Inhibition of Insulin Secretion by Betagranin, an N-terminal Chromogranin A Fragment*

Received for publication, January 26, 2007 Published, JBC Papers in Press, February 8, 2007, DOI 10.1074/jbc.M700788200

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Betagranin, an N-terminal fragment of chromogranin A, results from a proteolytic processing, and is co-secreted with insulin. While other chromogranin A-derived peptides negatively modulate hormone secretion, the role of betagranin in pancreatic β-cells is so far unknown. We have recently shown that pancreatic islet betagranin levels are down-regulated in obese, leptin-deficient mice. In the present study, we have investigated the distribution of betagranin in primary mouse islets and cells of the MIN6 line and have evaluated its effects on insulin secretion. We showed that betagranin co-localizes with insulin within secretory granules and strongly inhibited insulin secretion in response to both glucose and potassium, by blocking the influx of calcium. The data demonstrated a hitherto unknown inhibitory effect of betagranin on insulin secretion.

Betagranin is a 21-kDa peptide of the insulin granule, closely related to chromogranin A (CgA) of the adrenal medulla (1). It corresponds to the N-terminal fragment of CgA and results from post-translational proteolytic processing (2). CgA is an acidic glycoprotein that is ubiquitously expressed in the secretory granules of endocrine and neuroendocrine cells. Secretion of CgA and CgA-derived peptides in response to secretagogues usually parallels the secretion of the resident hormone (3). CgA contains multiple sites with two adjacent basic residues that are potential sites for proteolytic processing. The extent of CgA processing is highly tissue-specific (4). Betagranin is most abundant in parathyroid, pituitary, and endocrine pancreas (5). In the latter tissue, it is derived from CgA by a pathway indistinguishable from that converting proinsulin into insulin (2). Both betagranin (rat CgA 1–128) and pancreastatin (rat CgA 281–332) are localized within the insulin-containing β-cells, and represent the major and stable end products of CgA processing. Betagranin is released with insulin in response to insulinotropic stimuli, whereas pancreastatin is mainly released in the form of a 45 kDa intermediate, reflecting its slower conversion (6).

The role of betagranin in β-cells is still unknown. Other CgA-derived peptides are thought to function as modulators of endocrine cell secretory activity via autocrine, paracrine, or hormonal mechanisms (7, 8). Pancreastatin inhibits insulin secretion (9) and vasostatins, another form of N-terminal CgA fragments, inhibit constriction of isolated vessels (10, 11).

Sanchez et al. (12) have recently shown that the betagranin levels of pancreatic islets were significantly down-regulated in leptin-deficient mice compared with wild-type mice. Leptin deficiency results in severe obesity, hyperinsulinemia, and hyperglycemia, a syndrome resembling type 2 diabetes. Initially discovered for its central role in hypothalamic regulation of food intake and energy expenditure, meanwhile leptin has also been shown to have multiple peripheral actions (13, 14). Leptin treatment of wild-type mice and isolated rodent and human islets has been shown to inhibit insulin secretion (15–18), probably via both actions on the central nervous system and direct effects on β-cells (19). However, the latter effect remains to be demonstrated and the underlying mechanisms shown (20).

The present study investigated the distribution of betagranin in mouse insulin-producing cells and its effects on insulin secretion. Our results demonstrate that betagranin inhibits glucose-stimulated insulin secretion, suggesting an autocrine negative role in the control of insulin release.

EXPERIMENTAL PROCEDURES

Materials—MIN6 cells were obtained from Prof. P. Halban (University of Geneva). The mouse insulin enzyme-linked immunosorbent assay kit from Mercodia (Uppsala, Sweden) was used for quantification of secreted insulin. A detergent-compatible protein assay (Bio-Rad) was used to quantify the amount of total protein in samples. Endotoxin levels were measured using the kinetic chromogenic limulus amebocyte lysate test from Charles River Endosafe (Kent, UK), according to the manufacturer’s instructions. Rabbit antiserum raised against a C-terminal synthetic betagranin peptide (CAE VPS

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† Supported by grants from the Swiss National Science Foundation (31-0000-109420), the Juvenile Diabetes Research Foundation (1-2005-46 and 1-2007-158), Novo Nordisk, and the Geneva Program for Metabolic Disorders (GeMet).

‡ Supported by grants from the Swiss National Science Foundation (SNSF 31-0000-109420), the Juvenile Diabetes Research Foundation (1-2005-46 and 1-2007-158), Novo Nordisk, and the Geneva Program for Metabolic Disorders (GeMet).

§ Supported by grants from the Swiss National Science Foundation (SNSF 31-0000-109420), the Juvenile Diabetes Research Foundation (1-2005-46 and 1-2007-158), Novo Nordisk, and the Geneva Program for Metabolic Disorders (GeMet).

¶ Supported by grants from the Swiss National Science Foundation (SNSF 31-0000-109420), the Juvenile Diabetes Research Foundation (1-2005-46 and 1-2007-158), Novo Nordisk, and the Geneva Program for Metabolic Disorders (GeMet).

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Inhibition of Insulin Secretion by Betagranin

R.D. T. ME) was obtained from Eurogentec (Seraing, Belgium). Rabbit anti-calreticulin antiserum was kindly provided by Dr. K. H. Krause (Geneva University Hospital).

**Mice Treatment**—Wild-type female C57BL/6 mice and C57BL/6 lep/lep mice were given a standard diet (B+K Universal, Hull, UK) and water ad libitum. Groups of six mice were injected (intraperitoneal) daily during 10 days with 1 mg/kg body weight recombinant mouse leptin (PeproTech, London). Controls received 10 ml/kg body weight saline. Food intake and bodyweight were measured daily. Mice were killed with an overdose of carbon dioxide. Pancreatic islets were isolated by collagenase digestion as described in Ref. 17. Islets were snap-frozen in liquid nitrogen and stored at −80 °C until analysis. All animal studies were approved by the local ethical review committee and were carried out in accordance with the United Kingdom government regulations and the National Institutes of Health guidelines on the care and welfare of laboratory animals.

**Isolation of Pancreatic Islets**—Islets of Langerhans were isolated from control C57BL/6 mice by collagenase digestion and Ficoll purification, as described previously (21). The freshly isolated islets were immediately fixed for either immunofluorescence or immunoelectron microscopy, as described below.

**SDS-PAGE, Western Blotting, and Mass Spectrometry**—Proteins were separated by SDS-PAGE according to Laemmli (22). Polyacrylamide gels were either stained with the fluorescent stain Deep Purple (GE Healthcare, Uppsala, Sweden) or transferred onto a polyvinylidene fluoride membrane (Bio-Rad). For Western blot analysis, anti-betagranin (1:2000) and anti-calreticulin (1:5000) polyclonal rabbit antibodies were used. Horse-radish-peroxidase-conjugated goat anti-rabbit IgG (1:2000) was used as secondary antibody (Dako, Glostrup, Denmark). Blots were developed using the enhanced chemiluminescence detection system (Roche Applied Science). For protein identification by mass spectrometry, bands were excised from Deep Purple-stained SDS-PAGE gels and analyzed using a 4700 MALDI-TOF/TOF tandem mass spectrometer (Applied Biosystems) as described in Ref. 23.

**Cell Culture**—MIN6 cells (passage 11–21) were cultured in Dulbecco’s modified Eagle’s medium supplemented with 4.5 g/liter glucose, 15% (v/v) fetal bovine serum, 71 μM β-mercaptoethanol, and 100 units/ml penicillin and 100 μg/ml streptomycin. The cells were grown in a humidified atmosphere of 95% air/5% CO₂. Cells were passaged and harvested using trypsin/EDTA. Cell viability was assessed by the trypan blue dye exclusion method.

**Immunoelectron Microscopy**—For immunolabeling, pellets of freshly trypsinized cells and isolated pancreatic islets were washed twice in 0.1 M phosphate buffer and fixed 5 min at room temperature in 4% (w/v) paraformaldehyde and 0.1% (w/v) glutaraldehyde and then 60 min in 4% (w/v) paraformaldehyde, and cooled on ice. Small blocks of gelatin-embedded cells were embedded in 3 μl sucrose, frozen in liquid nitrogen, and sectioned with a cryoultramicrotome (Leica, Wetzlar, Germany), as reported previously (24, 25). Single immunolabeling for betagranin was carried out using a rabbit polyclonal serum (Eurogentec) diluted 1:50, and revealed with a goat anti-rabbit IgG serum coupled to 10 nm gold particles diluted 1:20 (British Biocell International, Cardiff, Wales). Double immunolabeling for betagranin and insulin was performed using the conditions described above plus a mouse monoclonal against insulin (courtesy of Dr. M. J. Storch), diluted 1:100, and revealed with a goat anti-mouse IgG serum coupled to 15-nm gold and diluted 1:20 (British Biocell International). Controls included exposure of the sections to only the gold-conjugated goat antibodies against either rabbit or mouse IgGs, whichever appropriate. None of these incubations resulted in a sizable, specific staining of the sections. All cryosections were screened and photographed in a CM10 electron microscope (Philips, Eindhoven, The Netherlands).

**Immunofluorescence**—MIN6 and pancreatic islets were fixed for 60 min at room temperature in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, washed three times in 0.1 M phosphate buffer, and processed for immunofluorescence labeling as previously described (26), using the same antibodies against betagranin and insulin described above for immunoelectron microscopy, but now diluted 1:100 and 1:400, respectively. As secondary antibodies, we used a goat antibody against rabbit IgGs coupled to Texas Red and diluted 1:200 and a rabbit antibody against mouse IgGs coupled to fluorescein and diluted 1:200, respectively. Controls included exposure of the cells and islet sections to only the secondary appropriate antibody. None of these incubations resulted in a sizable, specific staining of the sections. Immunolabelings were screened and photographed using a LSM 510 confocal microscope (Zeiss).

**Recombinant Mouse Betagranin**—Betagranin (mouse CgA 1–133) was expressed in Escherichia coli as a fusion protein with an N-terminal His-tag placed in front of a tobacco etch virus protease recognition site (27). Nickel affinity-purified betagranin was renatured by dialysis in the presence of reduced and oxidized glutathione (28). For removal of the His-tag, betagranin was cleaved with His-tagged tobacco etch virus protease (Invitrogen) according to the manufacturer’s instructions. Betagranin was then purified by subtractive nickel affinity chromatography followed by an anion exchange Q column (GE Healthcare, Uppsala, Sweden). An aliquot of purified recombinant betagranin was dialyzed overnight at 4 °C against 2 liters of phosphate-buffered saline.

**Insulin Secretion Assay**—For static measurements of insulin release, MIN6 cells were seeded at 3 × 10⁵ cells per well in 24-well cell culture plates. After 3 days of culture, cells were preincubated for 2 h at 37 °C in a Krebs-Ringer bicarbonate HEPES buffer (KRHB: 125 mM NaCl, 4.74 mM KCl, 1 mM CaCl₂, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, 5 mM NaHCO₃, 25 mM HEPES, pH 7.4, and 0.1% (v/v) bovine serum albumin) supplemented with 2.8 mM glucose. Cells were then incubated for 1 h at 37 °C in KRHB containing 2.8 mM glucose, followed by a second 1-h incubation in KRHB containing either 2.8 or 25 mM glucose or 50 mM potassium in the absence or presence of various concentrations of recombinant betagranin. The supernatant was recovered and the amount of insulin quantified by mouse insulin ELISA. Insulin secretion was expressed as percentage of the first 1-h basal insulin secretion of the corresponding well. For time course analysis, aliquots of 5 μl were taken from the supernatant during the second 1-h incubation...
Betagranin Is Decreased in Pancreatic Islets of lep/lep Mice and Restored upon Leptin Treatment—By Western blot analysis, we found that betagranin was strongly decreased in islets of lep/lep mice compared with lean controls (Fig. 1A). Treatment of the leptin-deficient mice with mouse leptin for 10 days resulted in a markedly reduced food intake and bodyweight compared with those of the saline treated mice (data not shown). These changes were paralleled by restoration of control betagranin levels in lep/lep mice.

Betagranin Co-localizes with Insulin in Pancreatic Islets—Immunolabeling of ultrathin cryosections showed that both betagranin (Fig. 1B) and insulin were expressed in dense core secretory granules. Dual immunolabeling using gold particles confirmed the co-localization of betagranin and insulin observed within the very same secretory granules by double immunolabeling, using large and small gold particles, respectively. E–G, double immunofluorescence analysis confirmed the co-localization of betagranin (F, red) and insulin (E, green) within most β-cells of isolated mouse islets. G, merge of E and F illustrating the overlap (yellow) of the two previous immunolabelings. Scale bar, 250 nm in B–D and 30 µm in E–G.
Inhibition of Insulin Secretion by Betagranin

**FIGURE 2.** Betagranin secretion in MIN6 cells is modulated by glucose. Western blot analysis detected the expression of betagranin (21 kDa) in a lysate of MIN6 cells cultured in the presence of 25 mM glucose (lane 1). For the analysis of secreted betagranin, MIN6 cells were incubated for 1 h in KRHB under stimulating (25 mM glucose, lane 2) and basal (2.8 mM glucose, lane 3) conditions. Betagranin secretion into the cell supernatant (sup) was modulated by the glucose concentration in the medium. The band at the apparent molecular mass of ~90 kDa in a MIN6 cell lysate (lane 1) represents chromogranin A, the precursor of betagranin.

**FIGURE 3.** Betagranin co-localizes with insulin in MIN6 cells. A and B, double immunolabeling of MIN6 cells showed that betagranin (small gold particles, some of which are pointed out by arrowheads) was confined to cell compartments that also contained insulin (large gold particles), including Golgi apparatus (A) and immature (A and B) and mature secretory granules (B). Note in A the absence of labeling over nucleus (n), mitochondria (m), and cytosol (c). C, immunolabeling for betagranin alone revealed that most of the protein was located around the dense core formed by the insulin-Zn\(^{2+}\) crystals in mature secretory granules. Note absence of labeling over the cell membrane (arrows). Scale bar, 250 nm. D–F, double immunofluorescence analysis confirmed the co-localization of betagranin (F, red) and insulin (E, green) within most cells of MIN6 cell cultures. G, merge of D and E illustrating the overlap (yellow) of the two previous immunolabelings. Scale bar, 16 μm.

**FIGURE 4.** A, Analysis of purified recombinant mouse betagranin (10 μg) by SDS-PAGE under reducing conditions showed a major band at the apparent molecular mass expected for the protein (21 kDa). Mass spectrometry identified the major band and the minor bands at 45 and 66 kDa as betagranin. B, Western blot analysis of purified betagranin (20 ng) confirmed the identity and the oligomeric pattern of the protein.

immunofluorescence labeling further showed that the co-localization of betagranin and insulin could be observed in virtually all β-cells of isolated mouse islets (Fig. 1, E–G).

Betagranin Is Expressed in MIN6 Cells and Co-localizes with Insulin—Following incubation in 25 mM glucose, Western blot analysis confirmed the presence of betagranin in total MIN6 cell lysates and culture supernatants (Fig. 2). Betagranin secretion from MIN6 cells was modulated by glucose as shown by the lack of immunodetectable protein in supernatants of cultures (Fig. 2). Betagranin secretion into the cell supernatant (sup) was modulated by the glucose concentration in the medium. The band at the apparent molecular mass of ~90 kDa in a MIN6 cell lysate (lane 1) represents chromogranin A, the precursor of betagranin.

Immunolabeling of ultrathin cryosections showed that betagranin immunoreactivity was confined to cell compartments that also contained insulin, including Golgi apparatus (Fig. 3A), as well as immature (Fig. 3A) and mature secretory granules (Fig. 3, B and C). In the latter type of granules, where betagranin was most abundant, the protein was found localized at the periphery of the dense core formed by the insulin-Zn\(^{2+}\) crystals (Fig. 3B), a location that became more apparent where only betagranin was immunolabeled using antibodies conjugated to small diameter gold particles (Fig. 3C). In contrast, no labeling was observed over nuclei, mitochondria and cell membranes (Fig. 3A).

Double immunofluorescence labeling further showed that the co-localization of betagranin and insulin could be observed in virtually all cells of monolayer MIN6 cultures (Fig. 3, D–F).

Double immunofluorescence analysis confirmed the co-localization of betagranin and insulin in MIN6 (Fig. 3, D–F).

Recombinant Betagranin—The expression of betagranin with a His-tag in E. coli allowed for an efficient purification of the protein. The integrity of the N terminus after proteolytic cleavage of the His-tag was confirmed by N-terminal protein sequencing (data not shown). The purified protein preparation was analyzed by SDS-PAGE (Fig. 4A). A major band was observed at the apparent molecular mass expected for betagranin (21 kDa). Two minor bands around 45 and 66 kDa were also observed. Analysis by tandem mass spectrometry identified all three bands as betagranin (data not shown), indicating that the
major band is the betagranin monomer and that the two minor bands correspond to betagranin dimers and trimers, respectively. Western blot analysis with anti-betagranin antibodies revealed the same oligomeric pattern (Fig. 4B). The endotoxin concentration in the protein preparation was 0.2 ng/ml as determined by the limulus amebocyte lysate test.

Betagranin Inhibits Insulin Secretion in MIN6 Cells—Exposure of MIN6 cultures to recombinant betagranin significantly reduced glucose-stimulated insulin secretion in a dose-dependent manner over a 1-hour period (Fig. 5A). Betagranin at the concentration of 125 nM inhibited glucose-stimulated insulin release by 

\[ \frac{75\%}{100\%} \]

Moreover, when betagranin was washed out from the medium after a 30-min incubation, MIN6 cells regained normal glucose responsiveness (data not shown). 125 nM betagranin inhibited potassium-stimulated insulin release by 

\[ \frac{70\%}{100\%} \]

In contrast, betagranin did not affect basal insulin secretion (Fig. 5C).

To investigate the dynamic of the inhibitory effect of betagranin, a time course analysis was performed. Fig. 6 shows the time-dependent effect of betagranin on stimulated insulin secretion. At the concentration of 125 nM, betagranin significantly reduced both glucose- and potassium-stimulated insulin release by roughly 40% already after 5 min. This effect remained stable after 10 min and significantly increased after 60 min.

The inhibitory activity of betagranin was not affected by dialysis of the protein (data not shown).

Betagranin Inhibits the Calcium Response of MIN6 Cells—The \( \text{Ca}^{2+} \) response to glucose and potassium was examined in control cells and in cells exposed to 125 nM betagranin (Fig. 7A). Control cells showed oscillations of \( \text{Ca}^{2+} \) upon elevation of the glucose concentration from 2.8 to 16.7 mM, whereas cells exposed to betagranin only slightly increased their \( \text{Ca}^{2+} \) until a plateau was reached. Here, \( \text{Ca}^{2+} \) oscillations were never observed. Stimulation by potassium induced a rapid and strong transient elevation in \( \text{Ca}^{2+} \), in control cells. In the presence of betagranin, a more sustained but much lower increase in \( \text{Ca}^{2+} \) was observed. The quantitative analysis of 30 traces from three independent experiments shown in Fig. 7B demonstrated that betagranin strongly inhibits glucose- and potassium-stimulated calcium influx in MIN6 cells. In addition, when betagranin was washed out via perfusion of the chamber, the cells exhibited a close to normal calcium response toward glucose and potassium (data not shown).

Betagranin Inhibits Insulin Secretion in the Perfused Pancreas—Isolated rat pancreases were perfused with a low (5.6 mM) and a high (16.7 mM) glucose containing buffer in the absence and presence of 10 nM betagranin. As shown in Fig. 8, when perfused with glucose alone, the pancreas showed the normal increase in insulin release with increasing glucose concentrations. Furthermore, insulin output readily decreased when glucose was lowered at the end of the experiment. During the addition of betagranin to the perfusate, insulin levels rapidly dropped irrespectively of whether insulin
output was stimulated by 5.6 or 16.7 mM glucose. This effect was fully reversible. As evaluated by measuring the areas under the curve, betagranin reduced insulin secretion by \( \frac{1}{2} \) of 60% under stimulation by 16.7 mM glucose (Fig. 8B).

**DISCUSSION**

The N-terminal sequence of CGA, which encodes betagranin, is highly conserved among species, suggesting an important, specific biological function (8). In pancreatic \( \beta \)-cells, betagranin is produced via the post-translational proteolytic processing of CGA and is co-secreted with insulin in response to insulinotropic stimuli. Still, betagranin role in \( \beta \)-cells has remained unknown so far.

Using a proteomic approach, Sanchez et al. (12) have recently shown that the levels of betagranin in pancreatic islets are significantly down-regulated in leptin-deficient \( \text{lep/lep} \) mice. Moreover, we showed that treatment of leptin-deficient mice with leptin completely restored normal betagranin levels. Using nanomolar concentrations of recombinant betagranin, we demonstrated that the peptide strongly inhibits insulin secretion of MIN6 cells, in response to both glucose and potassium. A comparable effect was also observed in an in vitro perfused rat pancreas model, consistent with the inhibitory role of other CGA-derived fragments on the secretion of insulin and other hormones (8, 9, 31). For technical reasons, our experiments tested a recombinant mouse betagranin in a mouse cell line and in the rat pancreas, limiting the comparison between the two models. However, the very high homology between mouse and rat betagranin (90% amino acid identity) probably legitimates this approach. Another issue is raised by our use of recombinant betagranin. We investigated the activity of this protein by measuring its endotoxin contaminants. At the highest betagranin concentration used in our secretion assays (125 nM), we found that the endotoxin concentration was 4000-fold lower than that reported to have an effect on insulin secretion (32).
Furthermore, dialysis did not affect the activity of betagranin, thereby excluding that the secretory effects we observed were due to small molecular weight molecules. Eventually, betagranin wash out experiments demonstrated the reversibility of the inhibitory effect of betagranin in both MIN6 cell cultures and the perfused pancreas, indicating that betagranin inhibited insulin secretion without compromising cell viability.

In static incubation experiments, betagranin inhibited both glucose- and potassium-stimulated insulin secretion, indicating that it did not interfere with either glucose uptake or the production of metabolic coupling factors required for glucose-stimulated insulin secretion. In addition, the inhibitory effect of the peptide was rapid in both MIN6 cell cultures and in the perfused pancreas. The rapidity of the inhibitory effect and its reversibility suggest that betagranin binds to the $\beta$-cell surface, possibly on ATP-dependent potassium channels ($K_{\text{ATP}}$) and/or on voltage-dependent calcium channels of the L-type. An effect on $K_{\text{ATP}}$ channels, which may be maintained in an opened configuration by betagranin, would interfere with $\beta$-cell depolarization, thus explaining the observed inhibition of calcium influx in MIN6 and, consequently, the inhibition of glucose-induced insulin secretion. Upon stimulation with high potassium, an effect on voltage-dependent calcium channels would be required. Such an effect is supported by recent studies with betagranin/vasostatin-derived small synthetic peptides, which have shown that the peptides exert negative inotropic effects in the isolated frog and rat heart (33–35), probably via an action of the peptides on L-type calcium channels.

A further mechanism involving a specific membrane receptor, as it has been shown for other physiological inhibitors of insulin secretion (36), is also conceivable for betagranin. Although high affinity binding sites have been reported on parathyroid cells (37), and on calf aortic smooth muscle cells (11), classical betagranin/vasostatin receptors remain to be identified in $\beta$-cells. Alternatively, it has been proposed that betagranin/vasostatin exerts its biological activity by hydrophobic binding to membrane phospholipids and thereby perturbing the structural microenvironment required for the proper functioning of classical membrane receptors (38, 39). Based on the inhibition of insulin secretion we observed, these membrane receptors could be those for other known physiological inhibitors of insulin secretion (36), and betagranin could affect their calcium channel gating properties. Alternatively, it is conceivable that betagranin interacts with the plasma membrane and directly affects the proper functioning of calcium channels. It remains to be analyzed whether the reported effects on the plasma membrane microenvironment show a time course and reversibility that is compatible with the inhibitory effect of betagranin on insulin secretion.

At any rate, our results clearly demonstrate an inhibitory effect of recombinant betagranin on glucose-stimulated insulin secretion in vitro, raising the question of whether such an effect is likely to represent the action of endogenous betagranin in vivo. First, we definitively demonstrate the presence of endogenous betagranin within secretory granules of pancreatic islets and MIN6 cells and show that betagranin co-localizes there with insulin. Second, we confirmed that endogenous betagranin is secreted by $\beta$-cells in response to glucose. Third, we document that nanomolar concentrations of recombinant betagranin inhibit glucose-induced insulin secretion. Even though the concentrations we tested are slightly higher than the plasma concentrations of chromogranin A measured in healthy individuals (40), the presumably higher intra-islet concentrations are those which, most likely, are relevant to account for the inhibition of secretion. While the intra-islet concentrations of betagranin are not known, they may be estimated to reach values in the nanomolar range just after exocytosis. The perfused pancreas is considered as a model that mimics physiological conditions more closely than cultures of MIN6 cells. In this model, inhibition of second phase secretion was achieved with concentrations of betagranin ~10-fold lowered than those that were used in static incubations of MIN6 cells. In addition, preliminary experiments have also shown slight inhibition of first phase insulin secretion (data not shown). The latter needs further confirmation and validation to understand the precise mechanisms of action and was beyond the scope of this study.

These features, and the fact that the N-terminal sequence of CgA, which encodes betagranin, is highly conserved among species, suggest that betagranin may represent an autocrine negative-feedback loop controlling insulin release of $\beta$-cells. A similar mechanism has been described for the highly conserved C-terminal CgA fragments catestatin and parastatin in catecholamine release from the adrenal medulla and in the secretion of parathyroid hormone from the parathyroid glands (41, 42).

In our experiments, recombinant betagranin was added to the incubation medium. Although this setting favors a mechanism of action via a target on the $\beta$-cell surface, the experiments do not rule out the possibility that endogenous, intracellular betagranin may inhibit the exocytosis of insulin-containing granules by virtue of its localization within these organelles. Interestingly, the expression of betagranin within pancreatic islets was modulated by leptin (Fig. 1A). The decrease in betagranin expression in leptin-deficient $\text{lep/lep}$ mice could indirectly result from a generalized leptin insufficiency and its associated metabolic abnormalities. Alternatively, leptin could promote betagranin expression via direct actions on the $\beta$-cell. The analysis of the actual mechanism involved was beyond the scope of this study. Nevertheless, it is tempting to speculate that betagranin contributes to the inhibitory action of leptin on insulin secretion (12). A growing body of evidence suggests a direct effect of leptin on $\beta$-cell insulin secretion. Thus, leptin has been reported to inhibit insulin secretion from tumor-derived $\beta$-cell lines (15, 43–45). Leptin-mediated activation of $K_{\text{ATP}}$ channels and phosphodiesterase 3B, as well as reduction in glucose transport, has been demonstrated (43, 46–48). While these appear to be acute effect of leptin, the modulation of betagranin expression by leptin may represent a more long-term effect.

In conclusion, betagranin is a consistent component of insulin-containing secretory granules, and recombinant betagranin is a potent inhibitor of glucose-stimulated insulin. The rapidity and the reversibility of the inhibitory effect on the one hand, and the reversible blocking of the MIN6 calcium response on the other hand, suggest an effect of the peptide on some ion channels of the $\beta$-cell surface. Because betagranin is naturally
Inhibition of Insulin Secretion by Betagranin

secreted by β-cells, the inhibition of insulin secretion by endogenous betagranin may represent an additional autocrine negative feedback loop whereby insulin release from β-cells is controlled.

Acknowledgments—We thank Yannick Brunner, Isabelle Wehrli, Dr. Michel Rossier, and Dr. Maud Frieden for technical assistance.

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