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Desensitization of insulin secretory response to imidazolines, tolbutamide, and quinine
I. Secretory and morphological studies

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Abstract

The desensitization of pancreatic B-cells against stimulation by insulin secretagogues that inhibit ATP-dependent K^+ channels (K_{ATP} channels) was investigated by measuring insulin secretion of perifused pancreatic islets. Additionally, the islet insulin content and the number of secretory granules per B-cell were determined. Prior to the measurement of secretion, islets were cultured for 18 h in the presence or absence of the test agents in a cell-culture medium containing 5 mM glucose. The effects of three imidazolines, phentolamine, alinidine, and idazoxan (100 μM each) were compared with those of the well-characterized sulfonylurea, tolbutamide (500 μM), and those of the ion channel-blocking alkaloid, quinine (100 μM). Insulin secretion was strongly reduced upon re-exposure to phentolamine, alinidine, tolbutamide, and quinine, whereas idazoxan, which stimulated secretion only weakly, had no significant effect. The imidazoline secretagogues phentolamine and alinidine induced a cross-desensitization against the stimulatory effect of tolbutamide and quinine. A long-term depolarization with 40 mM KCl was also able to induce a significant reduction of the secretory response to all of the above secretagogues. The insulin content of cultured islets was moderately, but significantly reduced by alinidine, whereas the reduction by phentolamine, tolbutamide, and quinine was not significant. In contrast to these observations, the ultrastructural examination revealed that tolbutamide-treated B-cells had a high degree of degranulation, whereas the other test agents and 40 mM KCl produced only a partial degranulation, except for phentolamine, which produced no significant degranulation at all. These results suggest that the desensitization of insulin secretion is a common property of all agents that stimulate insulin secretion by depolarisation of the plasma membrane. Depending on the specific secretagogue, additional mechanisms, proximal and distal to Ca^{2+} influx, appear to contribute to the desensitization (see Rustenbeck *et al.*, pages 1695–1703, this issue).

Keywords: Insulin secretion; Pancreatic islets; Desensitization; Imidazolines; Tolbutamide

1. Introduction

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 continually present or repeatedly applied. 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 [1,2]. Such a homologous desensitization has to be distinguished from a heterologous desensitization where application of one type of stimulus decreases also the response to other stimuli. This latter phenomenon is often due to effects at more distal steps in signal transduction, but may also involve early steps like changes in the G-protein subunit expression [3] proximal events like where signal pathways converge to elicit cellular responses such as secretion or contraction. Here, we studied the desensitization of insulin secretion by chronic exposure to pharmacological insulin secretagogues acting at B-cell ATP-dependent K^+ channels (K_{ATP} channels), comparing the effect of imidazolines with those of the sulfonylurea, tolbutamide and quinine.

Imidazolines are of interest as potential oral antidiabetic drugs because they enhance insulin secretion only in the presence of a stimulatory glucose concentration [4–6]. Like sulfonylureas, imidazolines inhibit the activity of the B-cell ATP-dependent K^+ channels (K_{ATP} channels) [7–9]. It is

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unclear at present whether imidazolines stimulate insulin secretion only by inhibition of K_{ATP} channels, probably partly due to a direct interaction with the pore-forming subunit [10,11], or by binding to a specific imidazoline receptor, which indirectly influences K_{ATP} channel activity and, possibly, elicits additional effects [12,13] by an as yet unknown signal transduction pathway. The hypothesis that a specific B-cell imidazoline receptor is involved in imidazoline-induced insulin secretion is based on the observations that enantiomeric compounds differ in their efficacy [14,15] and that an agonist-induced desensitization occurs after prolonged incubation in the presence of secretion-enhancing imidazolines [16].

Desensitization of insulin secretion, however, occurs also with other stimulators of insulin secretion. Most important is, of course, the desensitization following prolonged exposure to high glucose [17,18]. It is less well known that *in vitro* exposure of pancreatic islets to sulfonylureas such as tolbutamide or glibenclamide also causes a reversible impairment of insulin secretion [19,20]. This desensitization has been described to be selective for sulfonylureas [19,21,22] but other authors found that glucose-induced insulin secretion was impaired by previous long-term exposure of isolated islets to sulfonylureas [23,24]. The desensitization of insulin secretion is of clinical relevance, because it may be involved in the secretory abnormalities in type 2 diabetes [25,26] as well as in the secondary failure of sulfonylurea treatment of this disease [27,28]. To clarify whether imidazoline- and sulfonylurea-induced desensitization are different processes or are rather a common property of insulin secretagogues that block K_{ATP} channels, we compared the desensitization by imidazolines with that induced by tolbutamide. The ion channel-blocking action of tolbutamide is regarded to be specific for K_{ATP} channels and is mediated by a regulatory subunit of the K_{ATP} channel, termed sulfonylurea receptor [29,30].

Additionally, quinine was selected as a test agent. Quinine stimulates insulin secretion with considerable potency and efficacy [31,32], and hypoglycemia is a known side effect of quinine treatment [33,34]. The stimulatory effect of quinine is usually ascribed to its ability to block a number of potassium channels in the B-cell plasma membrane, most notably K_{ATP} channels, but also Ca^{2+} activated K^+ channels and delayed rectifier K^+ channels [35]. When it became clear that all of the above test agents induced a desensitization, the weakly effective imidazoline secretagogue, idazoxan [6,36] was included in this study.

2. Materials and methods

2.1. Materials

Phentolamine was kindly donated by Novartis (East Hanover, NJ, USA), alinidine by Boehringer Ingelheim (Ridgefield, CT, USA), and idazoxan by Research Biochemicals (Natick, MA, USA). Tolbutamide was purchased from Serva (Bay City, MI, USA) and quinine from Sigma (St. Louis, MO, USA). Collagenase P was obtained from Boehringer Mannheim (Indianapolis, IN). Cell-culture medium RPMI 1640 (without glucose) was from Life Technologies (Rockville, MD, USA) and fetal calf serum from Biochrom (Berlin, Germany). All other reagents of analytical grade were from Merck (West Point, PA, USA).

2.2. Tissue culture

Islets were isolated from the pancreas of albino mice by a conventional collagenase digestion technique. The freshly isolated islets were transferred into cell-culture medium RPMI 1640 with 10% fetal calf serum and cultured in a humidified atmosphere of 95% air and 5% CO_2. The RPMI medium had a lower glucose concentration than usual, namely 5 mM glucose, to minimize a possible depletion of the insulin stores. This was expected from the combined action of glucose and the insulin secretagogues, which were present during tissue culture to induce the desensitization. After 18 h of tissue culture, the secretory response to a renewed stimulation by the respective secretagogue was measured by perifusion of the preincubated islets. The interval between the end of the preincubation in the tissue culture medium and the start of the perifusion was about 30 min.

2.3. Measurement of insulin secretion and insulin content

Batches of 25 albino mouse islets that had been cultured either in the presence of one of the above-named insulin secretagogues or in RPMI 1640 alone were introduced into a purpose-made perifusion chamber thermostatically controlled at 37° and perifused with a HEPES-buffered Krebs-Ringer medium containing the respective secretagogue. The glucose concentration was 10 mM, because the stimulatory action of some imidazoline compounds such as alinidine or idazoxan is strongly dependent on an increased glucose concentration [6]. The insulin content in the fractionated effluate was determined by radioimmunoassay, using a locally produced antiserum, ^{125}I insulin (DuPont-NEN, Wilmington, DE, USA), and crystalline mouse insulin (Novo Biolabs, Danbury, CT, USA) as standard [37]. The percentage of changes in insulin secretion was calculated from the integrated insulin release over time. The insulin content of islets that had been cultured under the same condition as for the perifusion experiments was measured after washing the islets in fresh medium, homogenizing by hand on ice and desintegrating the secretory vesicles by freeze-thawing and sonication on ice.

2.4. Electron microscopy

Islets from 10 albino mice were pooled and cultured in batches of 50 islets under the same conditions as for the perifusion experiments. For each test agent, two preincuba-

tions were performed. At the end of the preincubation period, islets were fixed for electron microscopy [38]. In short, islets were immersed in a solution of para-formaldehyde (2%) and di-glutaraldehyde (2%) in cacodylate buffer (0.1 M, pH 7.3), post-fixed in osmium tetroxide (1%) for 1 h and embedded in epoxy resin. Ultrathin (50 nm) sections of the islets were cut by an ultramicrotome (Ultracut, Leica, Deerfield, IL, USA), placed on nickel grids and contrast-stained with uranyl acetate and lead citrate. Transmission electron microscopy was performed by using a Zeiss EM 9 electron microscope (Oberkochen, Germany). For each type of pretreatment five medium-sized islets (diameters about 150 μm) were analysed quantitatively, using adjacent sections. The analyzed islets contained 70–100 B-cells per section, which were chosen for the quantitation of the secretory granules. B-cells containing more than 50 secretory granules in the plane of section were regarded as being well granulated and prevailed in control-cultured islets.

2.5. Data handling and statistics

Additional calculations and statistics were performed by Prism and Instat software (GraphPad). Without further specifications *t*-test stands for Student's unpaired two-sided *t*-test.

3. Results

3.1. Desensitization of insulin secretion against renewed stimulation by the same secretagogue

In the first series of experiments it was tested how a long-term exposure of pancreatic islets to the test agents influenced the secretory response to a subsequent challenge with the same compound. For all experiments the compounds were used at their maximally effective concentration, which is ~100 μM for phentolamine, alinidine, and quinine, and ~500 μM for tolbutamide.

Both, phentolamine and alinidine strongly reduced the secretory response to renewed stimulation with the respective compound. (Fig. 1A and B). Interestingly, the phentolamine-induced increase of secretion by control-incubated islets receded in spite of the continued presence of phentolamine, in contrast to the nearly irreversible effect of phentolamine on freshly isolated islets. The amount of insulin released from phentolamine-desensitized islets was 32.2 ± 6.2% of the corresponding value of control-cultured islets. The perifusion with alinidine was followed by a depolarisation with 40 mM K^+ to ascertain that alinidine-desensitized islets were principally able to release stored insulin upon depolarization. The depolarization-induced secretion was reduced to 36.9 ± 5.7% of the value of control-cultured islets, whereas the alinidine-induced secretion was reduced to 19.2 ± 1.8% of the corresponding control value (Fig. 1B).

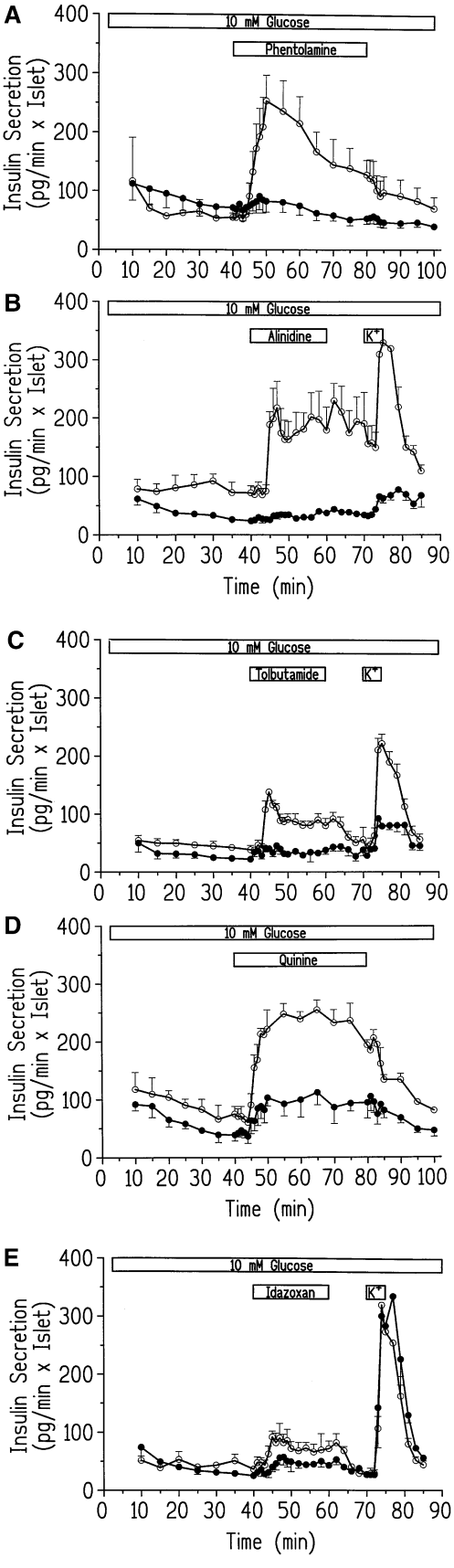
A desensitization was also induced by tolbutamide and quinine (Fig. 1C and D). As with phentolamine and alinidine, the increase of secretion upon re-exposure to these agents failed to achieve statistical significance. The amount of insulin released from desensitized islets during re-exposure to tolbutamide was 39.5 ± 7.9% of the control value and that of quinine-desensitized islets was 42.2 ± 10.1%. The stimulatory effect of a subsequent K^+ depolarization on the secretion of tolbutamide-desensitized islets was reduced to 56.1 ± 8.7% of the value of control-cultured islets (Fig. 1C).

In summary, all test agents significantly ($P \leq 0.02$) increased insulin secretion by control-cultured islets but achieved no or only a marginally significant increase (P between 0.08 and 0.66) when the islets had been previously exposed to the same compound. Relative to the secretory rate immediately before perifusion with the secretagogues, the increase of secretion with control-cultured islets was 258 ± 25% (mean value for all four test agents), whereas the corresponding increase achieved by desensitized islets was 152 ± 17%, which was significantly smaller ($P = 0.017$, *t*-test). The secretory rate immediately before perifusion with the secretagogues (i.e. in the presence of 10 mM glucose) was only non-significantly reduced by desensitization to 65.6 ± 14.5% of the normalized value of control-cultured islets (100 ± 15%). The rank order of desensitizing efficacy was alinidine > phentolamine > tolbutamide = quinine.

Idazoxan stimulated insulin secretion from control-cultured islets with fast onset and offset (Fig. 1E), but the magnitude of secretion enhancement was smaller than with freshly isolated islets [6], thus the increase in secretion achieved only marginal significance ($P = 0.06$, *t*-test). After culture for 18 h in the presence of 100 μM idazoxan a renewed stimulation with 100 μM idazoxan led to a secretory response, which was non-significantly reduced to 64.6 ± 13.2% of control. When the responses were compared as the relative increase over the last prestimulatory rate they were practically identical, 155.8 ± 15.8% for idazoxan-pretreated islets and 157.7 ± 15.3% for control-cultured-islets. It is noteworthy that there was a strong increase in secretion in response to a subsequent K^+ depolarization both with control-cultured and with idazoxan-pretreated islets (Fig. 1E), in clear contrast to the significantly reduced effect of the K^+ depolarization after desensitization by alinidine and tolbutamide (Fig. 1B and C).

3.2. Desensitization of insulin secretion by imidazolines against stimulation by tolbutamide and quinine

The question whether desensitization by the imidazolines was specific for the insulinotropic action of these compounds was addressed by culturing islets in the presence of either phentolamine or alinidine and measuring then the secretory response to a perifusion with the non-imidazoline secretagogues, tolbutamide, and quinine. After desensitization by phentolamine, both tolbutamide- and quinine-induced secretion were virtually abolished (Fig. 2A). Likewise, a desensitization by alinidine abolished tolbutamide-induced insulin secretion (Fig. 2B). During perifusion with quinine, the secretion of alinidine-desensitized islets was



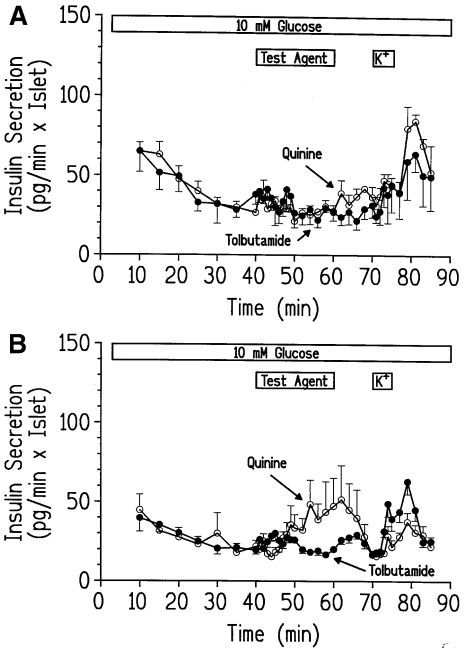


Fig. 2. Desensitization of islets cultured in the presence of phentolamine (A) or alinidine (B) to the stimulatory action of tolbutamide and quinine. Isolated mouse pancreatic islets were cultured for 18 h in cell-culture medium RPMI 1640 containing 5 mM glucose and either phentolamine or alinidine (100 μM each). Insulin secretion was then measured by perifusing the islets with a Krebs-Ringer medium containing 10 mM glucose and either 500 μM tolbutamide (closed circles) or 100 μM quinine (open circles). Neither of the compounds was able to significantly increase insulin secretion of imidazoline-pretreated islets. The data are means ± SEM of four experiments.

higher, but did not achieve significance with the given number of experiments (Fig. 2B). Only the subsequent K^+ depolarization produced a small, but significant increase of secretion. It thus appears that tolbutamide- and quinine-induced secretion is even more strongly affected by phentolamine- and alinidine-desensitization than by the desensitization evoked by tolbutamide and quinine themselves.

3.3. Desensitization of insulin secretion by a high potassium concentration

All test agents are known to depolarize the B-cell plasma membrane [39]. Thus the question arose whether a long-term depolarization as such would be sufficient to

Fig. 1. Desensitization of insulin secretion by culture of pancreatic islets in high concentrations of phentolamine (A), alinidine (B), tolbutamide (C), quinine (D), and idazoxan (E). Isolated mouse pancreatic islets were cultured for 18 h in cell-culture medium RPMI 1640 containing 5 mM glucose plus 100 μM of the named secretagogues (tolbutamide 500 μM) 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 (closed circles). The secretory response was compared with that of islets, which had been control-cultured (open circles). The data are means ± SEM of four experiments.

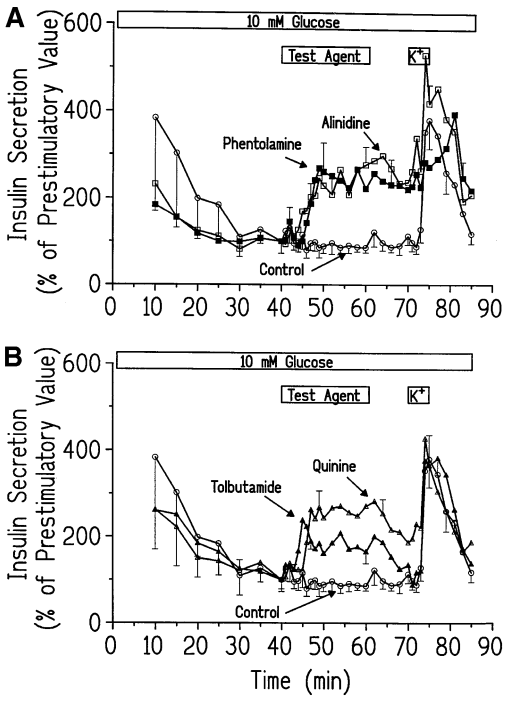


Fig. 3. Desensitization of insulin secretion by culture in high potassium. Isolated mouse pancreatic islets were cultured for 18 h in cell-culture medium RPMI 1640 containing 40 mM K^+. Insulin secretion was then measured by perifusing the islets with a Krebs-Ringer medium containing 10 mM glucose and 100 μM phentolamine or 100 μM alinidine (A) or 10 mM glucose and 500 μM tolbutamide or 100 μM quinine (B). Islets perifused with 10 mM glucose served as control. The data are means ± SEM of three experiments.

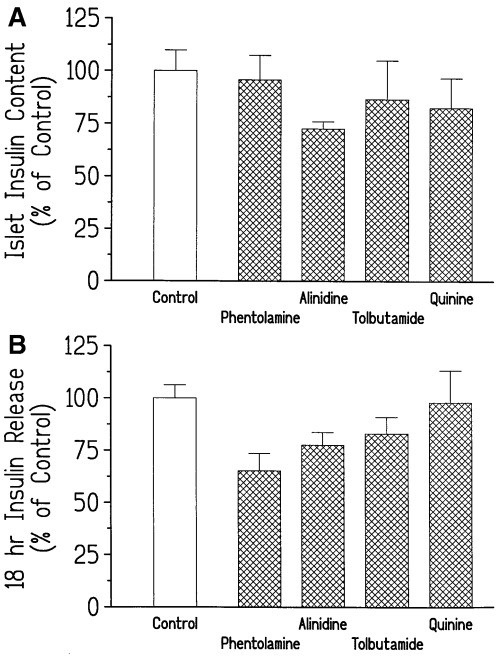


Fig. 4. Insulin content in islets (A) and cell-culture medium (B) after culture in the presence of insulin secretagogues or cell-culture medium alone (control). The conditions were the same as for the perifusion experiments, i.e. isolated mouse pancreatic islets were cultured for 18 h in cell-culture medium RPMI 1640 containing 5 mM glucose plus 100 μM of the named secretagogues (tolbutamide 500 μM) or no secretagogue (control culture). The insulin content of the islets was measured to check whether a depletion of insulin stores could explain the desensitization of secretion, the insulin content in the cell-culture medium was measured as an integral of secretion during the cell culture. The islet insulin content was significantly reduced by alinidine ($P = 0.03$, *t*-test), the insulin content in the medium was significantly reduced by phentolamine and alinidine ($P = 0.003$ and 0.02, respectively, *t*-test). The data are means ± SEM of six experiments.

induce a desensitization. To test this hypothesis, islets were cultured in RPMI cell-culture medium with a high K^+ concentration (40 mM) for 18 h. Then, the secretory response to phentolamine, alinidine, tolbutamide, and quinine was measured. The K^+-pretreated islets were still able to achieve a significant increase of secretion in response to the secretagogues and in response to a subsequent K^+ depolarization (Fig. 3A and B). Interestingly, the K^+ depolarization after perifusion with phentolamine elicited a markedly retarded secretory response (Fig. 3A). On the whole, the secretagogues increased secretion of the K^+-pretreated islets to 183 ± 8.6% of the prestimulatory level (mean value for all four test agents). When comparing this response to that achieved by the control-cultured islets shown in Fig 1A–D (mean increase to 258 ± 25%), a significant difference becomes apparent ($P = 0.032$, *t*-test). Thus, a prolonged depolarization by high K^+ is sufficient to desensitize insulin secretion.

3.4. Insulin content in pancreatic islets and cell-culture medium after induction of desensitization

The simplest reason for the observed decrease in secretory responsiveness would be a reduced insulin content of the pretreated islets. Therefore, isolated islets were cultured under the same conditions as for the secretion studies and their insulin content was determined at the end of the 18-h period. In addition, the insulin content in the cell-culture medium was determined as a measure of the secretion during the pretreatment period. The amount of insulin secreted by control-incubated islets during the pretreatment period corresponded to 9.7% of the islet insulin content and to a secretory rate of about 18 pg per islet and minute. Alinidine was the only compound that significantly reduced the insulin content of cultured islets (reduction by 27%, $P = 0.027$, *t*-test), whereas the reduction by phentolamine, tolbutamide, and quinine was not significant (Fig. 4A). Unexpectedly, none of the test agents increased the insulin content of the cell-culture medium over control levels, rather, phentolamine and alinidine caused a significant decrease by ~25% (Fig. 4B).

3.5. Content of insulin secretory granules after preincubation of pancreatic islets with imidazolines and other insulin secretagogues

The unexpectedly small changes in islet insulin content after pretreatment led to the question whether more subtle

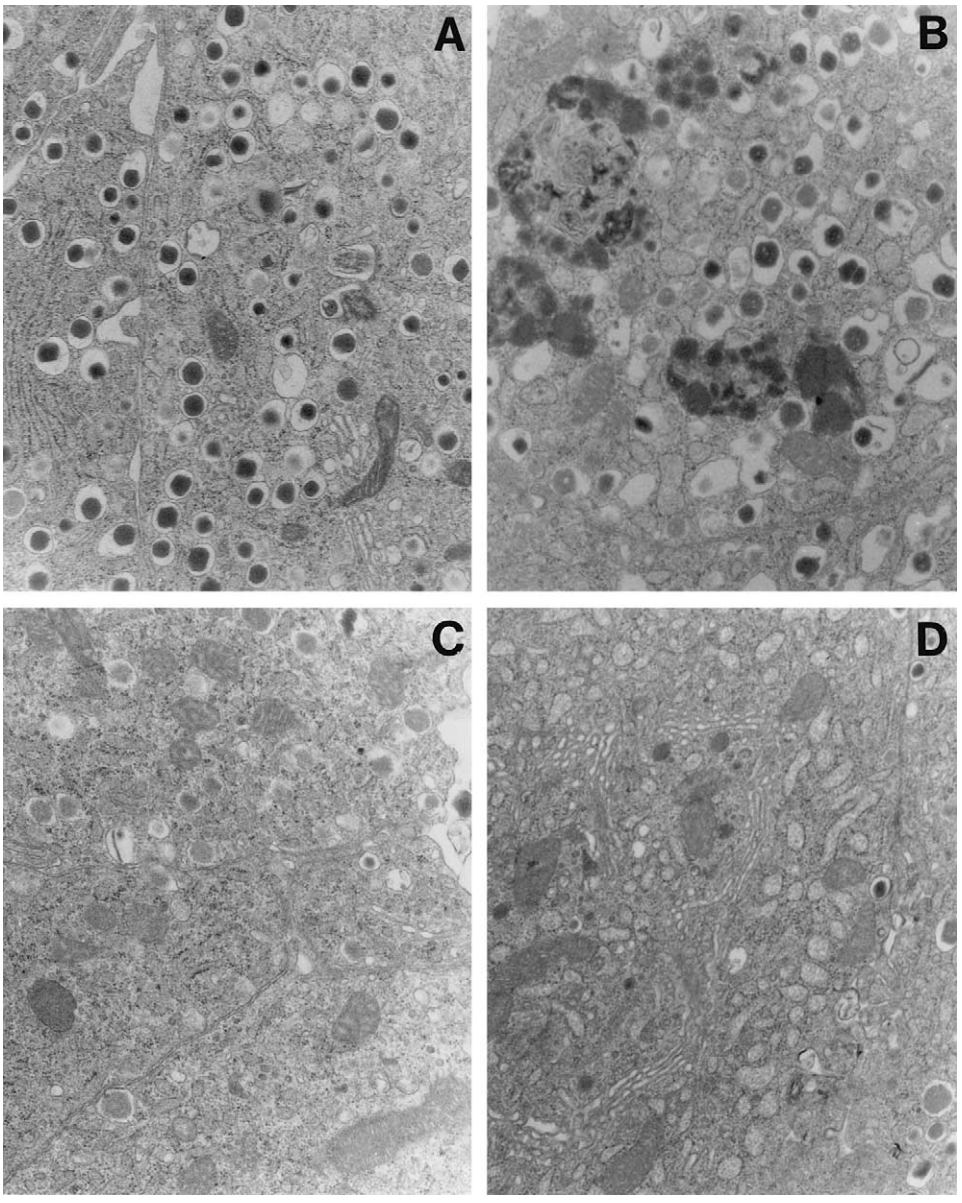


Fig. 5. Degranulation of pancreatic B-cells during induction of the desensitization. Ultrastructure of pancreatic islets after tissue culture for 18 h in RPMI 1640 with 5 mM glucose = control (A). Ultrastructure after culture in the presence of 100 μM phentolamine (B), 100 μM alinidine (C), or 500 μM tolbutamide (D). In contrast to the strongly degranulated B-cells with enlarged endoplasmic reticulum and Golgi complexes after tolbutamide treatment (D) the B-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). 18.400× magnification.

changes in the B-cell morphology, in particular in the granulation state, were induced by the secretagogues. This was assessed by using electron microscopy of islets that had been cultured under the same conditions as for the secretion measurements. Under control conditions all endocrine cell types in the isolated islets were ultrastructurally well preserved. Especially the B-cells were mostly well granulated and the cell organelles involved in the synthesis of insulin, such as the cisternae of the rough endoplasmic reticulum and the Golgi complex were developed to the same extent as in mouse B-cells under *in vivo* conditions (Fig. 5A). In secretagogue-incubated islets a small number of B-cells appeared damaged, but only after culture in the presence of idazoxan the percentage of damaged B-cells (~10%) appeared non-negligible. The other endocrine cell types of the islets were not affected by the secretagogues.

Surprisingly, islets that had been cultured in the presence of phentolamine still had a high content of secretory granules in the B-cells (Fig. 5B), not significantly different from that of control-cultured islets (Table 1). Like in controls, a high number of these secretory granules was situated adjacent to the plasma membrane. However, there was one feature that was unique for phentolamine-desensitized islets: a number of secondary lysosomes was present in the

Table 1
Degranulation of B-cells in pancreatic islets exposed to various types of K_{ATP} channel blockers

Phentolamine	Alinidine	Idazoxan	Tolbutamide	Quinine
15.2 ± 2.5	65.8 ± 3.9	49.1 ± 7.3	86.2 ± 2.8	64.6 ± 6.7

Isolated mouse pancreatic islets were cultured for 18 h in tissue culture medium RPMI 1640 containing 5 mM glucose and 100 μM of the named secretagogues (tolbutamide 500 μM). Islets were then fixed, embedded in epoxy resin, and examined by electron microscopy. Given is the percentage (mean ± SEM) of degranulated B-cells in 5 islets of medium size, 100% meaning that every single B-cell in an islet was degranulated and 0% meaning that none of the B-cells in an islet was degranulated). Under control conditions (no secretagogue added to the tissue-culture medium) the percentage of degranulated B-cells was 14.2 ±2.2%, after culture in the presence of 40 mM K^+ 58.3 ± 6.4%. Except for phentolamine, all secretagogues significantly increased the number of degranulated B-cells ($P \leq 0.01$, *t*-test). Degranulation by tolbutamide was significantly more extensive than that by the other secretagogues ($P < 0.02$, *t*-test).

cytoplasm, often associated with secretory granules (Fig. 5B). B-cells from islets that had been cultured in the presence of alinidine (Fig. 5C), idazoxan, or quinine were partially degranulated (Table 1), but had no other functional characteristics distinguishing them from control-incubated islets. The same applied for islets preincubated in a high K^+ concentration (40 mM). Tolbutamide-treated islets showed a strong degranulation of the B-cells (Table 1) and at the same time a cystic enlargement of the rough endoplasmic reticulum and a well-developed Golgi complex, all of which are indicative of a marked stimulation (Fig. 5D). Although there were only few secretory granules left in tolbutamide-pretreated B-cells, a number of small clear vesicles was present in the cytoplasm, often situated in the vicinity of the dilated cisternae (Fig. 5D).

4. Discussion

Four of the five insulin secretagogues selected for this study (the imidazolines phentolamine and alinidine, the sulfonylurea tolbutamide and the cinchona alkaloid quinine), induced a desensitization of insulin secretion in that secretion in response to one of these compounds was smaller when the islets had been previously exposed to the same drug. A desensitization could not be verified after pretreatment with the imidazoline idazoxan, which is a weakly effective insulin secretagogue. Although it is conceivable that each type of secretagogue (imidazoline, sulfonylurea, quinine) activated a specific mechanism of desensitization, the results of the present secretion measurements are more easily compatible with the notion that the prolonged stimulation by these secretagogues produced a generalized desensitization. This view is based on three observations: (i) secretion in response to high K^+ subsequent to re-exposure to alinidine and tolbutamide was clearly reduced; (ii) there was a cross-desensitization in that imidazoline-desensitized islets were also refractory against stimulation by tolbutamide and quinine; (iii) a prolonged depolarization by high K^+ mimicked at least part of the secretagogue-induced desensitization.

The most straightforward explanation for a generalized desensitization of secretion would be that insulin stores became exhausted because of the long-lasting stimulation of secretion during pretreament. This is rendered unlikely by the observations that (i) the insulin content in the preincubation media was not increased, but rather unchanged or even slightly reduced and that (ii) the insulin content of the islets was only moderately reduced. These observations however, are in contrast to the ultrastructural appearence of the desensitized B-cells. Here, desensitization by all agents except for phentolamine led to a significant degranulation, which was most prominent with tolbutamide.

It appears that there is a systematic discrepancy between the results obtained from measurements of insulin content and those of B-cell degranulation, at least after stimulation by sulfonylureas. Similar to our data, determination of the insulin content of glibenclamide- and tolbutamide-desensitized rat islets gave values of about 80% of control [28]. The insulin content of glibenclamide-desensitized MIN6 cells was 77% of control [40] and that of tolbutamide-desensitized BRIN-BD11 cells was found to be unchanged [41]. In contrast, morphological examinations of B-cells exposed to high concentrations of sulfonylureas described them to be largely degranulated, suggesting a nearly complete release of stored insulin [42–44]. In an early *in vivo* study, where both the insulin content and the degranulation in response to sulfonylureas were measured concomitantly at various time points, degranulation and loss of insulin were found to correlate, but the degranulation clearly preceded the loss of insulin [45]. For example, after glibenclamide treatment the insulin content was virtually unchanged when the number of secretory granules was halved and was still ~50% when the number of secretory granules had already reached its minimal value of 10%. A similar course of events may have occurred during the pretreatment until to the onset of the desensitization.

The desensitization may well have begun early during the 18 h pretreatment period such that the initial increase in insulin release was effectively offset. In this context the earlier observation that the stimulatory effect of tolbutamide *in vitro* turned into an inhibition after about 1 h [21] may be regarded as the onset of the tolbutamide-induced desensitization. Actually, the first sign of an inhibitory action of tolbutamide may appear as early as 6 min after exposure to the drug [46]. The observation that phentolamine and alinidine led even to a decrease of secreted insulin during the pretreatment, but not to a compensatory increase in islet insulin content seems puzzling. An explanation is offered by the occurrence of secondary lysosomes in phentolamine-desensitized B-cells, which points to an intracellular degradation of insulin by crinophagy [47]. However, there was no crinophagy apparent in alinidine-desensitized B-cells, which were significantly degranulated in contrast to phentolamine-pretreated B-cells.

It is noteworthy that tolbutamide induced a significantly more extensive degranulation than the other secretagogues including high K^+, suggesting that mechanisms in addition to depolarization and Ca^{2+} influx may contribute to the tolbutamide-induced secretion [48,49]. Thus, a lack of releasable insulin may play a role in the tolbutamide-induced desensitization, but certainly not in the phentolamine-induced desensitization. With regard to the other secretagogues, it may be possible that a subfraction of readily releasable secretory granules [44,50] was critically reduced, leading only to minor changes in the total insulin content. At this point, however, the originally opposite concepts of B-cell exhaustion (lack of insulin) vs. B-cell desensitization (functional change in signal transduction) converge.

Investigations on the mechanism of glucose-induced desensitization have also shown that the desensitization was not sufficiently explained by a global exhaustion of insulin stores [51–53]. Because the common pathway of nutrient stimuli and the secretagogues used in the present study consists in the depolarization by block of K^+ efflux and the resultant Ca^{2+} entry into the B-cells, a critical role for Ca^{2+} to induce the desensitization would not be unexpected. This view is supported by our observation that culture in high K^+ was sufficient to desensitize insulin secretion, even though the decrease in responsiveness was somewhat smaller than after culture with the secretagogues. It is also compatible with the cross-desensitization of phentolamine- and alinidine-pretreated islets against stimulation by tolbutamide or quinine. Such a cross-desensitization after phentolamine exposure was not observed in an investigation using BRIN-BD11 cells [54]. This may be due either to the lower concentration of phentolamine in these experiments or to peculiarities of this cell line, since our measurements of $[Ca^{2+}]_i$ in phentolamine-desensitized B-cells strongly support the notion of a cross-desensitization by phentolamine [39].

The observation that phentolamine but not idazoxan induced a marked desensitization concurs with earlier investigations on imidazoline-induced desensitization [16]. Because at that time idazoxan was found not to stimulate insulin secretion and not to antagonize the effects of other imidazolines in islets [5,55] it was suggested that idazoxan was not a ligand for the putative islet imidazoline receptor [16]. In contrast, the desensitization by those imidazolines that were effective insulin secretagogues and K_{ATP} channel blockers such as efaroxan and phentolamine was regarded to represent an agonist-induced desensitization where prolonged receptor occupancy had led to a specific down-regulation in the signal transduction pathway coupled to the hypothetical islet imidazoline receptor [16,56].

In later investigations, however, idazoxan was found to have a significantly blocking effect on K_{ATP} channel activity in pancreatic B-cells [36] and to elicit a marked depolarization of the B-cell plasma membrane [6]. These observations were compatible with competition binding to nonadrenergic imidazoline binding sites at insulin-secreting cells where idazoxan showed the same biphasic displacement of labeled clonidine as displayed by the imidazolines with undisputed insulinotropic efficiency [36]. They also fit to the present result that idazoxan leads to a partial degranulation of B-cells. Thus, the weak insulinotropic effect of idazoxan [6,57] appears to be due to the fact that for some reason idazoxan elicits only a modest increase of $[Ca^{2+}]_i$ [6,58]. Correspondingly, the virtually unchanged secretory responsiveness after idazoxan preincubation, in particular the preserved response to K^+ depolarization, may be explained not so much by the low affinity of binding to the putative B-cell imidazoline receptor, but rather by the small insulinotropic effect as such.

Quinine was included in this study because its insulinotropic effect is clearly a consequence of its block of K^+ permeability [32,59], and because it is unlikely to interact with binding sites for sulfonylureas and imidazolines other than the K_{ATP} channel. The ability of quinine to induce a desensitization of secretion was thus a decisive result to point to a more generalized mechanism of desensitization than originally expected. The moderate extent of desensitization, similar to the one induced by exposure to 40 mM K^+ may indicate that mechanisms in addition to an increase in $[Ca^{2+}]_i$ contribute to the desensitization produced by the other secretagogues. Our observation that pretreatment with the secretagogues was accompanied by occasional B-cell damage is reminiscent of the recent report that prolonged *in vitro* exposure to tolbutamide promoted apoptosis in cultured islets [60]. However, the B-cell damage, which was not seen after sulfonylurea exposure *in vivo* [61] does not seem to be relevant for the reduced secretory responsiveness, because (i) the number of affected B-cells was generally small; and (ii) there was no correlation between the degree of desensitization produced by a secretagogue and the extent of B-cell damage.

To summarize, the present observations permit the conclusion that any measure that leads to a prolonged depolarization of the B-cells will eventually induce a desensitization of insulin secretion. With respect to glucose-induced insulin secretion this conclusion lends support to the view that the well-known oscillatory pattern of glucose-induced changes in membrane potential and $[Ca^{2+}]_i$ may serve to delay the onset of desensitization [62]. With respect to pharmacological secretagogues, additional effects are likely to contribute to the desensitization and to diminish the secretory responsiveness beyond the extent established by long-lasting depolarization alone, depending on the specific secretagogue. To clarify these mechanisms, desensitization-induced changes in K_{ATP} channel function and $[Ca^{2+}]_i$ were investigated as detailed in the companion paper [39].

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