude that partial B-cell insensitivity to glucose in STZ alters time dynamics of the insulin response, includes loss of a priming effect, is influenced by hyperglycemia, and is nutrient but not glucose specific. Results in DMT indicate that increased demands on B-cell secretion can lead to hyperresponsiveness to nonmetabolic secretagogues as well as loss of induction of priming by glucose without concomitant loss of sensitivity to ambient glucose. DIABETES 1986; 35:44–51.

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Non-insulin-dependent diabetes mellitus (NIDDM) is associated with abnormalities of insulin secretion that are not readily explainable from a decrease in B-cell number in the endocrine pancreas. Glucose-induced insulin secretion is thus impaired in human NIDDM[1,2] and abolished in animal models of NIDDM.[3–5] Other secretagogues, such as arginine or isoproterenol, may exert "normal" responses in human NIDDM.[6–8] However, abnormal responses are revealed when these secretagogues are tested in animal models of NIDDM in the absence of glucose or together with low concentrations of the hexose. In these situations, the non-glucose secretagogues thus evoke exaggerated insulin responses.[3,4] Thus, two abnormalities in insulin secretion can be defined, at least in animal models of NIDDM: first, insensitivity to glucose and second, hyperresponsiveness to certain other secretagogues.

Several questions regarding these two abnormalities remain unanswered. First, it is not known whether time dynamics of the glucose-induced insulin response are altered when partial insensitivity to glucose exists. Second, the possible influence of diabetes on a priming effect of glucose previously documented to operate under normal physiologic conditions[9–11] has not been thoroughly tested. Third, the degree of specificity of the insensitivity as well as of the hyperresponsiveness of the diabetic B-cell is incompletely clarified. Fourth, it is not known at which stages of glucose intolerance that these two abnormalities are present. The present study aimed to address these questions. To this end, we have measured B-cell secretion in response to nutrient and nonnutrient secretagogues during varying degrees of demands on the B-cell and at varying levels of glycemic decompensation. These variations were achieved by using rats in which diabetes had evolved as a consequence of

streptozocin injection during neonatal age and in which hyperglycemia could then be reduced by fasting or insulin treatment. Additionally, a situation of increased B-cell demands with normoglycemia upheld was studied in nondiabetic rats that had been treated with dexamethasone.

MATERIALS AND METHODS

Animals. Lactating Sprague-Dawley rats with their offspring were obtained from Anticimex, Stockholm, Sweden, on the day after delivery. On the same day, male and female pups were injected with 90 mg/kg of streptozocin (STZ, kindly supplied by Dr. William E. Dulin, Upjohn Company, Kalamazoo, Michigan). STZ was dissolved immediately before intraperitoneal (i.p.) injection in citrate buffer (0.05 mol/L, pH 4.5) to yield a concentration of 9 mg/ml. Litter sizes were between 10 and 15 animals. Pups from control litters received citrate buffer alone. Two days after injections, a specimen of whole blood was obtained by skin incision for the determination of blood glucose by a glucose-oxidase method using reagent strips (Reflotest) read for absorbance in a reflectance meter (Reflomat, Boehringer-Mannheim, FRG). Animals were included in the study if their blood glucose was ≥10 mmol/L. Control litters were normalized to the size of the litters containing diabetic animals. Animals were weaned between 21 and 28 days of age, after which they were fed a standard pelleted diet (Anticimex, Stockholm, Sweden). A 12-h dark period from 6 p.m. to 6 a.m. was always strictly enforced.

Experimental protocols. STZ-treated and control animals of both sexes were used for experiments between 6 and 10 wk of age. All series of experiments included animals from more than one litter to minimize the influence of litter-dependent variation on the results. Some animals received pretreatment before the actual experiments. The pretreatment protocols used were fasting for 36 h or insulin therapy or dexamethasone treatment. Weights of the animals decreased during fasting, increased during insulin therapy, and were unchanged during dexamethasone treatment (data not shown). During insulin therapy, 4–8 IU of heat-treated Ultralente insulin (Novo Research Company, Bagsvaerd, Denmark) was administered subcutaneously (s.c.) in two daily injections. The first injection was given 48 h before the experiment and the last injection at 4 p.m. on the day preceding the experiment. Dexamethasone treatment consisted of 25 μg of dexamethasone-21-phosphate administered daily, s.c., in 0.1 ml of saline. The first injection was given 4 days before the experiment and the last injection at 4 p.m. on the day preceding the experiment.

Perfused pancreas preparation. At 10 a.m. on the day the animals were killed, a blood glucose determination was obtained by skin incision. The animals were then anesthetized by the i.p. injection of 100 mg/kg body wt of pentobarbital. The pancreas was isolated free from adjacent organs as described.[12] The pancreas was perfused through the abdominal aorta with a Krebs-Henseleit-bicarbonate buffer[13] containing 20 g/L of bovine albumin and, when not otherwise indicated, 3.9 mmol/L of glucose. A 30-min period (not recorded in the figures or tables) of perfusion with this medium was allowed before the administration of substances to be tested. D-Glyceraldehyde, α-ketoisocaproic acid, and 3-isobutyl-1-methylxanthine (IBMX) were obtained from Sigma Co. (St. Louis, Missouri). These substances were dissolved in buffer medium immediately before use. Flow rates were between 2.5 and 3.0 ml/min and were stable throughout the experiments. Samples were collected at times indicated in the figures into prechilled tubes, then frozen and stored at −20°C.

Extraction of insulin. On completion of the perfusion protocol, the pancreata were rapidly frozen. They were then kept at −70°C until extraction of insulin was performed as previously described.[14]

Assays. Insulin was measured radioimmunologically using charcoal addition to separate free and bound antibody.[15] The sensitivity of this assay was 8 mU/L and the intraassay coefficient of variation was ±10%.

Presentation of results. Results are expressed as mean ± SEM. Tests of significance were carried out using Student's *t*-test for paired or unpaired differences as evident or as indicated in text and tables. Multiple tests of significance between series of observations were avoided to uphold the reliability of P-values.

RESULTS

Blood glucose, weight, and pancreatic content of insulin in STZ-injected rats. Rats injected with STZ at neonatal age exhibited moderate but significant hyperglycemia between 6 and 10 wk of age (Table 1, compare lines 1 and 5). The state of diabetes did not affect growth, since body weight was similar to control animals of the same age. Mean pancreatic insulin content was reduced to one-third of that of control animals.

TABLE 1
Blood glucose, weight, and pancreatic insulin content of animals under study (number of observations is in parentheses)

Rats	Weight (g)	Blood glucose (mM)	Pancreatic insulin (mU/pancreas)
STZ-diabetic			
Fed, untreated	216 ± 7 (41)	9.2 ± 0.8† (36)	589 ± 108‡ (15)
Fasted, untreated	172 ± 9 (6)	3.5 ± 0.4* (6)	777 ± 152 (5)
Fed, insulin treated	206 ± 6 (3)	3.5 ± 0.9* (3)	875 ± 151 (3)
Fed, dexamethasone treated	162 ± 12 (5)	18.3 ± 1.2§ (4)	168 ± 39§ (4)
Control			
Fed, untreated	226 ± 10 (30)	5.3 ± 0.2 (21)	1800 ± 149 (19)
Fed, dexamethasone treated	193 ± 6 (14)	5.4 ± 0.3 (14)	1047 ± 104 (8)

*$P < 0.05$, †$P < 0.005$, and ‡$P < 0.001$, significance of difference versus fed control.
§$P < 0.01$, significance of difference with or without dexamethasone treatment.

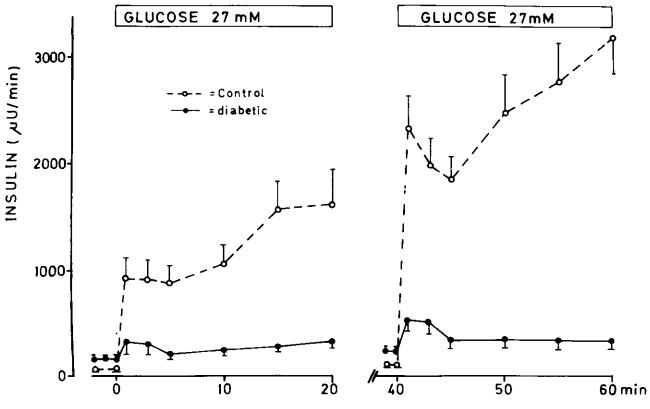

FIGURE 1. Glucose-induced insulin secretion from pancreata of STZ-diabetic and nondiabetic rats. Mean ± SEM of eight experiments in each series.

"Basal" rates of insulin secretion. Insulin secretion was elevated from pancreata of fed STZ-diabetic animals during perfusion with 3.9 mM glucose. Secretion rates from these animals (after the usual 30 min of preperfusion with the same medium) were thus 153 ± 26, N = 32 versus 57 ± 9, n = 28 μU/ml for corresponding control animals, $P < 0.005$.

Insulin responses to glucose. Exposure to 27 mM glucose for 20 min induced a significant ($P < 0.01$) but markedly diminished insulin response in diabetic animals relative to that obtained in pancreata from nondiabetic animals (Figure 1 and Table 2). The response was less diminished early rather than later during stimulation. Early insulin secretion (min 0–5, designated first phase) was thus decreased to 21%, whereas subsequent secretion (min 5–20, designated second phase) was decreased to 9% of release from nondiabetic animals.

In the nondiabetic animals, a second stimulation period with 27 mM glucose elicited twofold more insulin release than the first stimulation period (Figure 1 and Table 2). This priming effect of glucose was not observed in pancreata from the diabetic animals, in which the total response during the second stimulation with glucose was not significantly different from that obtained during the initial stimulation. Early insulin release was also less impaired than later release during the second stimulation with 27 mM glucose. First phase thus constituted 15% and second phase only 5% of the corresponding response in control pancreata.

Since glucose-induced insulin release is reportedly delayed in human diabetes,[1,2] the early time course of glucose-induced insulin secretion was examined in further detail. As shown in Figure 2, no evidence could be found for any delay in the insulin response to glucose in the diabetic pancreata. Thus, both onset (within 20 s after the rise in glucose concentration) and peak attainment (within 60–80 s) of response were similar to observations in control pancreata.

Effects of fasting and insulin therapy. Blood glucose was lowered in the diabetic animals to investigate the importance of hyperglycemia and/or decreased demands on B-cell secretion response to glucose. This was achieved either by 36 h of fasting or by insulin therapy. The fasting period lowered blood glucose of diabetic rats to levels below those of fed, nondiabetic rats (Table 1). "Basal" insulin release was reduced below limits of detection both from diabetic and nondiabetic pancreata (Figure 3). As expected from previous investigations (reviewed in ref. 16), the stimulatory effect of 27 mM glucose was much reduced in the fasted, nondiabetic rats compared with the response in the fed state (Figure 3 and Table 2). Such reduction by fasting was much less apparent in the diabetic state and, consequently, the response in the diabetic pancreas was increased relative to the nondiabetic, fasted control: first phase to 70% and second phase to 34% of control.

In the nondiabetic, fasted animals a second administration of 27 mM glucose induced a 2.3-fold higher response than the initial challenge (Figure 3 and Table 2). This priming effect was equivalent in percentage increase to the effect seen in the fed state. In diabetic animals, a priming effect was ob-

TABLE 2
Glucose-induced insulin release

	Insulin (μU/min)					
	First glucose challenge (min 0–20)			Second glucose challenge (min 40–60)		
Conditions	0–5 min	5–20 min	0–20 min	40–45 min	45–60 min	40–60 min
Fed rats						
Nondiabetic	3439 ± 685	20,769 ± 3699	24,208 ± 4350	7783 ± 1253	40,249 ± 4283	48,031 ± 5236*
STZ-diabetic	712 ± 270	1859 ± 523	2572 ± 780	1186 ± 384	2019 ± 1187	3205 ± 1532
Fasted rats						
Nondiabetic	526 ± 119	3948 ± 1613	4474 ± 1662	2256 ± 801	9099 ± 4380	11,356 ± 5153*
STZ-diabetic	369 ± 79	1351 ± 162	1720 ± 234	1346 ± 323	2761 ± 635	4107 ± 914*
Insulin-treated rats						
STZ-diabetic	288 ± 287	3895 ± 1980	4195 ± 2282	2883 ± 1171	8328 ± 3591	11,217 ± 4691*
Dexamethasone-treated rats						
Nondiabetic	3617 ± 889	10,752 ± 2982	14,365 ± 3652	4150 ± 1058	14,241 ± 4008	18,391 ± 5049
STZ-diabetic	0 ± 0	0 ± 0	0 ± 0	99 ± 60	280 ± 183	379 ± 242

Results are derived from Figures 1 and 3–5 and are expressed as mean ± SEM of incremental responses to each of two administrations of 27 mM glucose.
*$P < 0.05$ or less, significance of paired differences, first versus second glucose challenge.

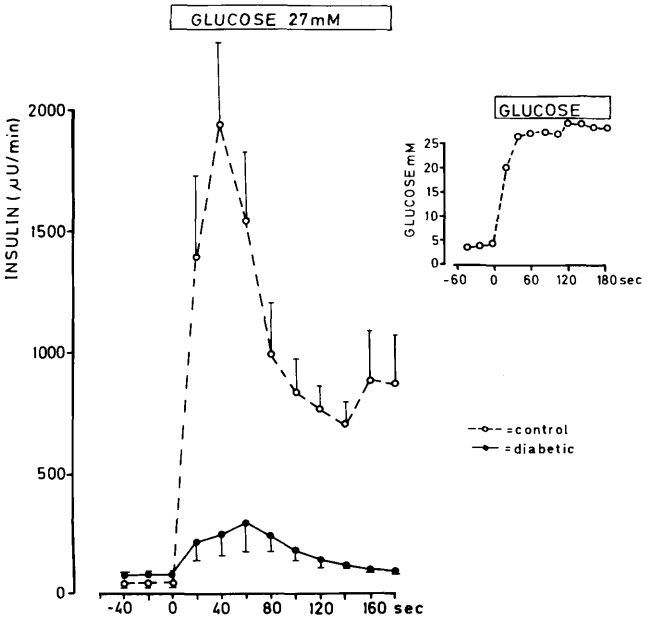

FIGURE 2. Early time course of glucose-induced insulin secretion from pancreata of STZ-diabetic and nondiabetic rats. Insert depicts mean perfusate concentrations of glucose. Mean ± SEM of six experiments.

served in the fasted state, where it amounted to a 2.4-fold enhancement of glucose-induced insulin release; however, the effect was absent in the fed state as already noted (Figure 1 and Table 2).

The effects of insulin treatment were studied in the diabetic animals only. Treatment reduced blood glucose in the diabetic rats to levels below those of fed, nondiabetic rats (Table 1). "Basal" insulin release from pancreata of the insulin-treated rats was reduced below the detection limit. The insulin response during initial stimulation with 27 mM glucose was predominantly second phase (Figure 4 and Table 2). A priming effect of previous glucose was seen on restimulation; this effect was similar (2.3-fold) to that observed after fasting in the diabetic animals.

Effects of dexamethasone treatment on glucose-induced insulin release. Glucocorticoid therapy is known to induce insulin resistance. The effect of increased demands on B-cell secretion thereby created were studied in nondiabetic and diabetic rats. Nondiabetic, dexamethasone-treated rats had levels of blood glucose before experiments that were similar to those of untreated rats (Table 1, compare lines 6 and 5). However, in diabetic animals, dexamethasone treatment further elevated preexisting hyperglycemia (Table 1, compare lines 4 and 1). In nondiabetic animals, dexamethasone treatment enhanced "basal" insulin secretion rates to 158 ± 35 μU/min compared with 57 ± 9 μU/min in the untreated state ($P < 0.001$; Figure 5). Such an effect by treatment was not seen in the diabetic animals.

The early insulin response to glucose was similar in pancreata from nondiabetic, dexamethasone-treated and untreated control rats (Figure 5 and Table 2). However, glucocorticoid treatment decreased only second phase insulin release, which was 52% of that in untreated rats. Previous exposure to glucose failed to induce a priming effect, i.e., a second challenge with 27 mM glucose induced a response

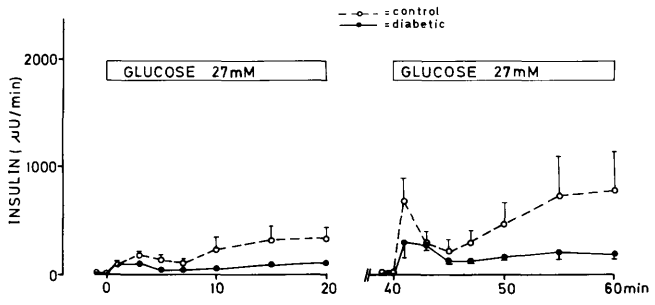

FIGURE 3. Effects of 36 h of fasting on glucose-induced insulin secretion from pancreata of STZ-diabetic and nondiabetic rats. Mean ± SEM of five and six experiments, respectively.

that was similar in magnitude to that of the initial stimulation (Figure 5 and Table 2).

In dexamethasone-treated, diabetic rats, an insulin response to glucose was abolished. Also, previous exposure to glucose failed to induce a significant priming effect of glucose (Figure 5).

Specificity of the insensitivity to glucose: effects of glyceraldehyde, KIC, and IBMX. The ability of nutrient and nonnutrient secretagogues to induce insulin release was compared in pancreata from fed control and diabetic rats. D-glyceraldehyde induced a diphasic insulin response in control but only a monophasic and reduced response in diabetic animals (Figure 6 and Table 3). First-phase response from the diabetic pancreata was thus reduced to 22% and second phase to 3% of that found in control pancreata. Similar patterns of response were found with KIC; a diphasic response in nondiabetic pancreata was reduced essentially to a monophasic response in the diabetic pancreata (Figure 7 and Table 3). First phase was thus reduced to 29% and second phase to 12% of that seen in nondiabetic pancreata (Figure 7 and Table 3).

In contrast to the reduced responses obtained with glyceraldehyde and KIC, the administration of the nonmetabolizable phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine (IBMX), induced an impressive insulin response from diabetic pancreata (Figure 8 and Table 3). The response consisted of a marked first-phase response followed by a declining second phase. The total response was 3.8-fold larger than that observed in control pancreata.

To determine whether a hyperresponse to IBMX could be also induced by increased demands on B-cell secretion in the absence of overt hyperglycemia, we tested the effects of IBMX in pancreata from nondiabetic rats that had been treated with dexamethasone for 4 days. This treatment en-

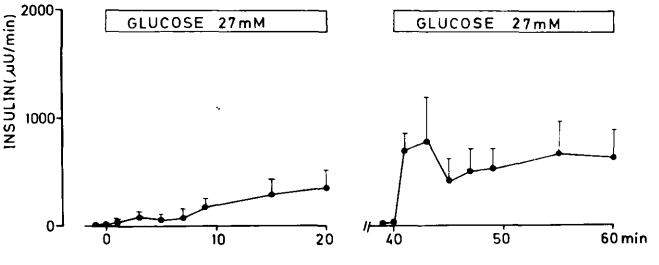

FIGURE 4. Effects of insulin therapy on glucose-induced insulin secretion in STZ-diabetic rats. Mean ± SEM of three experiments.

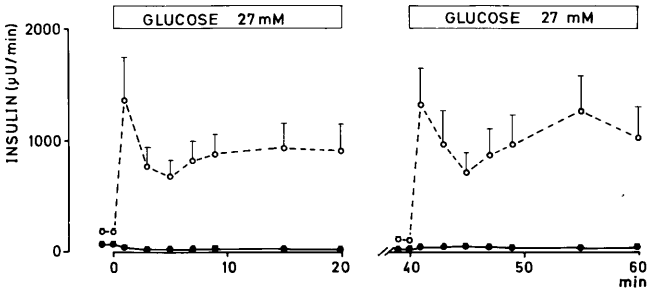

FIGURE 5. Effects of dexamethasone treatment on glucose-induced insulin secretion from STZ-diabetic (●——●) and nondiabetic rats (○-------○). Mean ± SEM of five and six experiments, respectively.

hanced the response to IBMX 2.5-fold relative to the response from pancreata of untreated animals (Figure 8 and Table 3).

DISCUSSION

The present study, as well as previous reports,[3,4] demonstrates B-cell insensitivity to ambient glucose in perfused pancreata from rats that have been rendered mildly diabetic after STZ injection during neonatal age. In contrast to others,[3,4] we observe that glucose induces definite release from our diabetic pancreata; however, insensitivity was present as judged, for example, by the fact that the response was reduced out of proportion to the decrease in pancreatic insulin content of the diabetic animals. The reason that we, but not others, obtain some response is not immediately apparent, since blood glucose levels and pancreatic insulin content at the time animals were killed were similar between the present and previous[3,4,17] studies. Our results indicate, however, that a partial response to glucose is coupled to a mild degree of diabetes, since the response was completely lost when hyperglycemia in the diabetic rats was augmented by previous glucocorticoid treatment.

Our results indicate that B-cell insensitivity is specific for nutrient secretagogues but not for glucose or for glycolytic intermediates. Hence, total insulin responses were severely reduced to a similar extent in response to glucose, D-glyceraldehyde, and to the metabolite of leucine, KIC. Temporal profiles of release were also altered in a similar fashion by diabetes. As with glucose, second-phase insulin release was thus reduced more than first phase during the stimulation with all three nutrients. Our data are at variance with those of others,[4] who have reported that responses to glyceraldehyde and KIC are not diminished in pancreata from STZ-diabetic animals. The cause of the discrepancy is not clear. It may be related to differences in experimental protocol and the fact that we used younger animals than in the previous study. Furthermore, it should be noted that the mean pancreatic insulin content was somewhat higher in the diabetic pancreata of the previous than of the present study (50% versus 33% of control). Although this observation may reflect a moderate difference in residual B-cells, such difference seems insufficient to explain the widely divergent effects of glyceraldehyde and KIC in the previous and present study.

Our observation that the diabetic pancreas is hyperresponsive to IBMX confirms previous results.[18] We furthermore demonstrate that a marked hyperresponse can also be evoked in nondiabetic rats after treatment with dexamethasone. This finding may also have relevance for the in vivo situation, since a hyperresponse to another nonmetabolizable secretagogue, isoproterenol, has been demonstrated after dexamethasone treatment in man.[19] Our finding of hyperresponse to IBMX in dexamethasone-treated rats has several implications. First, the abnormality is not peculiar to the STZ-diabetic state. Second, the abnormality can be induced after a relatively short period of time. Third, persistent or prolonged hyperglycemia is not necessary for inducing the hyperresponse. Fourth, the abnormality is not tightly coupled to a reduced acute insulin response to glucose, since, as previously mentioned, the latter was relatively well preserved after dexamethasone treatment.

It seems unlikely that direct effects of dexamethasone on insulin release could explain our results. In our experiments, the last injection of dexamethasone was given on the day preceding operation and perfusion, making it probable that blood levels of dexamethasone were low or absent before perfusion. Also, while glucocorticoids in vitro have been reported to inhibit insulin release induced by many secretagogues, metabolizable as well as nonmetabolizable,[20] we found some inhibition of glucose-induced insulin secretion but enhanced stimulation with IBMX.

It is interesting to note that "basal" release of insulin was enhanced both in STZ- and dexamethasone-treated rats. Stimulation of basal release may be secondary to the degree of demands on B-cell secretion, since it was high in the aforementioned states but low after fasting and after insulin therapy. The mechanisms behind the regulation of basal release remain, however, to be determined.

A special concern of the present study was to study not only the effect of ambient but also of previous exposure to glucose. Two modalities of the latter, priming effect of glucose can be recognized, i.e., induction and expression of the effect. Induction requires the presence of glucose or other nutrients,[11,21-23] whereas expression of priming is nonspecific, since it enhances the signal of secretion evoked by any secretagogue[22] by an action exerted probably distal to the

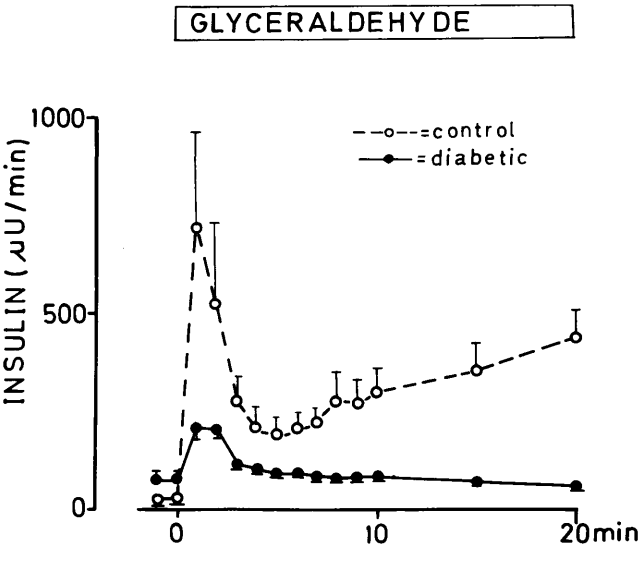

FIGURE 6. Effects of glyceraldehyde on insulin secretion from STZ-diabetic and nondiabetic rats. Mean ± SEM of six experiments in each series.

TABLE 3
Insulin release induced by nonglucose secretagogues

Rats	Secretagogue	Insulin release (μU/min) 0–5 min	5–20 min	0–20 min
Nondiabetic	D-Glyceraldehyde 5 mM	1593 ± 506	5180 ± 901	6774 ± 1371
STZ-diabetic	D-Glyceraldehyde 5 mM	343 ± 84	135 ± 366	478 ± 398*
Nondiabetic	KIC	2622 ± 456	3135 ± 812	5757 ± 1138
STZ-diabetic	KIC	758 ± 110	385 ± 292	1143 ± 323*
Nondiabetic	IBMX	2267 ± 494	4074 ± 688	6742 ± 1164
Control, dexamethasone-treated	IBMX	8152 ± 1257	8504 ± 1368	16,656 ± 2354†
STZ-diabetic	IBMX	8364 ± 1474	17,670 ± 3011	25,574 ± 3864*

Results are derived from Figures 6–8 and are expressed as mean ± SEM of incremental responses.
*$P < 0.005$ or less, significance of difference versus nondiabetic rats.
†$P < 0.005$, significance of difference versus untreated rats (line 5).

generation of "second messengers."[21–23] Our results indicate that induction of priming is impaired in the STZ-induced diabetic state. Hence, whereas a second stimulation with glucose evoked a twofold higher response than the initial glucose challenge in control rats, this priming effect was not observed in the diabetic rats. This "defect" was present also in dexamethasone-treated rats despite the fact that these animals did not display overt hyperglycemia. The latter findings indicate that increasing demands on B-cell performance may exhaust the capacity of glucose to induce priming already before overt hyperglycemia ensues.

If increased demands exhaust inducibility of priming, decreased demands should restore it. In line with this notion are the priming effects seen after fasting or insulin therapy in the diabetic rats. Both fasting and insulin therapy thus revived the ability of glucose to induce priming, i. e., now the second pulse of glucose stimulated insulin secretion 2–3-fold more than the first pulse. Since this effect was seen not only during insulinopenia as achieved by fasting, but also after previous hyperinsulinemia and weight gain as achieved by insulin therapy, it seems unlikely that the return of a priming effect was secondary to reversal of generalized intracellular starvation. It is less easy to interpret the response to ambient glucose after fasting. It thus cannot be ascertained whether the response to ambient glucose was regained or not in the diabetic rats, since we do not know to which extent the well-known[16] inhibitory influence of fasting on insulin secretion from normal rats was also operative in the diabetic rats.

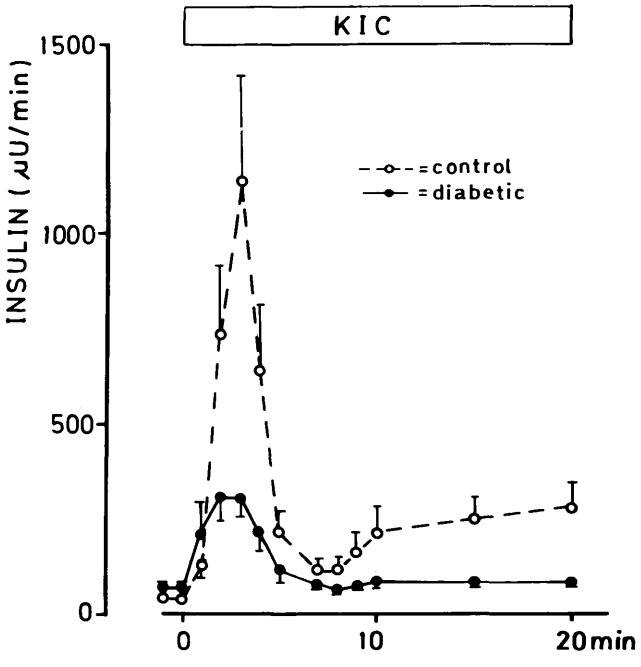

FIGURE 7. Effects of KIC on insulin secretion from STZ-diabetic and nondiabetic rats. Mean ± SEM of six and seven experiments, respectively.

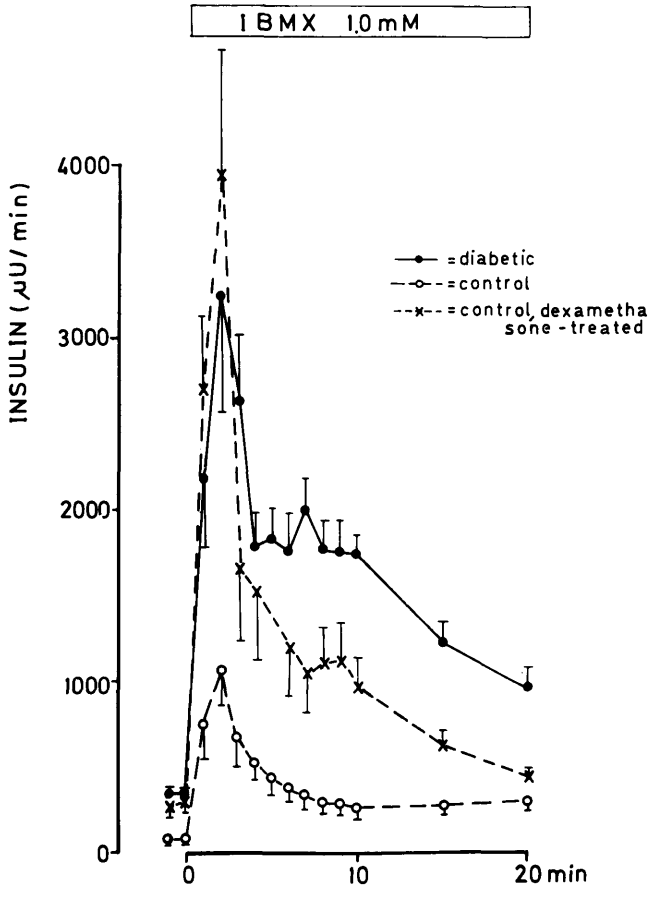

FIGURE 8. Effects of IBMX on insulin secretion from STZ-diabetic, from nondiabetic, and from nondiabetic, dexamethasone-treated rats. Mean ± SEM of six, seven, and six experiments, respectively.

The other modality of priming, i.e., expression of the effect, may not be exhausted in a diabetic state; instead, some evidence indicates that hyperresponse to nonnutrients, such as IBMX and arginine, could reflect a primed state of the diabetic B-cell and may, in part, depend on common mechanisms. Thus, endogenous substrates may play a role both in expression of priming and in the diabetic hyperresponse. This notion is supported by several observations. First, it is clear that the insulin secretory process requires energy.[24] Furthermore, glucose can impart lasting elevations of fuel substances in islets. Thus, we have previously found that ATP levels are elevated long after the cessation of exposure to glucose.[25] Accumulation of glucose into glycogen in B-cells has also been demonstrated after 4 h of i.p. glucose administration in *ob/ob* mice[26] and after 8 h of glucose infusion in normal rats.[27] Glycogen also accumulates in diabetic states[28] and during treatment with corticosteroids.[29] Finally, glycogen deposition has also been associated with an enhanced response to theophylline.[30]

The relevance, if any, of our findings for the understanding of type II diabetes in humans needs to be considered. The present study has used an animal model of NIDDM that may mimic the moderate reduction of B-cell capacity seen in human NIDDM[31] but not other aspects of the disease. If so, similarities and differences between model and human NIDDM may provide clues to those features of abnormal insulin secretion that are associated or not with decreased B-cell capacity. Lack of data in type II diabetic subjects makes it difficult to analyze the priming effect of glucose in this way. With regard to ambient glucose, results in the STZ-diabetic model and in type II diabetes are analogous insofar that the insulin response to glucose is depressed out of proportion to the reduction of B-cell capacity.[1,2] However, although the normal time dynamics of glucose-induced insulin secretion were altered in the diabetic animals, the alterations encountered were the opposite of the delayed response observed in human type II diabetes.[1,2] Thus, in our experiments, second-phase insulin release was more affected than first-phase release, and within first phase, no delay could be observed in the onset of glucose action in the diabetic pancreata. According to previous reasoning, the delayed insulin response to glucose could thus be thought to be an intrinsic feature of type II diabetes. Alternatively, extrapancreatic factors regulated by glucose may modulate second-phase insulin release in human type II diabetes. Glucose could thus be envisaged to affect neuronal input to the islets of Langerhans and/or to release hormones that, in turn, could stimulate insulin secretion. The present findings suggest that the diabetic B-cell would be responsive to such putative secretagogues. Further studies comparing secretory profiles in vivo and in vitro in animal models of NIDDM could resolve this question.

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