

Overstimulation and β -Cell Function

Valdemar Grill and Anneli Björklund

Previous and present evidence ascribes an important role to overstimulation of β -cells for the secretory abnormalities associated with type 2 diabetes. The abnormality most clearly linked to overstimulation is the elevated ratio of circulating proinsulin to insulin. Evidence obtained in human pancreatic islets suggests that aberrations in insulin oscillations that occur in type 2 diabetes could at least in part be linked to abnormalities in cytoplasmic Ca^{2+} oscillations induced by overstimulation. Furthermore, in a transplantation model, we have obtained evidence for long-lasting, perhaps irreversible, effects of overstimulation, implying that this is a causative factor for the well-recognized deterioration of insulin secretion with increasing duration of type 2 diabetes. The mechanisms behind the effects of overstimulation are only partly clarified, but it is clear that reduced insulin secretion after overstimulation is only partly explained by decreased insulin stores. In cultured human pancreatic islets, overstimulation by high glucose leads to a rise in cytoplasmic Ca^{2+} levels, which persists after normalization of the glucose levels. Persistent elevation of cytoplasmic Ca^{2+} may trigger apoptosis, thus participating in long-term irreversible deterioration of β -cell function. These data provide sufficient rationale for clinical studies to test the beneficial effects of relative β -cell rest in type 2 diabetic patients. *Diabetes* 50 (Suppl. 1): S122–S124, 2001

In 1986, Leahy et al. (1) reported that 48 h of marked hyperglycemia in normal rats, achieved by massive glucose infusions, produced almost total insensitivity to glucose when insulin release was subsequently measured from the perfused pancreas. This desensitizing effect was specific for glucose and other secretagogues, such as arginine, eliciting normal or even exaggerated responses. Desensitization was reversible within 24 h. One of us (V.G.) later showed that if glucose-induced insulin secretion during glucose infusion was blocked by the simultaneous infusion of diazoxide, no later desensitization occurred; if anything, insulin responses to glucose were enhanced (2) (Fig. 1). Because the levels of hyperglycemia were kept similar with

or without diazoxide, it was concluded that the desensitizing effect was due to overstimulation of the β -cells rather than to effects of hyperglycemia per se.

We studied the desensitizing effect of overstimulation further in vitro (3). It was found to be induced in perfused islets by only a few hours of glucose stimulation. The decline of insulin secretion during prolonged in vitro stimulation mimicked that first described by Bolaffi et al. (4) as the third phase of insulin secretion, was proportionate to the degree of stimulation, and could be totally prevented by blocking glucose-induced insulin secretion with diazoxide.

Which mechanisms explain desensitization by overstimulation and its prevention by diazoxide? Diazoxide blocks glucose-induced insulin secretion by opening K^+ -ATP channels (5). An effect of overstimulation on channel activity could therefore be envisaged. Our experiments showed, however, that the protective effects of diazoxide were only indirectly related to the action of the drug on K^+ -ATP channels, since the drug was not protective against desensitization induced by stimulation with high concentrations of potassium, which depolarize the cell membrane by mechanisms not involving the K^+ -ATP channel (3). Furthermore, the results with diazoxide were mimicked by somatostatin (6), which inhibits insulin secretion by interfering with G-proteins in the β -cell membrane rather than interacting with the K^+ -ATP channel.

A common denominator of the desensitized state, both in vivo and in vitro, is the reduction of insulin content in islets or perfused pancreas. Indeed, insulin depletion has been proposed to fully explain the decrease in glucose-induced insulin secretion (7), in which case the term “desensitization” may not be appropriate. However, several of our observations indicate that the protective effect of diazoxide is only partly explained by effects on insulin content. In this context, experiments in rat islets using cooling to block glucose-induced insulin secretion were particularly instructive. Cooling below 30°C inhibits exocytosis of insulin but only marginally decreases glucose-induced Ca^{2+} inflow (8,9) and does not block the generation of second messengers such as cAMP (10). We found that cooling during glucose stimulation, while blocking insulin secretion and maintaining islet insulin content, only partially protected against desensitization; only after exclusion of Ca^{2+} from the incubation media did cooling completely protect against desensitization (3) (Fig. 2). These results support the notion that persistent inflow of Ca^{2+} and/or cellular events that follow it—distinct from exocytosis—are deleterious for β -cell function and participate in desensitization.

As further support, we found that culturing human islets for 48 h at a high glucose concentration completely desensitizes glucose-induced insulin secretion and that this is accompanied by profound alterations in Ca^{2+} fluxes (11). Prominent abnormalities were 1) absence of a Ca^{2+} response to glucose, 2) doubling of the postculture “resting” level of cyto-

From the Department of Internal Medicine (V.G.), Section of Endocrinology, University Hospital of Trondheim, Trondheim, Norway; and the Department of Molecular Medicine (A.B.), Karolinska Institute, Stockholm, Sweden.

Address correspondence and reprint requests to Valdemar Grill, Department of Internal Medicine, Section of Endocrinology, University Hospital of Trondheim, N-7006, Trondheim, Norway. E-mail: valdemar.grill@medisin.ntnu.no.

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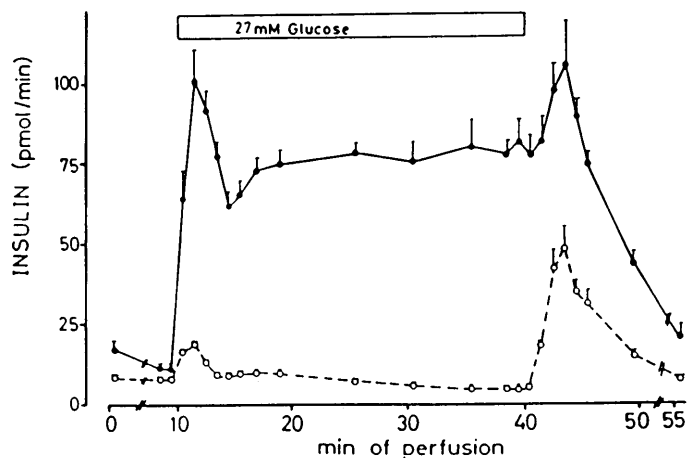


FIG. 1. Effects of diazoxide infusion (●) on subsequent glucose-induced insulin release. Glucose was infused for 48 h to achieve and maintain hyperglycemia (>20 mmol/l). Blood glucose levels were adjusted to comparable levels in the rats that were co-infused with diazoxide. ○, No previous diazoxide. Results are derived from Sako and Grill (2).

plasmic Ca^{2+} , and 3) reduction of slow (0.2–0.5 min) Ca^{2+} oscillations. The increase in “resting” Ca^{2+} was partly normalized by previous diazoxide treatment, which also normalized the oscillatory activity. However, diazoxide did not restore the Ca^{2+} rise in response to glucose. These results indicate that overstimulation has profound effects on Ca^{2+} fluxes, which may participate in desensitization; however, at least in human islets, long-term exposure to elevated glucose may also cause other alterations in the regulation of cytoplasmic Ca^{2+} that are related to glucose per se or its metabolism. Other studies in human islets have shown that culture at high glucose for several days leads to downregulation of insulin biosynthesis, possibly secondary to decreased expression of

the relevant transcription factors (12). Still other studies have reported decreased glucose metabolism (13). The role of overstimulation versus effects of glucose per se could not, however, be assessed in these experiments.

We have obtained strong evidence that the abnormally increased secretion of proinsulin in type 2 diabetes can be explained to a large part by overstimulation of β -cells. Culture of human pancreatic islets for 48 h at 27 mmol/l glucose thus markedly increased the ratio of proinsulin to insulin in islets and in the secreted products both during and after culture; the increased ratio of both stored and secreted products was completely normalized by blocking insulin secretion with diazoxide (14). These results are very similar to those in the pancreas of 90% pancreatectomized rats (15). Also, treatment of type 2 diabetic patients with diazoxide improves insulin secretion (16), an effect not secondary to changes in hyperglycemia per se (17). Such results do not a priori rule out other influences on proinsulin-to-insulin ratios, such as that of fatty acids (14) or, in type 1 diabetes, of cytokines (18). Also, although no positive evidence for genetic influences have so far been found, they cannot be completely ruled out.

The effects of overstimulation so far described were induced in vitro or in vivo by exposure to elevated glucose up to 48 h, and desensitization was readily reversible. The question remains whether chronic overstimulation over months and years may produce irreversible damage to β -cells. In support of this, we have obtained evidence for a lasting effect of diazoxide treatment on β -cell function in a rat transplantation model (19). Islets from normal rats were transplanted under the kidney capsule in streptozotocin-induced diabetic syngeneic recipients. Diazoxide treatment for 8 weeks improved transplant function not only during but also after cessation of the treatment, implying that overstimulation had permanently damaged the transplanted β -cells in control animals. As to possible mechanisms for such an effect, one should consider the possibility of “ Ca^{2+} toxicity,” i.e., negative

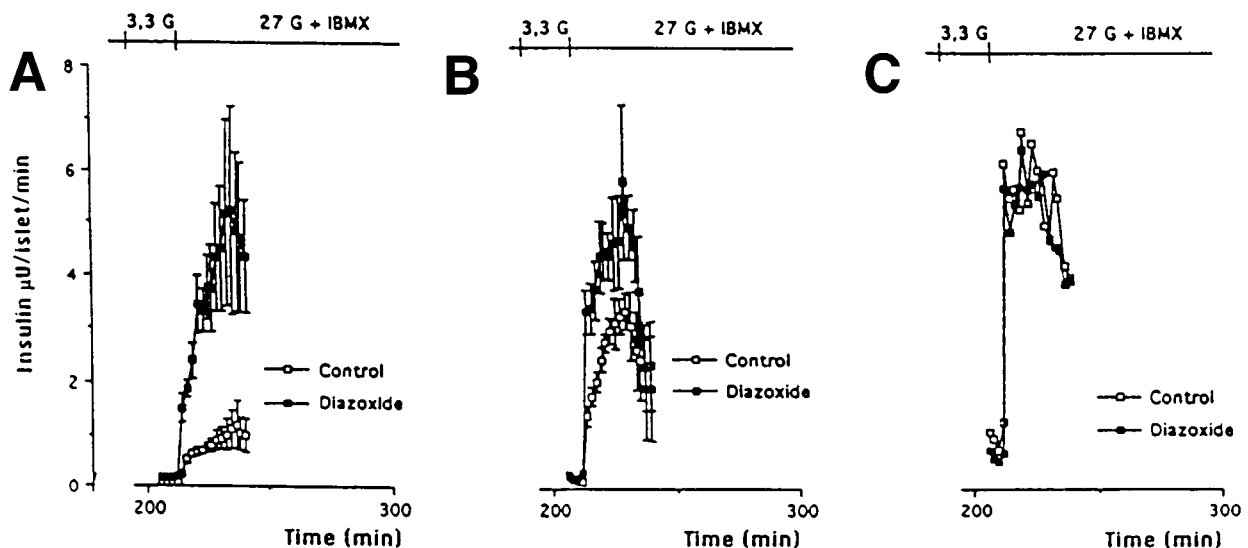


FIG. 2. Evidence that a protective effect of diazoxide against desensitization during high glucose exposure is partly linked to reduced Ca^{2+} inflow. Rat pancreatic islets were perfused for 180 min with 27 mmol/l glucose in the presence of 0.2 mmol/l 3-isobutyl-1-methylxanthine (IBMX) in the presence or absence of 325 $\mu\text{mol/l}$ diazoxide. Islets were then restimulated at the same glucose and IBMX concentrations in the absence of diazoxide. In A, islets were perfused for the 180-min period at 37°C, in B at 22°C, and in C also at 22°C, with the omission of added Ca^{2+} to the Krebs-Ringer bicarbonate medium. All panels show means \pm SE of the restimulated responses (which were always performed at 37°C in the absence of diazoxide). Results are derived from Björklund and Grill (3).

effects of continuous inflow of Ca^{2+} that may activate Ca^{2+} -dependent intracellular proteases, thereby triggering apoptosis (20,21). Such a notion would be in line with our findings that culture of human islets for 48 h at high glucose leads to persistent elevation of cytoplasmic Ca^{2+} and that diazoxide partly restores the Ca^{2+} levels (11).

Negative effects of overstimulation need not necessarily be linked with marked hyperglycemia. Insulin resistance increases demands on β -cell secretory capacity and leads by several mechanisms to increased insulin biosynthesis and β -cell replication and neogenesis. The potential for increasing β -cell capacities is age and species dependent; it is probably also genetically determined. The question arises whether long-term increased demands for insulin secretion can negatively affect β -cells by overstimulation. Results in animal models are equivocal (22,23), probably reflecting species differences. Epidemiological data in humans give some support for negative effects. Thus, in Pima Indians, obesity duration is a risk factor for diabetes and low insulin secretion (24); such was also the case in a Swedish study (25). The mechanisms behind these negative effects are not clear but could be similar to those operative during overstimulation by hyperglycemia.

The notion of overstimulation as a negative factor for β -cell function and perhaps survival has additional clinical implications. Inducing better control by intensive insulin treatment in type 2 diabetic patients has repeatedly been shown to improve insulin secretion (26). Furthermore, as already mentioned, short-term treatment with diazoxide improves insulin secretion in type 2 diabetic subjects (16), and this effect may be independent of an effect on blood glucose (17). Also, data from type 1 diabetic subjects indicate long-term beneficial effects on residual insulin secretion by intensive insulin (27) and by diazoxide (28) treatments. Although it is usually assumed that such beneficial outcomes in type 1 diabetes are due to effects on the autoimmune process, it cannot be excluded that the more general mechanisms described above are at play. Current treatment of type 2 diabetes extensively uses sulfonylurea compounds that stimulate insulin secretion through a glucose-like effect on the K^+ -ATP channel. It seems possible that long-term treatment with sulfonylureas could overstimulate β -cells, resulting in negative consequences. Clinical studies in type 2 diabetic patients are needed in which such possible effects are examined in patients randomized to either sulfonylureas or insulin treatment. Such a multicenter study is underway in Sweden, with 2-year results to be reported at the end of 2001.

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