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Commentary

# Desensitization of insulin secretion

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**Abstract**

Desensitization of insulin secretion describes a reversible state of decreased secretory responsiveness of the pancreatic β-cell, induced by a prolonged exposure to a multitude of stimuli. These include the main physiological stimulator, glucose, but also other nutrients like free fatty acids and practically all pharmacological stimulators acting by depolarization and $Ca^{2+}$ influx into the β-cell. Desensitization of insulin secretion appears to be an important step in the manifestation of type 2 diabetes and in the secondary failure of oral antidiabetic treatment. In this commentary, the basic concepts and the controversial issues in the field will be outlined. With regard to glucose-induced desensitization, two fundamentally opposing concepts have emerged. The first is that desensitization is the consequence of functional changes in the β-cell that impair glucose-recognition. The second is that long-term increased secretory activity leads to a depletion of releasable insulin, often in spite of increased insulin synthesis. The latter concept is more appropriately termed β-cell exhaustion. The same dichotomy applies to the desensitization evoked by pharmacological stimuli: again the relative contributions of a decreased insulin content versus alterations in signal transduction are in dispute. The action of tolbutamide on β-cells may be an example of desensitization caused by a lack of releasable insulin since the signaling mechanisms are nearly unchanged, whereas the action of phentolamine, an imidazoline, induces a strong desensitization without reducing insulin content or secretory granules, apparently by abolishing $Ca^{2+}$ influx. With pharmacological agents it seems that both, alterations in signal transduction and decreased availability of releasable insulin, can contribute to the desensitized state of the β-cell, the relative contribution being variable depending upon the exact nature of the secretory stimulus. 

*Keywords:* Insulin secretion; Pancreatic islets; Desensitization; $K_{ATP}$ channels; Cytosolic calcium concentration; Sulfonylureas; Imidazolines

## 1. Definition

The term desensitization of insulin secretion is used to describe a state of decreased responsiveness to physiological or pharmacological stimuli of insulin secretion. This state is induced by prolonged exposure to effective concentrations of insulinotropic stimuli, in particular glucose, and is readily reversible after discontinuation of the exposure. Closely related, but not identical is the concept of glucose toxicity. Originally, the decrease in secretory responsiveness was believed to be a consequence of glucose toxicity, which in contrast to desensitization infers a damaging effect, leading not only to functional changes but also to structural alterations in β-cells [1,2]. Once manifest, glucose toxicity is of limited reversibility and may be the reason for the loss of β-cell mass in advanced type 2 diabetes [3,4]. In the last few years, the emerging concept of desensitization of insulin secretion has attracted considerable interest because it may be relevant for the natural history of type 2 diabetes and also for the loss of efficacy of treatment with oral antidiabetic agents [5,6].

## 2. Relevance of desensitization for type 2 diabetes

The predominant view on the pathogenesis of type 2 diabetes is that resistance of the insulin-sensitive tissues to the effects of insulin plays the leading role. The hyperinsulinemia often present at the onset of clinically overt type 2 diabetes is interpreted as the endocrine pancreas trying to compensate for the primary defects in adipose tissue [7]. Thus, the progression from relative to absolute insulin deficiency by decreasing insulin secretory capacity appears as the result of a detrimental long-term increased workload of the β-cells [4].

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*Abbreviations:* $[Ca^{2+}]_i$, cytosolic free calcium concentration; CCK/PP, cholecystokinin-pancreozymin; FFA, free (unesterified) fatty acids; GLP, glucagon-like peptide; $K_{ATP}$ channel, ATP-sensitive $K^+$ channel; Kir, inwardly rectifying $K^+$ channel; $PIP_2$, phosphatidylinositol bisphosphate; PDX-1 (=IDX-1), pancreatic duodenal homeobox 1; PKA, protein kinase A; PKC, protein kinase C; and SUR, sulfonylurea receptor.

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However, it was observed that the secretory capacity of β-cells was diminished in first-degree relatives of individuals with type 2 diabetes even before the onset of obesity and insulin resistance [8]. Thus, insulin resistance may lead to overt diabetes only in those persons whose β-cells have a genetically limited capacity to compensate for an increased workload [9]. Also, qualitative secretion abnormalities may play a role like an impaired acute insulin secretory response to glucose (a clinically useful index of first phase insulin secretion), which leads to a decreased efficiency of insulin action in humans [10]. Secretion abnormalities of the β-cell in type 2 diabetes are, therefore, not only secondary to insulin resistance but are likely to constitute an independent pathogenetic factor.

On the other hand, it is beyond doubt that hyperglycemia as such, irrespective of its cause, is detrimental for the functional and, eventually, for the structural integrity of the β-cell. Hence, once prolonged periods of high blood glucose levels have occurred in the course of type 2 diabetes, a desensitization of the β-cells and a further decrease of glucose tolerance seem inevitable. It is unknown whether physiological enhancers of insulin secretion, such as the incretin hormones GLP-1 or CCK/PP, contribute to the desensitization. It is clear, however, that a desensitization to pharmacological stimulators of insulin secretion occurs *in vitro* and *in vivo* as will be discussed below. The secondary failure of treatment with oral antidiabetic agents, requiring change to insulin treatment, may reflect a chronic desensitization to the pharmacological stimuli and/or a progression of the underlying β-cell defects. Therefore, studying the mechanisms of desensitization of insulin secretion is obviously of major relevance for understanding the causes of type 2 diabetes and for the further development of pharmacotherapeutic concepts of how best to treat this disease.

## 3. Inducers of desensitization

In this commentary, a compound that is able to increase insulin secretion in the absence of any other stimulatory agent will be called a stimulator of insulin secretion. An enhancer of insulin secretion is a compound that can increase insulin secretion only when a certain stimulus for insulin secretion is already present. Physiologically, stimulators of insulin secretion are nutrients, i.e. they generate reducing equivalents that can be used for the synthesis of ATP [11,12]. Among the non-nutrient stimulators of insulin secretion, the group of pharmacological agents that act by inhibition of the $K_{ATP}$ channel is the most prominent. The common mode of action of nutrient and non-nutrient stimuli is by the depolarization of the plasma membrane, which triggers an influx of $Ca^{2+}$ across the plasma membrane, thereby activating the exocytotic machinery of the β-cell (Fig. 1). Enhancers act by modulating the efficiency with which an increase in $[Ca^{2+}]_i$ is

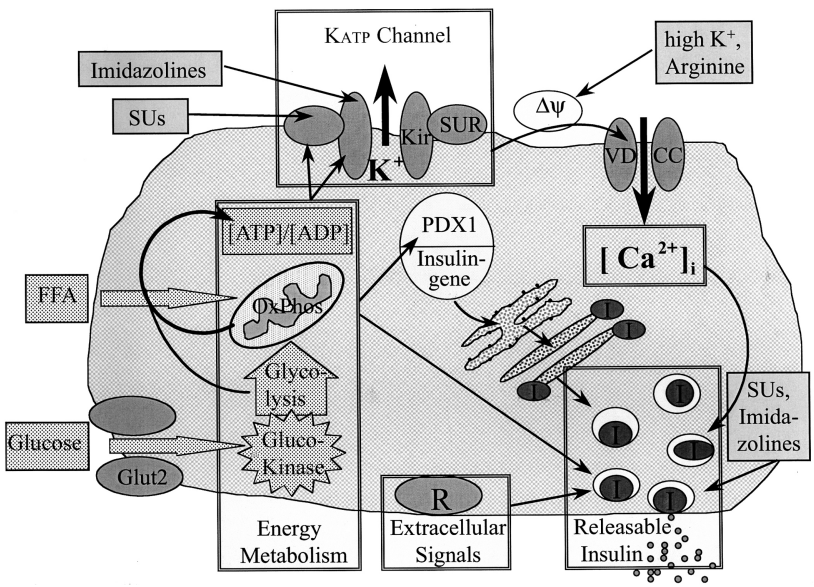


Fig. 1. Schematic drawing of the stimulus–secretion coupling in a pancreatic β-cell, placing the desensitizing stimuli and the sites of desensitization into the context of the consensus hypothesis of insulin secretion. In this widely accepted hypothesis, an increase in energy metabolism leads to an increased ATP/ADP ratio, thus closing the $K_{ATP}$ channel and depolarizing the plasma membrane. The ensuing opening of voltage-dependent $Ca^{2+}$ channels increases the cytosolic $Ca^{2+}$ concentration whereby the secretory granules are enabled to fuse with the plasma membrane. The nutrient stimuli and their entry into energy metabolism are depicted by a dotted pattern. The pharmacological stimuli are given in boxes outside the β-cell; their sites of action are marked by an arrow. The double-lined boxes mark the possible sites of desensitization as discussed in the text. The arrow connecting energy metabolism to the nucleus symbolizes the glucose-dependent regulation of insulin gene transcription and that from energy metabolism to the secretory granules the $K_{ATP}$-independent augmentation pathway of insulin secretion. Abbreviations: ΔΨ, membrane potential; FFA, free fatty acids; I, insulin; OxPhos, oxidative phosphorylation; PDX-1, pancreatic duodenal homeobox 1; R, any receptor mediating cell–cell or cell–matrix interactions or autocrine or paracrine feedback loops that are relevant for secretion; SUs, sulfonylureas; and VDCC, voltage-dependent $Ca^{2+}$ channel.

translated into an increase in exocytosis, e.g. by activating PKA or PKC. With the possible exception of fatty acids, inducers of desensitization are stimulators of secretion.

### 3.1. Nutrient stimuli

#### 3.1.1. Glucose

It was only in the second half of the 1980s that the decreased secretory response of pancreatic islets after a prolonged exposure to a stimulatory glucose concentration was recognized to be a (patho)physiologically relevant phenomenon and not just an *in vitro* artifact reflecting an unspecific loss of β-cell function. This loss of responsiveness could be shown to occur in batch-incubated islets, perifused islets, and in the perifused pancreas [13–15] and was termed "third phase of insulin secretion" by Grodsky to emphasize that this was a regular characteristic of the β-cell response to a continued glucose challenge [16]. As is generally known, the first phase response is a fast and transient increase in insulin secretion visible only when the glucose concentration is raised in a square wave-like manner. After a nadir of secretory activity at about 15 min, the slowly increasing second phase release develops, which reaches a maximum after 2–4 hr. Thereafter, secretion decreases in spite of the unchanged presence of glucose, reaching a steady-state level of about 20% of the second phase peak value 8–12 hr after the onset of the glucose stimulation. This third phase of secretion then can be maintained for more than 48 hr [16].

The reversibility of a glucose-induced desensitization was found to be surprisingly rapid *in vivo* [17] and *in vitro* [18]. *In vitro*, it took only 5 min for islets desensitized by a 6-hr exposure to high insulin secretion to normalize insulin secretion and $K^+$ and $Ca^{2+}$ fluxes. Even in 24-hr-desensitized islets these functions were again normal after the same time. Reversibility required much more time (1 hr) when glucose-desensitized islets were transferred to a Krebs-Ringer medium with a basal glucose concentration, instead of a cell culture medium with the same glucose concentration [18]. Thus far, the question as to which (nutrient?) factors in addition to glucose are responsible for the different recovery rates has not been pursued further.

With regard to the specificity of glucose desensitization, two aspects have to be distinguished: (i) are islets that have been desensitized by glucose also desensitized against other stimuli, and (ii) are non-glucidic stimuli also able to induce a desensitization against glucose? A 48-hr *in vitro* exposure of human islets to 16.7 mM glucose resulted in a strongly reduced response to a renewed glucose stimulus and also in a significantly reduced response to tolbutamide, whereas the response to arginine was not diminished significantly [19]. Similar results were obtained with islets from rats chronically infused with glucose [20,21]. Vice versa, islets exposed for 48 hr to arginine or tolbutamide in the presence of a non-stimulatory glucose concentration had a significant loss of responsiveness to glucose [19]. Thus, the desensitization does not appear to be strictly specific for the desensitizing agent, but shows a graded preference.

With regard to the mechanism of desensitization, two fundamentally opposing points of view have emerged. The first is that glucose-induced desensitization is a consequence of functional changes in the β-cell that affect glucose-recognition (glucose "non-sense" of the β-cell [22]); the second is that the long-term increased secretory activity leads to a depletion of releasable insulin ("overworked" or "exhausted" β-cell [23]). It should be noted that not only does exposure to high glucose concentrations induce a loss of secretory responsiveness, but prolonged exposure to a low glucose concentration (3.3 mM) also leads to a near complete loss of insulin secretion upon stimulation by high glucose [24]. Usually, the diminished presence of an agonist leads to a hypersensitivity of the response-generating system. This illustrates that one should be cautious when extrapolating mechanisms valid for receptor desensitization to the desensitization of stimulus–secretion coupling in pancreatic β-cells.

#### 3.1.2. Free fatty acids

The serum levels of FFA are often elevated in obese type 2 diabetic individuals. It is widely known that FFA impair insulin action [25], but they also affect glucose-induced insulin secretion. FFA have an acute enhancing effect on insulin release as was found *in vivo* [26] and *in vitro* [27]. The effects of chronically elevated levels of FFA appear to be complex: a chronic exposure to FFA by infusion *in vivo* has been found to induce a desensitization of glucose-induced insulin secretion [28,29], but there are also reports from *in vivo* and *in vitro* experiments that suggest a permissive or even enhancing effect of FFA on insulin secretion [30,31]. There are multiple steps in stimulus–secretion coupling of β-cells that are affected by FFA and that could contribute to FFA-induced desensitization of glucose-induced insulin secretion, the most consistent observation being a reduction of insulin content (see below).

### 3.2. Pharmacological stimuli

#### 3.2.1. Sulfonylureas

Compared to the attention gained by glucose-induced desensitization, the observation that exposure of pancreatic islets to sulfonylureas such as tolbutamide (Fig. 2, top panel) or glibenclamide also causes a reversible impairment of insulin secretion [32,33] is less well known, even though an inhibitory effect of sulfonylureas was noted about 30 years ago [34]. The sulfonylurea-induced desensitization has been described as being selective for sulfonylureas [35,36], but other authors found that prolonged exposure of isolated islets to sulfonylureas reduced glucose-induced insulin secretion by about 50% [22,37–39].

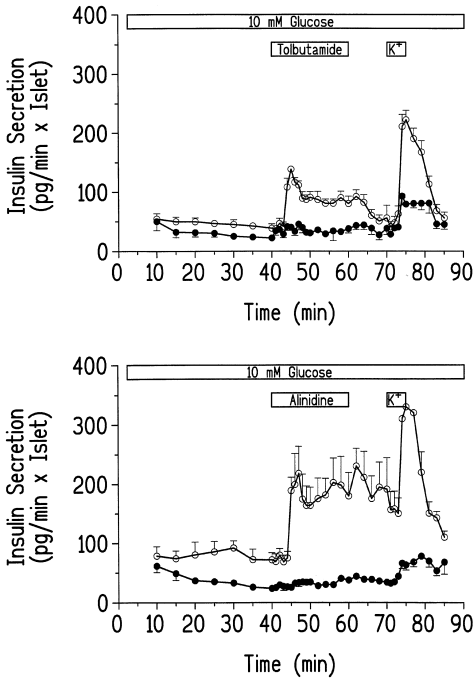


Fig. 2. Desensitization of insulin secretion by culture of pancreatic islets in high concentrations of tolbutamide (top panel) or alinidine (bottom panel). Isolated mouse pancreatic islets were cultured for 18 hr in cell culture medium (RPMI 1640) containing 5 mM glucose plus a 100 μM concentration of the named secretagogues or no secretagogue (control culture). Insulin secretion was then measured by perifusing the islets with a Krebs-Ringer medium containing 10 mM glucose and the same compound to which they had been exposed previously (●). The secretory response was compared with that of islets that had been control-cultured (○). The data are means ± SEM of 4 experiments.

It is a well-known feature in pharmacology that a response to a stimulatory agent may decrease in magnitude or may even cease to occur when the agent is present continually or applied repeatedly. When the response can still be elicited by other stimuli, it is assumed that the desensitization to the first stimulus is due to a dissociation between receptor occupancy and subsequent response-generating elements in the signal transduction pathway [40]. Such a homologous desensitization has to be distinguished from a heterologous desensitization where application of one type of stimulus also decreases the response to other stimuli. This latter phenomenon is usually due to effects at more distal steps in signal transduction where signal pathways converge to elicit cellular responses such as secretion or contraction. In practice, it may prove to be quite difficult to determine to which degree a desensitization caused by pharmacological stimulators of insulin secretion is homologous or heterologous in nature.

In a recent investigation employing glibenclamide to desensitize MIN6 cells, a diminished presence of SUR1 and, hence, $K_{ATP}$ channels in the plasma membrane was observed [41]. At first sight, a reduced receptor availability in response to constant receptor occupancy would fit well to a homologous desensitization, but the functional consequences thereof, partial depolarization of the β-cell and an increase in the resting cytosolic $Ca^{2+}$ concentration, do not. Correspondingly, an increased instead of a decreased basal rate of secretion was found, combined with a strongly reduced secretory response not only to sulfonylureas but also to glucose [39]. Such a cross-desensitization is more typical for heterologous desensitization and again, as with nutrient-induced desensitization, the problem arises as to whether (i) a depletion of insulin stores or (ii) functional changes in signal transduction is responsible for the loss of secretory responsiveness.

### 3.2.2. Imidazolines

The desensitization of insulin secretion by prolonged exposure to insulin secretagogues has been used as an experimental tool to characterize the mode of action of a newly defined group of pharmacological stimulators of insulin secretion, the imidazolines. These compounds, most of which were synthesized to act as α-adrenoceptor ligands, are of interest as potential oral antidiabetic drugs since some of them enhance insulin secretion only in the presence of a stimulatory glucose concentration [42–44]. Imidazolines inhibit $K_{ATP}$ channels [45], probably due to a direct interaction with the pore-forming subunit [46,47], and some, but not all, exert a number of additional effects that may contribute to the enhancement of insulin secretion such as release of $Ca^{2+}$ from internal stores or activation of protein kinases [48–51].

The β-cell imidazoline binding sites proved to be different from the $I_1$ and $I_2$ imidazoline receptor subtypes characterized in other tissues [52,53]. The hypothesis that a specific β-cell imidazoline receptor, tentatively named the $I_3$ receptor [54,55], is involved in imidazoline-induced insulin secretion is based on the observations that enantiomeric compounds differ in their efficacy [56] and that an agonist-induced desensitization occurs after prolonged incubation in the presence of secretion-enhancing imidazolines [52]. The desensitization by imidazolines was described to be specific, since the pretreated islets still responded to high glucose and to diazoxide, whereas the typical effect of imidazolines to overcome the diazoxide block of secretion was lost. Only imidazolines that stimulated insulin secretion, such as phentolamine and efaroxan, but not an apparently inactive imidazoline, e.g. idazoxan, induced desensitization [52].

Except for one very recently published report on an imidazoline derivative [57], all insulinotropic imidazolines investigated thus far block $K_{ATP}$ channels and increase the cytosolic $Ca^{2+}$ concentration, thus sharing essential parts of the β-cell signaling cascade with the sulfonylureas. Bearing this in mind, it is not surprising that an 18-hr exposure of isolated islets to the imidazolines phentolamine and alinidine desensitized them not only against re-exposure to these compounds, but also against stimulation by tolbutamide and quinine [58]. Likewise, the response to a $K^+$ depolarization was blunted (Fig. 2, bottom panel). The immunoreactive insulin content of the desensitized

islets was not reduced significantly by exposure to the imidazolines, but ultrastructural examination revealed a significant degranulation [58]. Thus, the same problem arises as with nutrients and sulfonylureas: to distinguish β-cell exhaustion from β-cell desensitization.

*3.2.3. Other depolarizing pharmacological stimulators of secretion*

BTS 67 582 is an investigational antidiabetic agent that lowers blood glucose in normal and diabetic animals [59,60]. Structurally, there are similarities to the imidazoline group of insulin secretagogues. Again, drug-induced desensitization was used as a tool to delineate signaling pathways that mediate the insulinotropic effect. BTS 67 582 was reported to desensitize insulin secreting BRIN BD11 cells against stimulation by BTS 67 582 itself and against tolbutamide, but not against the imidazoline efaroxan. Vice versa, tolbutamide induced a cross-desensitization against BTS stimulation, but not against efaroxan, suggesting that BTS shared the insulinotropic mechanisms of sulfonylureas but not of imidazolines [61,62].

A depolarization of the β-cells with a high $K^+$ concentration is the pharmacological maneuver by which an increase of insulin secretion is unquestionably mediated by influx of $Ca^{2+}$ through voltage-dependent $Ca^{2+}$ channels, possible further effects being secondary to the $Ca^{2+}$ influx. This is of importance in view of the current uncertainty about how much of the secretion elicited by sulfonylureas (and imidazolines) is due to $K_{ATP}$ channel-independent effects distal to $Ca^{2+}$ influx [48,63,64]. It was shown that a prolonged exposure of isolated islets to a high $K^+$ concentration desensitized them to a subsequent glucose stimulus [65] and also to stimulation by a number of imidazolines, tolbutamide, and quinine [58]. These observations suggest that a prolonged exposure to any insulin secretagogue, the actions of which include depolarization and $Ca^{2+}$ influx, should induce a more-or-less marked desensitization against secretagogues that stimulate β-cell exocytosis by eliciting $Ca^{2+}$ influx. Thus, it is surprising that the imidazoline efaroxan, which is known to block $K_{ATP}$ channels [66] and to elicit a substantial increase in $[Ca^{2+}]_i$ by opening voltage-dependent $Ca^{2+}$ channels [67], did not desensitize against stimulation by tolbutamide and BTS 67 582 [62].

The insulinotropic effect of arginine is usually explained by an electrogenic uptake of this amino acid into the β-cell, which leads to a substantial depolarization of the plasma membrane when arginine concentrations are above 5 mM [68]. Thus, the mechanism of action is very similar to a $K^+$ depolarization, but effects of arginine in addition to the depolarization cannot be excluded. Similar to islets desensitized by high $K^+$, islets cultured in the presence of 10 mM L-arginine showed a strongly reduced response to glucose and tolbutamide [19]. Interestingly, an acute stimulation by arginine gave unchanged or only moderately reduced secretory responses with islets desensitized by a number of insulin secretagogues [19,20,69,70].

## 4. Possible sites of desensitization

After having presented the essential phenomenology of desensitization by nutrient stimuli and depolarizing pharmacological stimuli, we shall now consider the possible mechanisms by which these agents induce the desensitization. The order of presentation follows the sequence of events as proposed by the consensus hypothesis of insulin secretion (Fig. 1), i.e. first data concerning changes in energy metabolism will be discussed, then data concerning the $K_{ATP}$ channel as a transducer of metabolic events into ionic currents, followed by a discussion on the regulation of the cytosolic calcium concentration. Finally, we have to ask whether the availability of releasable insulin can be the limiting step in desensitized islets by reviewing the data on the insulin content and granulation state of β-cells.

### *4.1. Energy metabolism*

It is the energy metabolism of the β-cell that constitutes the unique signal recognition apparatus for glucose, setting β-cells apart from other endocrine cells which, like pancreatic α-cells, may express $K_{ATP}$ channels and voltage-dependent $Ca^{2+}$ channels, but do not respond to an increase in glucose concentration with an increase in secretory activity [71–73].

Mouse islets cultured at 6.7 mM glucose for 6 days showed the same sigmoid concentration-dependency of glucose oxidation as freshly isolated islets. After culture at 28 mM glucose, the curve was more hyperbolic, amounting to a sensitization of oxidation to low glucose concentrations; also, the maximal rates were increased [74]. It was found that the activity of high $K_m$ glucose phosphorylation was increased under this condition [74], probably due to an increased glucokinase expression. These early observations were confirmed in a more recent study employing purified β-cell aggregates [75]. A 9-day exposure of these aggregates to 20 mM glucose led to a state of metabolic and biosynthetic activation and of glucose hypersensitivity, as measured by glucose utilization, glucose oxidation, and NADH levels. These characteristics were maintained for at least 2 hr after changing to a low glucose concentration [75]. In islets isolated from rats that had been glucose-infused for 7 days, glucose utilization was increased by about 30%, but glucose oxidation was unchanged as compared with control islets [20].

Human islets exposed *in vitro* for 7 days to either 5.6 or 11 mM glucose showed the same rates of glucose oxidation. After exposure to 27 mM glucose, oxidation was reduced by about 25% [76]. More recently, unchanged rates of glucose utilization and oxidation were found in human islets exposed to high (27 mM) and basal (6 mM)

glucose [70]; there was even a tendency for glucose oxidation to be increased after exposure to high glucose. In conclusion, it seems that if the desensitization of β-cells by glucose is due to a specific defect in glucose-sensing [22], there is no obvious defect where one would look first for it: in the glucose-fueled energy metabolism.

Concerning the mechanism of FFA-induced desensitization, the interference of FFA with glucose oxidation and utilization appears to be a particularly relevant mechanism in view of the physiological role of β-cells as sensors and integrators of the fuel supply. The oxidation of glucose was reduced by FFA, apparently by up-regulation of a glucose–fatty acid cycle [77]. The signaling link between lipid and carbohydrate metabolism may be malonyl-CoA, which is presumed to be a general signal of abundance and to regulate the balance between FFA and glucose oxidation [78].

Not much is known about the energy metabolism of β-cells during desensitization by pharmacological stimulators. Islets that had been exposed to glibenclamide for 7 days had a reduced glucose oxidation rate and enlarged mitochondria [79], similar to mitochondria in islets that had been cultured at a low glucose concentration [80]. The question is whether these alterations are causally linked to the loss of secretory responsiveness. The observation that NAD(P)H fluorescence was not different from control when glibenclamide-desensitized islets were stimulated with 15 mM glucose [39] suggests that secretagogue-induced desensitization is not necessarily linked to changes in energy metabolism.

### 4.2. $K_{ATP}$ channels

It is generally recognized that in β-cells the $K_{ATP}$ channel transduces an activation of energy metabolism into a depolarization of the plasma membrane, the precise mechanisms being still a matter of debate [81,82]. Thus far, no data exist which show that $K_{ATP}$ channels react less efficiently to glucose-derived ATP during glucose-induced desensitization or that they are more sensitive to the opening effect of ADP. Also, there is no evidence that channel-opening mediators are produced under this condition. $K_{ATP}$ channels may well play a role in FFA-induced desensitization. Long chain fatty acyl-CoA esters at reasonably low concentrations have an opening effect on the β-cell $K_{ATP}$ channel [83], thereby leading to a hyperpolarization and inhibition of $Ca^{2+}$-triggered insulin secretion. This opening effect is probably exerted at the pore-forming subunit, Kir6.2 [84].

If a desensitization by sulfonylureas were to conform to the definition of a homologous desensitization, the $K_{ATP}$ channels in desensitized β-cells should have lost their susceptibility to being blocked by sulfonylureas. Such a behavior of $K_{ATP}$ channels, which should result in a sulfonylurea-resistant hyperpolarization of the β-cell plasma membrane, has not been observed. Rather, a partial depolarization was observed after treatment of MIN6 cells with a high glibenclamide concentration (10 μM) for 14 days [41], and after desensitization of normal mouse islets with a therapeutically relevant glibenclamide concentration (10 nM for 20 hr [39]). Two explanations are readily apparent as to how $K^+$ conductance was decreased in the glibenclamide-desensitized β-cell: either $K_{ATP}$ channels present in the plasma membrane were blocked by sulfonylurea accumulated within the cell or the number of $K_{ATP}$ channels in this location was decreased. Employing an immuno-gold technique to determine the intracellular distribution of SUR1, Kawaki *et al.* [41] concluded that the latter explanation was appropriate. Results obtained with glibenclamide may not be representative of sulfonylureas as a whole. A desensitization induced by the prototypic first-generation sulfonylurea, tolbutamide, did not lead to a persistent depolarization (Fig. 3A), similar to a desensitization with high $K^+$ [85], and did not increase basal insulin secretion [39]. Glibenclamide, but not tolbutamide, is known to accumulate intracellularly, which may well be the reason for the apparently divergent effects.

A prolonged exposure to tolbutamide or to a high $K^+$ concentration is accompanied by a prolonged increase in $[Ca^{2+}]_i$. Thus, there seems to be a discrepancy between the observation of an essentially unchanged $K_{ATP}$ channel function when insulin secretion was desensitized by these maneuvers [58,85] and a recent report that an increase in $[Ca^{2+}]_i$ for 30 min led to functional modification of the $K_{ATP}$ channel, i.e. opening nucleotides and tolbutamide lost their efficiency, while the blocking effect of ATP was retained [86]. These $Ca^{2+}$-induced changes, which corresponded to a functional uncoupling of SUR1 and Kir6.2 subunits of the channel, were apparently mediated by the actin cytoskeleton and could be prevented by $PIP_2$ and ATP [86]. Perhaps, the concentrations of ATP and $PIP_2$ remained sufficiently high during the tolbutamide- and $K^+$-induced desensitization to prevent such a $Ca^{2+}$-induced uncoupling or, at least, to permit a fast reversal.

Interestingly, there was one agent, the imidazoline idazoxan, where prolonged exposure did not affect basal channel activity, but led to a marked loss of channel blocking efficiency upon re-exposure [85] (Fig. 4). This characteristic, which was neither shared by two other imidazolines, phentolamine and alinidine, nor by tolbutamide and quinine (Fig. 4), may actually correspond to a homologous desensitization.

### 4.3. Cytosolic $Ca^{2+}$ concentration ($[Ca^{2+}]_i$)

In the original version of the consensus hypothesis of glucose-induced insulin secretion, an increase of $[Ca^{2+}]_i$ is the only and immediate signal for the activation of the exocytotic machinery of the β-cell. Such an exclusive role of $[Ca^{2+}]_i$ was no longer tenable after the observation that the stimulation of insulin secretion by nutrient secretagogues involves signaling pathways other than those causing

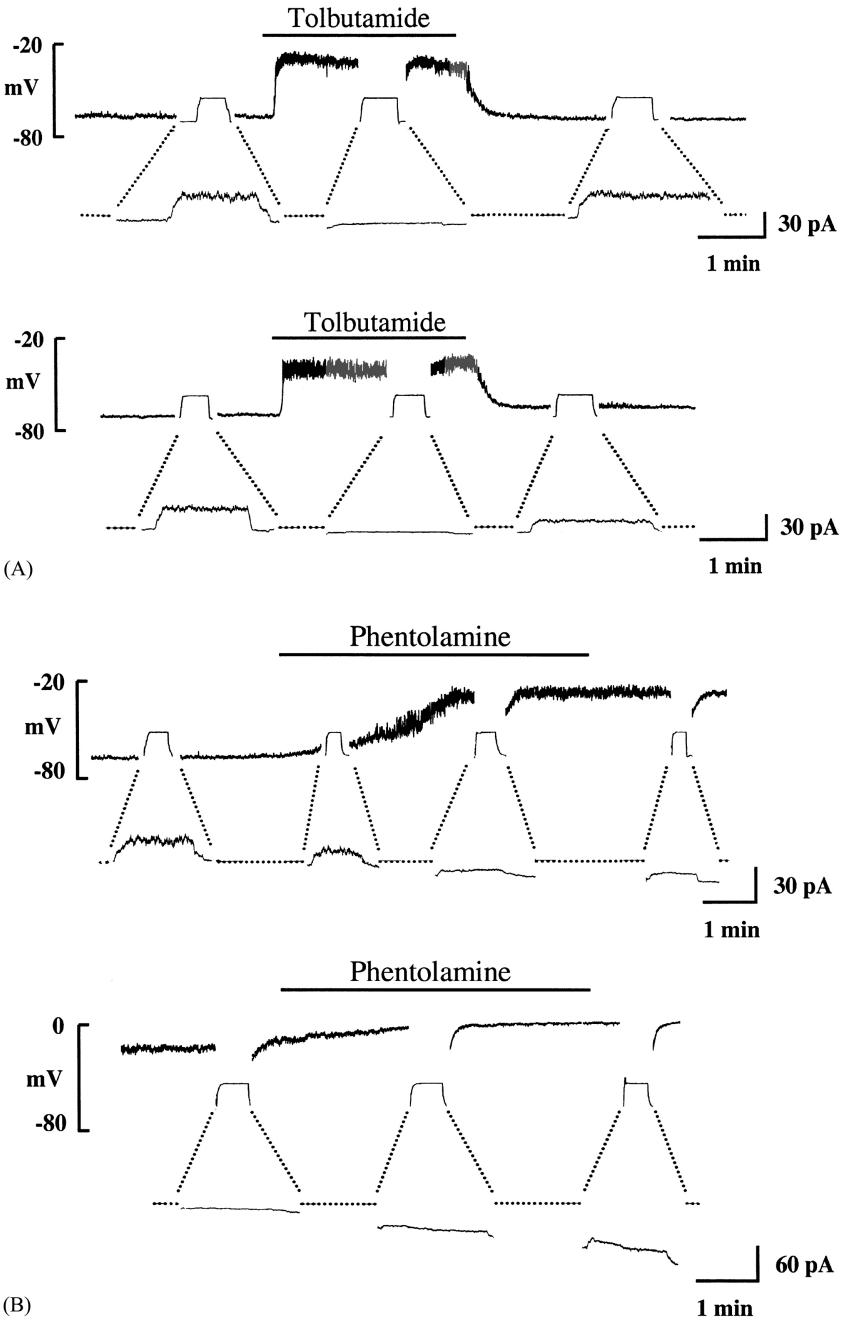


Fig. 3. Changes of membrane potential and whole-cell currents in pancreatic β-cells in response to 500 μM tolbutamide (A) or 100 μM phentolamine (B). The upper graphs show the response of control-cultured β-cells, the lower graphs the response of β-cells desensitized by culturing in the presence of the respective secretagogue. The membrane potential (upper traces) was measured using the whole-cell configuration of the patch clamp technique under current clamp condition. Before, during, and after exposure to the test agents, the whole-cell current (lower traces) was measured by switching to voltage clamp at a holding potential of −50 mV.

a depolarization-induced increase of $[Ca^{2+}]_i$ [87–89]. Experimentally, it was even possible to elicit insulin secretion in the absence of an increase in $[Ca^{2+}]_i$ [90]. Physiologically, a rise in $[Ca^{2+}]_i$ can be regarded as a *conditio sine qua non*, even if the extent of insulin secretion is strongly affected by the additional signaling mechanisms of the ''augmentation pathway'' [91]. The same reasoning applies to sulfonylureas and imidazolines, which may exert relevant effects on insulin secretion at steps distal to the increase of $[Ca^{2+}]_i$ [48,63,92], but which lose insulinotropic efficiency when $Ca^{2+}$ influx is blocked.

Thus, changes in $Ca^{2+}$ signaling might well be a mechanism whereby glucose or depolarizing pharmacological stimulators of insulin secretion induce a desensitized state of the β-cells. Indeed, in human islets exposed for 48 hr to 27 mM glucose, three abnormalities in $[Ca^{2+}]_i$ were observed: (i) the basal levels of $[Ca^{2+}]_i$ were increased markedly, (ii) the initial increase of $[Ca^{2+}]_i$

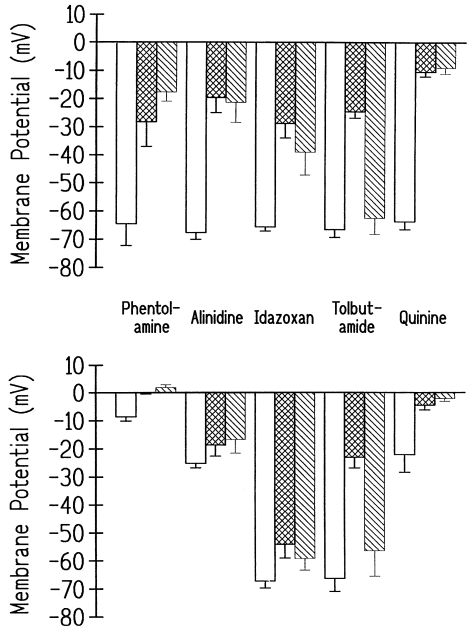


Fig. 4. Comparison of the effects of depolarizing insulin secretagogues on membrane potential of control-cultured (upper panel) and secretagogue-desensitized (lower panel) β-cells. Isolated β-cells were cultured for 18 hr in cell culture medium (RPMI 1640) containing 5 mM glucose and, additionally, 100 μM of the named secretagogues (tolbutamide, 500 μM) or no secretagogue (control culture). The effect of the secretagogues on the membrane potential of these cells was then measured, as depicted in Fig. 3. The open bars represent values measured immediately before exposure to the secretagogue, the cross-hatched bars values during exposure, and the lined bars values after return to basal extracellular medium. The data are means ± SEM of 4–6 experiments.

following renewed glucose exposure was strongly reduced, and (iii) the oscillatory pattern of $[Ca^{2+}]_i$ during glucose exposure was disturbed [70]. Similar but less extensive changes were found in rodent islets [93]. Energy metabolism is widely held responsible for the generation of $[Ca^{2+}]_i$ oscillations [70] and might thus be altered in a more subtle way than could be found by measuring global parameters of glucose metabolism (see above). Since the arginine-induced rise in $[Ca^{2+}]_i$ was essentially unchanged in glucose-desensitized islets, voltage-dependent $Ca^{2+}$ channels are unlikely to play a major role in glucose-induced perturbations of $[Ca^{2+}]_i$ [70].

The same conclusion was reached with respect to sulfonylurea-induced desensitization. In glibenclamide-desensitized MIN6 cells, voltage-dependent (L-type) $Ca^{2+}$ channels did not differ in their electrophysiological properties from the $Ca^{2+}$ channels in control-incubated MIN6 cells [41]. Desensitization by three different imidazolines, tolbutamide, and quinine led to a quite variable response of $[Ca^{2+}]_i$ upon renewed exposure to the respective secretagogue. In phentolamine-desensitized β-cells, there was a complete lack of $[Ca^{2+}]_i$ increase, whereas in tolbutamide-desensitized β-cells there was only a minor reduction in the peak value of the tolbutamide-induced increase of $[Ca^{2+}]_i$, the responses to the other secretagogues ranging in between these extremes [85].

Since an 18-hr desensitization by high $K^+$ (40 mM) had only negligible effects on the $[Ca^{2+}]_i$ increase induced by renewed exposure to a high $K^+$ concentration, the prolonged increase of $[Ca^{2+}]_i$ during the desensitization incubation is unlikely to cause lasting changes in $Ca^{2+}$ channel function [85]. The reason for the depressed $[Ca^{2+}]_i$ response after desensitization by secretagogues such as phentolamine or quinine seems to be that there is a persistent depolarization of the β-cells (Fig. 3B). In fact, the magnitude of a depolarization-induced $[Ca^{2+}]_i$ increase after preincubation with the various secretagogues correlated very well with the membrane potential of such cells prior to renewed stimulation. The variable degree of depolarization, in turn, fitted well to the relative extent of inhibition of $K_{ATP}$ channel activity in the secretagogue-desensitized β-cells [85].

In conclusion, perturbations of $[Ca^{2+}]_i$ in desensitized β-cells are likely to reflect changes at preceding steps of stimulus–secretion coupling, rather than changes in the $Ca^{2+}$ transport mechanisms themselves. In particular, there is no evidence that the function of the L-type $Ca^{2+}$ channel is directly affected by the desensitization elicited by long-term exposure to glucose, sulfonylureas, or other pharmacological stimulators of insulin secretion.

### 4.4. Content of releasable insulin

Investigations on the mechanism of glucose-induced desensitization have repeatedly led to the conclusion that the desensitization was not sufficiently explained by a global exhaustion of insulin stores [18,20,94]. Nevertheless, reductions in islet insulin content by more than 80% have been reported to occur after *in vivo* and *in vitro* glucose exposure [20,65,70]. Such a massive decrease in insulin content suggests that β-cell exhaustion could well be a factor to consider in glucose-induced desensitization.

However, the relation between insulin content and desensitization seems to be complex. The biphasic time course of loss and replenishment of insulin stores of glucose-infused rats was not in parallel with the steadily increasing desensitization [20]. In single β-cells exposed for 9 days to various glucose concentrations, those β-cells that had been exposed to a moderately desensitizing glucose concentration (10 mM) had a higher insulin content than those exposed to a non-desensitizing (6 mM) or a strongly desensitizing (20 mM) glucose concentration [75]. In the latter study, however, no other reason could be identified for the desensitization than an imbalance between the increased rate of insulin synthesis and the even greater increase in the rate of insulin release, confirming observations made by Andersson *et al.* some 20 years earlier [95]. On the other hand, the reported rapid reversibility after a 24-hr glucose desensitization [17,18] argues against a major role of exhaustion in the decrease of secretory responsiveness.

A reconciliation between the hypotheses of glucose-specific desensitization and of β-cell exhaustion could be offered by the observation that in human islets, exposure to high glucose leads to a decrease of insulin gene transcription, due to a decreased binding of the glucose-dependent transcription factor PDX-1 [96]. On the other hand, however, most of the available evidence suggests that the reduction of β-cell insulin content by glucose-induced desensitization is accompanied not by a decreased, but rather by an increased rate of insulin synthesis. To sum up, it is undeniable that a decrease of insulin content occurs in glucose-induced desensitization, but it is also undeniable that there are clear dissociations between decreased insulin content and decreased secretory responsiveness.

There is no debate that insulin content is decreased by chronic exposure to FFA. The reason for this is not so much an acute stimulatory effect of FFA on secretion, but rather an increase in the basal rate of secretion without a concomitant increase in insulin synthesis [30]. FFA were reported to decrease insulin synthesis at the level of transcription by decreased expression of IDX-1/PDX-1 [97], but also to inhibit at the level of translation [31]. Mitochondrial oxidation seems to be a prerequisite for the effect of FFA on insulin synthesis [97]. It should be noted, however, that in the latter investigation insulin secretion was not decreased by FFA (palmitate, in that case) in spite of a strongly decreased insulin content.

With respect to drug-induced desensitization of insulin secretion, it was found repeatedly that the insulin content after *in vitro* desensitization was either not reduced or only moderately reduced. The insulin content of glibenclamide- and tolbutamide-desensitized rat islets was given as about 80% of control [38], the insulin content of glibenclamide-desensitized MIN6 cells was 77% of control [41], and that of tolbutamide-desensitized BRIN-BD11 cells was found to be unchanged [61]. In line with these observations, the insulin content of isolated islets after *in vitro* desensitization by imidazolines, tolbutamide, and quinine ranged between 75 and 100% [58]. In one report where a desensitization was induced by a comparatively low concentration of tolbutamide (50 μM) in the presence of a stimulatory glucose concentration (10 mM), a marked reduction of 45% was noted, but again it was concluded that this would not fully account for the extent of desensitization [39]. In an *in vivo* study using rats infused for 48 hr with tolbutamide and glucose, the reduction in secretion under a number of conditions (except for arginine stimulation, see Section 3.2.3.) was similar to the reduction of insulin content, which was about 50% [69].

In addition to the measurement of immunoreactive insulin, the amount of releasable insulin remaining after induction of desensitization was assessed by electron and light microscopy. Electron microscopy showed that culturing of mouse islets in the presence of 28 mM glucose for 7 days strongly reduced the number of secretory granules as compared with culturing in 5.5 or 3.3 mM glucose [98]. In contrast to the β-cells exposed to non-stimulatory glucose concentrations, those exposed to 28 mM glucose had a well-developed rough endoplasmic reticulum and an elaborate Golgi complex, a characteristic of increased biosynthetic activity [98]. Similarly, β-cells in tolbutamide-desensitized islets were found to be largely degranulated [58,99].

Islets cultured in the presence of the prototypic imidazoline, phentolamine, had a high content of secretory granules in the β-cells, not significantly different from that of control-cultured islets (Fig. 5). In these cells but not in control-incubated β-cells, a number of secondary lysosomes were present in the cytoplasm of phentolamine-desensitized β-cells, often associated with secretory granules (Fig. 5). β-Cells from islets cultured in the presence of alinidine (Fig. 5), idazoxan, quinine, or a strongly depolarizing $K^+$ concentration (40 mM) (not shown) were partially degranulated. The appearance of the endoplasmic reticulum and Golgi complex of these β-cells was unchanged, in marked contrast to tolbutamide-exposed β-cells.

The ultrastructural observation of a nearly complete tolbutamide-induced degranulation is in clear conflict with the above-mentioned measurements of immunoreactive insulin content, which showed either no or, at best, a partial loss of insulin. However, it is consistent with earlier morphological investigations, which found β-cells exposed to high concentrations of sulfonylureas to be largely degranulated [99–102]. In a study where both the insulin content and the degranulation in response to various sulfonylureas administered *in vivo* were measured, degranulation and loss of insulin were found to correlate, degranulation preceding the decrease in insulin content [103].

In view of the extent of the tolbutamide-induced degranulation, compared with that caused by the imidazolines or high $K^+$ [58], it is tempting to speculate that this may be a consequence of the reported direct effect of sulfonylureas on the secretory granules [63,104]. The difficulty with this argument is that similar direct effects have also been described for imidazolines [48,92]. Another ultrastructural feature that sets sulfonylurea-desensitized β-cells apart from β-cells desensitized by other depolarizing secretagogues is that of a cystically enlarged rough endoplasmic reticulum and of prominent Golgi stacks, indicating an increased biosynthetic activity as is typically evoked by exposure to high glucose (Fig. 5). The recent observation that the signaling pathway for the glucose stimulation of insulin synthesis is different from that of glucose-induced insulin secretion (Fig. 1), in that an increase in $[Ca^{2+}]_i$ plays no role, but rather a phosphoinositide-3-kinase- and stress-activated protein kinase 2 (SAPK2/p38)-mediated effect on the transcription factor PDX-1 is involved [105], explains why sulfonylureas do not stimulate insulin synthesis [41,99]. It remains puzzling why they mimic

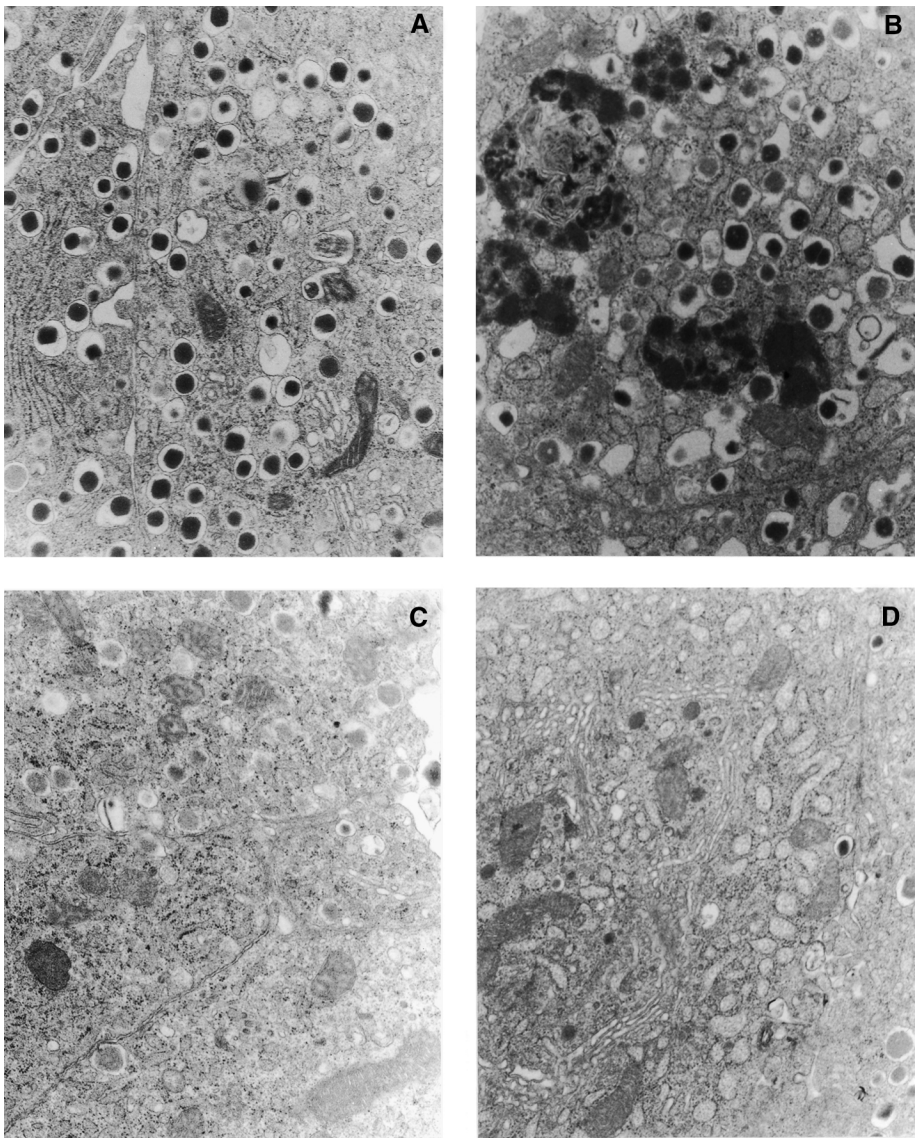


Fig. 5. Degranulation of pancreatic β-cells by induction of desensitization. Isolated pancreatic islets were cultured for 18 hr in RPMI 1640 with 5 mM glucose (A; control) or in the presence of 100 μM phentolamine (B), 100 μM alinidine (C), or 500 μM tolbutamide (D). In contrast to the strongly degranulated β-cells with enlarged endoplasmic reticulum and Golgi complexes after tolbutamide treatment (D), the β-cells were well granulated after phentolamine treatment and had prominent secondary lysosomes (B), whereas alinidine led to a partial degranulation, but no other characteristic features (C). For a quantitative evaluation, each β-cell was classified as being either well granulated or degranulated to account for the marked heterogeneity of the β-cell response to the stimuli [123]. The percentage (mean ± SEM) of degranulated β-cells was determined in 5 islets of medium size and was 15 ± 2 for phentolamine, 66 ± 4 for alinidine, and 86 ± 3 for tolbutamide. Under control conditions, the percentage of degranulated β-cells was 14 ± 2; after culture in the presence of 40 mM $K^+$, it was 58 ± 6. Magnification: 18,400×.

the effects of glucose stimulation on the ultrastructural appearance of the β-cell, whereas other depolarizing secretagogues do not.

In the past few years, there has been a significant advance in the knowledge of the exocytotic machinery in the β-cell [106–109]. However, this is yet to have an impact on research on desensitization, e.g. it would be interesting to know whether changes in function or abundance of a $Ca^{2+}$-activated protein (like synaptotagmin) occur under conditions where the $Ca^{2+}$ increase is largely undisturbed but secretion is inhibited markedly, as is the case with tolbutamide [85].

### 4.5. Extracellular signals

Changes in the β-cell environment can contribute to a reversible decrease in secretory responsiveness. It is well known that β-cells within islets have a greater secretory activity than single β-cells. This secretion-enhancing effect is at best partially due to the presence of non-β-cells in islets, since it can also be seen in pseudo-islets grown from MIN6 cells [110]. Thus, changes in β-cell to β-cell contacts or β-cell–matrix interactions could be an additional mechanism whereby a prolonged stimulation leads to desensitization. The observation that the secretory

responsiveness of β-cells was dependent upon the type of extracellular matrix they were attaching to, suggests that integrin-mediated signaling is important for insulin secretion [111], and it might be worthwhile studying the effect of chronic exposure to glucose and other secretagogues on this pathway. Also, human β-cells were shown to express an extracellular calcium-sensing receptor that seems to deliver some form of negative feedback to the secretory machinery [112]. A prolonged stimulation of secretion may well activate this mechanism, since secretory granules contain large quantities of calcium. A feedback signaling of insulin itself on insulin secretion was already postulated 20 years ago [113]. Originally, it was supposed that a negative feedback was exerted, but recent results suggest that insulin receptor signaling may be relevant for the β-cell to maintain the typical secretion pattern in response to glucose [114,115].

## 5. Prevention of desensitization

The initial evidence that the decreased secretory responsiveness after exposure to high glucose is functional in nature and not due to glucose toxicity was obtained by using diazoxide to uncouple secretion from the presence of high glucose. Diazoxide, when present during the exposure to high glucose, preserved the ability of the β-cells to respond adequately to a renewed glucose stimulus [116] and increased the response to a barium or arginine stimulus [65]. That diazoxide was not able to protect against a desensitization induced by 30 mM $K^+$ [65] is not unexpected since $K^+$ can depolarize β-cells whether or not $K_{ATP}$ channels are opened. However, the opening effect of diazoxide on β-cell $K_{ATP}$ channels may not be the only mechanism by which diazoxide prevents desensitization. In the *in vitro* studies, diazoxide was used at a concentration at which it is maximally effective to open $K_{ATP}$ channels, but at which it has also direct effects on mitochondria [117]. Effects of diazoxide, in addition to an opening of $K_{ATP}$ channels, may be relevant, since the diazoxide-induced block of secretion and the ensuing preservation of β-cell insulin content can only partially explain the preserved and occasionally even enhanced secretory responsiveness [65]. On the other hand, block of secretion with somatostatin led to similar results as with diazoxide, suggesting a decisive role of the secretory rate to induce desensitization [118]. Another agent described to prevent glucose-induced desensitization is the biguanide metformin [119]. Interestingly, the response to a renewed glucose stimulus was preserved, in spite of similarly reduced insulin contents in metformin-exposed and in control-desensitized islets. Of note, these effects were produced at therapeutically relevant concentrations of metformin [119].

In certain animal models of type 2 diabetes, an exaggerated β-cell responsiveness exists before the development of obesity, glucose intolerance, and manifest hyperinsulinemia [120]. Here, inhibition of secretion by diazoxide delayed the onset of diabetes [120]. Type 2 diabetes in humans is a progressive disease, even when treated with insulin or oral antidiabetic agents. When treatment with oral antidiabetics or insulin starts, there is a clear improvement in metabolic control, but upon continued treatment metabolic control is lost nearly linearly over the years. There is no doubt that current forms of treatment of type 2 diabetes are far from satisfactory [121,122]. A transient improvement of the endogenous secretory capacity was achieved in type 1 and type 2 diabetic patients by diazoxide treatment [118]. The hypothesis that β-cells require rest for the maintenance of proper function leads to the disquietening question as to whether oral antidiabetic agents that stimulate insulin secretion may contribute to the progressive loss of β-cell functions. The data provided by the UK Prospective Diabetes Study thus far do not suggest an accelerated loss of secretion, but the issue deserves further attention. In Sweden, a multicenter study specifically designed to clarify whether sulfonylureas are detrimental for the endogenous secretory capacity is under way.

## 6. Concluding remarks

First, there is convincing evidence that insulin stores are reduced after desensitization by various stimuli, but there is no study showing that a graded reduction in insulin content leads to a correspondingly graded reduction in secretion. In view of numerous discrepancies between insulin content and secretory responsiveness, the question as to whether the desensitization is due to exhaustion of the β-cell is still open.

Second, radioimmunological determination of the total islet insulin content may not always give meaningful information as to what amount of insulin is available for secretion. Measurements of the granulation state, in combination with immunohistochemistry, should permit one to check for possible subpopulations of secretory granules and for a role of heterogeneity of the β-cells.

Third, a molecular characterization of the functional state of the β-cell exocytotic machinery during desensitization has become a possibility. Such an investigation appears particularly desirable in cases where there is a discrepancy between a preserved $[Ca^{2+}]_i$ increase and an inhibited insulin release.

Fourth, care should be taken to choose models that are as close as possible to physiological conditions. For most experiments where a primary culture of no longer than 2 or 3 days is needed, normal β-cells or islets appear as the preferable model.

Finally, it is not yet clear whether glucose-induced desensitization is a correlate of the secretion abnormalities in type 2 diabetes. The same applies for the relation

between desensitization by sulfonylureas and secondary failure of sulfonylurea treatment. In addition to a lack of hypoglycemic potency, a lack of desensitizing effect would be an advantageous feature for potential antidiabetic drugs.

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