Decreased Response of Mobile Calcium in Pancreatic Islets of Fasted Rats to Glucose Stimulation and Calcium Manipulation

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SUMMARY

The effect of fasting on mobile calcium in the B-cells of rat pancreatic islets was investigated in view of a possible role of calcium in the fasting-induced impairment of insulin secretion. Mobile calcium (GBHA-Ca), an ionized or readily ionizable calcium fraction, was determined histochemically with glyoxal bis (2-hydroxyanil). Fasting (24–72 h) strongly decreased the GBHA-Ca content of islets in situ (55–60%).

Incubation of isolated islets at 2.5 mM glucose in the presence of 2.5 mM Ca^{2+} resulted after 15 min in stable GBHA-Ca levels, which were 25% lower in fasted than in fed islets. Glucose (15 mM) caused the GBHA-Ca content of fed islets to decrease rapidly and to rise again after 30 min. These changes did not occur after 24 or 72 h of fasting. GBHA-Ca appeared not to be displaceable with La^{3+}.

At 2.5 mM glucose, withdrawal of Ca^{2+} rapidly reduced GBHA-Ca in fed and fasted islets. Glucose (15 mM) inhibited this rapid fall, and this inhibitory effect was particularly evident in fed islets.

Washing and preincubation in the absence of Ca^{2+} (2.5 mM glucose) largely depleted fed and fasted islets of GBHA-Ca. Reintroduction of Ca^{2+} at 2.5 mM glucose only partially restored the GBHA-Ca levels of fed and fasted islets. By contrast, 15 mM glucose restored the characteristic pattern of GBHA-Ca in fed islets as seen in nondepleted islets, but in fasted islets at lower levels as previously seen.

Thus, fasting decreased the GBHA-Ca content and its response to glucose stimulation. It is suggested that GBHA-Ca, which is presumably mainly localized in the secretory granules, plays a role in the initiation of insulin secretion. DIABETES 32:235–240, March 1983.

Calcium is an important regulator of the insulin-releasing process.[1–4] Raising the glucose concentration from a nonstimulatory to a stimulatory one causes, after a transient decrease, an increase of the Ca^{2+} efflux,[5,6] whereas, at the same time it also enhances Ca^{2+} uptake.[7] Moreover, the glucose-induced rise of the cAMP level[8,9] causes redistribution of calcium from intracellular stores.[10] The net effect of glucose stimulation on islet calcium content and its physicochemical state is still unclear.

Calcium is heterogeneously distributed in different cellular compartments: cytosol, mitochondria, granules, and endoplasmic reticulum. The major fraction of intracellular calcium is precipitated or bound to intracellular constituents (proteins, phospholipids, metabolic intermediates), and only a very small fraction is thought to be ionized.[11–13] The bound calcium can be categorized as restricted and readily diffusible, depending on binding at high or low affinity to intracellular components.[11–14]

Previously we have shown that glyoxal bis (2-hydroxyanil) only stains ionized or readily ionizable calcium (mobile calcium) in gelatin films.[15] It also stains in islets a calcium fraction, which is responsive to glucose stimulation.[16] Fasting decreases the secretory response[8,9] and also decreases the GBHA-stainable calcium fraction of islets in situ.[17] The present study aimed to investigate the effect of fasting on the response of this calcium fraction in incubated islets to glucose stimulation and manipulation of extracellular calcium.

MATERIALS AND METHODS

Isolation and incubation of islets. Male Wistar rats of about 350 g, fed or fasted for 24 and 72 h, were used. Islets of another group of rats were degranulated by oral administration of tolbutamide (500 mg/kg body wt) twice daily for 3 days.[17] All experiments were started at about 8 a.m. The islets for each experiment were isolated from two pancreata of each nutritional category with partially purified collagenase (Sigma type I, Sigma, St. Louis, Missouri) as described earlier.[8] Pools of 600 islets were collected and preincubated for 15 min at 37°C in Krebs-Ringer bicarbonate buffer (KRB) containing 2.5 mg bovine serum albumin per milliliter, 2.5

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Received for publication 27 May 1982 and in revised form 1 October 1982.

mM glucose, and 2.5 mM $CaCl_2$. When islets were preincubated or incubated in the "absence" of calcium, $CaCl_2$ was omitted from the medium. In case of preincubation in the absence of calcium the islets were first washed (6 ×) with $CaCl_2$-free buffer. This buffer still contained 40 μM calcium as determined by a fluorometric micromethod.[18]

Insulin secretion. Insulin secretion was measured in a separate series of experiments by serial sampling from vessels containing 25 islets of either fed, 24-h-, or 72-h-fasted rats. Insulin was measured by radioimmunoassay using rat insulin as a standard and polyethylene glycol for separation of free and antibody-bound insulin.[19]

Tissue preparation and staining. After incubation the islets were freed of buffer, frozen in liquid nitrogen, and subjected to freeze-substitution in acetone containing 1% oxalic acid.[16] For the study of islets in situ, pieces from the tail of the pancreas were frozen and subjected to freeze-substitution. Subsequently, the tissue was embedded in paraffin, sectioned at 15 μm, deparaffinized, and stained for 10 min with glyoxal bis (2-hydroxyanil) (GBHA) for "mobile" calcium.[15,17]

Quantitative evaluation of GBHA-stained islet sections. The staining intensities of B-cells in sections of islets in situ or isolated islets were determined by measuring the transmission at 497 nm with a Leitz orthoplan microscope with a 40× objective equipped with a Leitz compact photometer. Absorbance values were obtained by feeding the transmission values to a programmable calculator (Texas Instruments Ti 59) plus printer. Four sections from each incubated batch of 25 islets were stained, and in each section 10 plug measurements were made. For each experiment the islet batch means were taken and used for the calculation of the general mean ± SEM of the different experiments. Differences were tested for statistical significance with the unpaired Student *t* test.

In contrast to our previous study on mobile calcium,[16] in this study we corrected the absorbance by 0.043 ± 0.001 for nonspecific absorbance. This blanc value was obtained by treating tissue with staining solution devoid of GBHA.

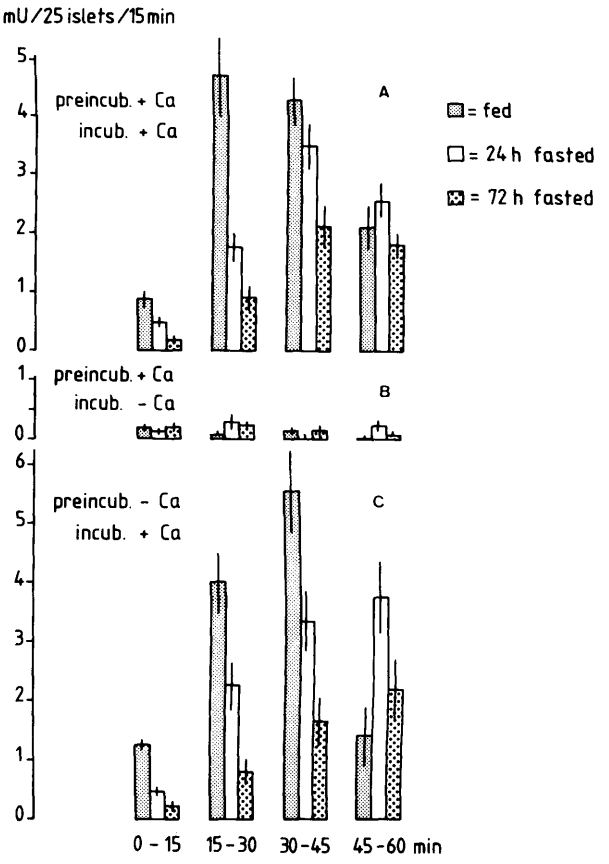

FIGURE 1. Effect of fasting and calcium manipulation on insulin secretion at 15 mM glucose over four periods of 15 min. Batches of 25 islets were preincubated at 2.5 mM glucose in the presence of 2.5 mM Ca^{2+} and incubated at 15 mM glucose in the presence of Ca^{2+} (A) or in the absence of Ca^{2+} (B). Preincubation was in the absence of Ca^{2+} at 2.5 mM glucose and incubation was at 15 mM glucose in the presence of Ca^{2+} (C). Means of nine experiments ± SEM.

RESULTS

Effect of calcium manipulation on insulin secretion of islets of fed and fasted rats. Insulin secretion of fed islets, preincubated in the presence of $CaCl_2$ and incubated at 15 mM glucose in the presence of $CaCl_2$, showed a marked increase in the 15–30-min period. This high secretion was maintained in the 30–45-min period but decreased considerably (50%) in the 45–60-min period (Figure 1A). The secretion of 24-h- and 72-h-fasted islets was strongly inhibited in the first and second 15-min period and that of 72-h-fasted islets also in the third 15-min period. However, the difference in secretion between fed and fasted islets disappeared in the 45–60-min period as a result of a decrease of the secretion of fed and 24-h-fasted islets to the same level as that of 72-h-fasted islets.

When the islets were preincubated in the presence of $CaCl_2$ and then incubated at 15 mM glucose in the absence of $CaCl_2$, the insulin secretion of fed, 24-h-, and 72-h-fasted islets was completely suppressed (Figure 1B).

When islets were preincubated in $CaCl_2$-free buffer and subsequently incubated at 15 mM glucose in the presence of $CaCl_2$ (Figure 1C), the secretion patterns of fed, 24-h-, and 72-h-fasted islets were, in the first three 15-min periods, roughly comparable with those shown in Figure 1A. In each of these periods fasted islets secreted less insulin than did fed islets. However, in the fourth period the secretion of fed islets, but not that of 24-h- and 72-h-fasted islets, decreased strongly. In that period 24-h-fasted islets secreted even significantly more insulin than fed islets.

Islets incubated at 2.5 mM glucose at either of the three calcium conditions used secreted only small amounts of insulin, which did not differ significantly (results not shown).

Mobile calcium content of isolated islets and pancreatic islets in situ. To calculate the GBHA-Ca content of islet tissue, staining intensity was compared with a standard curve, which was made with $CaCl_2$-containing gelatin films.[16] Figure 2 shows the relationship between the amount of Ca^{2+} and the staining intensity. The absorbance of freshly isolated islets of fed rats amounted (after subtraction for nonspecific absorbance of 0.043 ± 0.001) to 0.130 ± 0.003, which corresponds to 0.47 μg Ca^{2+}/cm^2. Since the sections are 15 μm thick, islet tissue of fed rats contains 7.8 mmol Ca^{2+} per liter. The absorbance of islets in pancreatic tissue (in situ) of fed, 24-h-, and 72-h-fasted rats amounted to 0.137 ± 0.005, 0.060 ± 0.002, and 0.055 ± 0.001, respectively (mean ± SEM of 4 rats, from each of which 25 islets were measured).

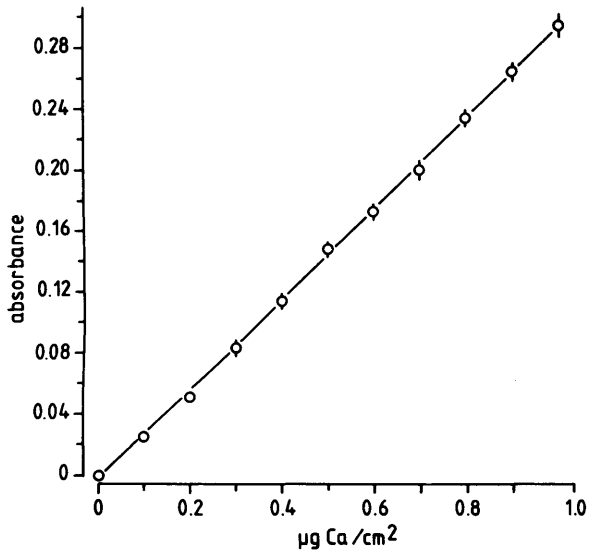

FIGURE 2. Relationship between absorbance of GBHA-stained gelatin films and the amount of $CaCl_2$ in the films. Each point is the mean ± SEM of eight films.

Effect of isolation of islets from pancreatic tissue on the mobile calcium content. Pancreata of fed and 24-h-fasted rats were divided into two parts. One part of the tissue was immediately frozen in liquid nitrogen for measurement of the mobile calcium content of islets in situ; islets from the other part were isolated with collagenase. The mobile calcium content of islets of fed rats virtually did not change during the isolation procedure, whereas that of the islets of 24-h-fasted rats increased considerably (Figure 3). During harvesting and incubation at 2.5 mM glucose the mobile calcium content of both categories of islets did not change.

Effect of fasting on mobile calcium during incubation at low and high glucose in the presence of calcium. After isolation and preincubation for 15 min in the presence of 2.5 mM $CaCl_2$ and 2.5 mM glucose, the amount of mobile calcium in 24-h- and 72-h-fasted islets was lower than in fed islets. On incubation at 2.5 mM glucose the mobile calcium content of all three categories of islets decreased gradually during the initial 15 min and then remained constant for the next 45 min. Between 30 and 60 min islets of 24-h- and 72-h-fasted rats contained 25% less mobile calcium than fed islets (Figure 4A).

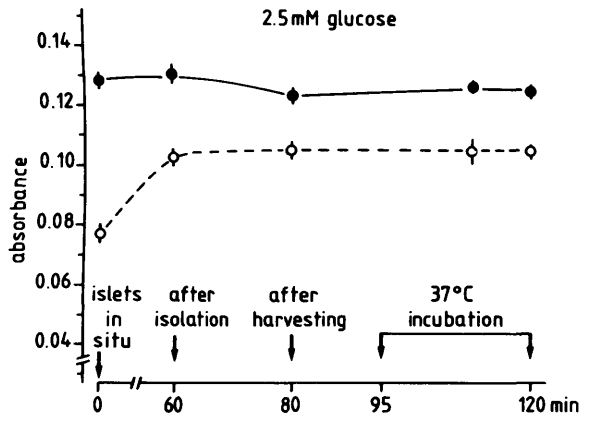

FIGURE 3. Effect of islet isolation on the GBHA-Ca content of islets of fed (●) and 24-h-fasted (○) rats. Mean ± SEM of 5 pancreata or 5 batches of 25 islets.

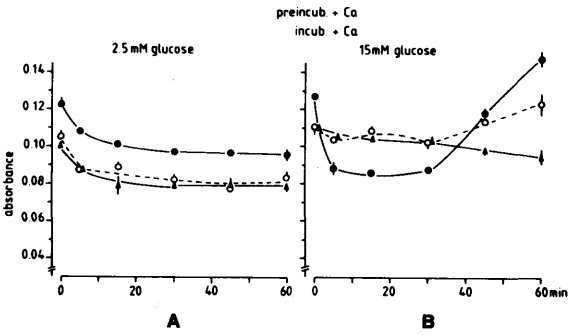

FIGURE 4. Effect of glucose stimulation on GBHA-Ca content of isolated islets of fed (●), 24-h- (○), and 72-h-fasted (△) rats in the presence of Ca^{2+}. Islets were preincubated at 2.5 mM glucose in the presence of Ca^{2+} and incubated at 2.5 mM (A) or 15 mM (B) glucose in the presence of Ca^{2+}. Mean ± SEM of four (2.5 mM) and six (15 mM) separate experiments.

In fed islets, stimulation with 15 mM glucose markedly increased the fall of mobile calcium as seen at 2.5 mM glucose over the 0–30-min period. Over the 30–60-min period 15 mM glucose caused the mobile calcium to rise above the initial value. Both effects of glucose stimulation (initial fall and the subsequent rise) were markedly inhibited in islets of fasted rats. Differences related to the duration of food deprivation were only apparent during the second period (30–60 min). This rise was partially inhibited after 24 h of fasting and fully abolished after a 72-h fast.

Effect of fasting on the depletion of mobile calcium content in the absence of extracellular calcium. Fed, 24-h-, and 72-h-fasted islets were preincubated at 2.5 mM glucose and 2.5 mM $CaCl_2$ and subsequently incubated in $CaCl_2$-free medium containing 2.5 or 15 mM glucose (Figure 5). At 2.5 mM glucose, removal of calcium caused a marked decrease of mobile calcium in the first 5 min, which was approximately of the same magnitude in all categories of islets (Figure 5A). Then, in islets of fed and 24-h-fasted rats, mobile calcium continued to decline slowly, reaching the same low level at 60 min as seen in islets of 72-h-fasted rats from 5 min onward. As shown in Figure 5B, 15 mM glucose largely

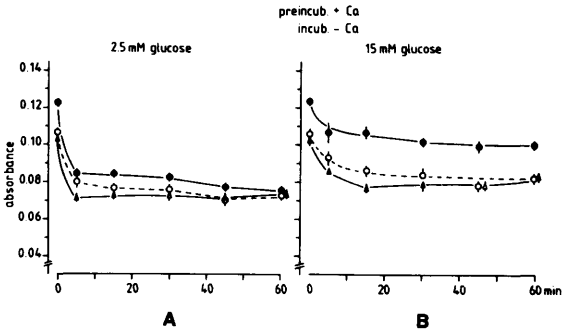

FIGURE 5. Effect of glucose stimulation on GBHA-Ca content of islets of fed (●), 24-h- (○), and 72-h-fasted (△) rats in the absence of Ca^{2+}. Islets were preincubated at 2.5 mM glucose in the presence of Ca^{2+} after which this medium was rapidly replaced (at time zero) by a Ca^{2+}-depleted medium with either 2.5 mM (A) or 15 mM (B) glucose. Mean ± SEM of six separate experiments.

inhibited the fall of mobile calcium in all categories of islets during the entire period of incubation. The time courses of GBHA-Ca are identical, and the only difference is the level of mobile calcium in fed and fasted islets. However, the effect of inhibition of the fall of mobile calcium by 15 mM glucose (fall at 15 mM glucose minus fall at 2.5 mM) is greater in fed than in fasted islets.

Effect of fasting on the recovery of mobile calcium content of calcium-depleted islets on reintroduction of extracellular calcium. When fed, 24-h-, and 72-h-fasted islets were washed (6 ×) and preincubated with $CaCl_2$-free buffer containing 2.5 mM glucose, the mobile calcium content of all three categories of islets decreased to the same low level (0.039 ± 0.001, Figure 6). In fed islets the mobile calcium content decreased from 0.127 ± 0.003 (Figures 4 and 5) to 0.039 ± 0.001, a decrease of 70%. Addition of calcium (2.5 mM $CaCl_2$) to the calcium-depleted islets incubated at 2.5 mM glucose resulted in a rapid but only partial restoration of the mobile calcium content; after 15 min a constant level was attained, which was higher in fed than in fasted islets.

At 15 mM glucose, reintroduction of calcium caused a complete restoration of the mobile calcium content within 5 min in fed islets. Then it declined, and from 15 min onward the same time course is seen as in nondepleted fed islets (Figure 4B). However, when calcium at 15 mM glucose was added to 24-h- and 72-h-fasted islets, the rise in the initial 5 min was 50% less than in fed islets. Their GBHA-Ca patterns, but not their levels as seen in non-Ca-depleted islets (Figure 4B), were restored. During the whole period of incubation, the mobile calcium content was higher than at 2.5 mM glucose.

Localization of GBHA-Ca. To test whether the GBHA-Ca fraction is located intracellularly and/or extracellularly, normal fed islets were incubated for 0, 30, and 60 min in the presence of $CaCl_2$ at 2.5 or 15 mM glucose. Subsequently, the islets were either immediately frozen in liquid nitrogen or washed for 60 min at 1°C in 5 mM $LaCl_3$[20] and then frozen in liquid nitrogen, after which both groups were processed for GBHA-Ca. Washing with lanthanum has no effect on the GBHA-Ca content of islets incubated at low glucose or on the glucose-induced changes of GBHA-Ca (Figure 7).

Previously, we have shown that degranulation of islets in vivo with tolbutamide caused almost complete disappearance of GBHA-Ca of the islets.[17] The possible significance of the granules in the GBHA-Ca changes was studied by subjecting degranulated islets to conditions analogous with those used for normal islets. The GBHA-Ca content of degranulated islets is low; the absorbance amounted to 0.029 ± 0.001. The aldehyde fuchsin positive granulation amounted to 10 ± 1% of control islets. Incubation of these islets for 5, 15, 30, 45, or 60 min, in the presence of $CaCl_2$ at 2.5 or 15 mM glucose, did not significantly alter the GBHA-Ca content (not shown). Washing with and incubation in buffer without added calcium also did not influence the low GBHA-Ca content of degranulated islets (Figure 8). When normal fed islets are treated in this way, the GBHA-Ca content de-

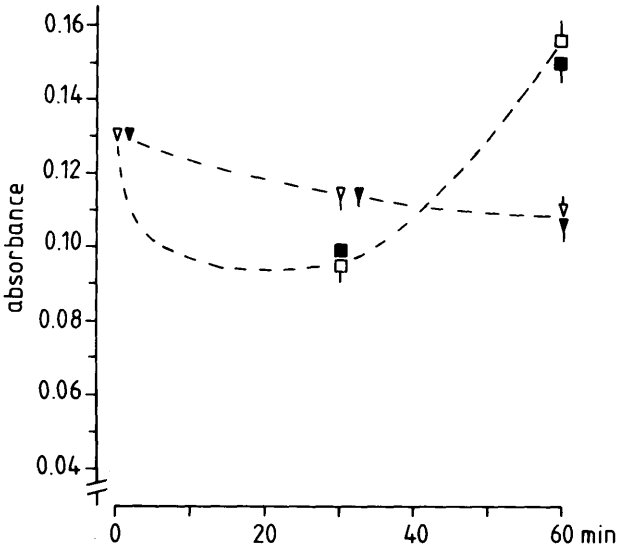

FIGURE 7. Effect of lanthanum washing on the GBHA-Ca content of isolated islets of fed rats. Islets were preincubated at 2.5 mM glucose in the presence of 2.5 mM $CaCl_2$ and incubated at 2.5 mM (▽, ▼) or 15 mM glucose (□, ■) in the presence of $CaCl_2$. Then the islets were either immediately frozen (□, ▽) in liquid nitrogen or incubated for 60 min in 5 mM $LaCl_3$ (in Tris-HCl buffer pH 7.4) (■, ▼), and then frozen in liquid nitrogen after which both groups were processed for GBHA staining. The dashed lines are drawn on the basis of a large series of experiments. Mean ± SEM of four separate experiments.

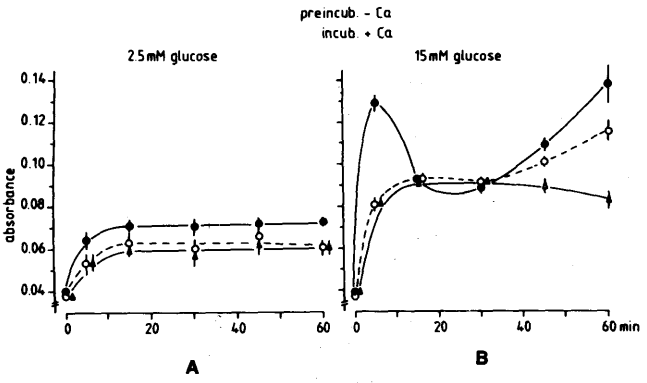

FIGURE 6. Effect of glucose stimulation on GBHA-Ca in Ca-depleted islets of fed (●), 24-h- (○), and 72-h-fasted (△) rats after reintroduction of Ca^{2+} in the medium at time zero. Islets preincubated at 2.5 mM glucose in the absence of Ca^{2+} and incubated at 2.5 mM (A) or 15 mM (B) glucose in the presence of Ca^{2+}. Mean ± SEM of four (2.5 mM) and six (15 mM) separate experiments.

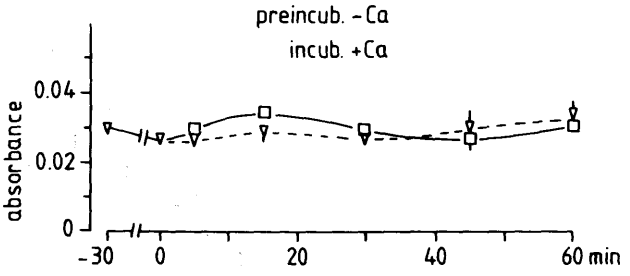

FIGURE 8. Effect of calcium depletion and reintroduction of calcium on the GBHA-Ca content of degranulated islets. Degranulated islets were isolated from tolbutamide-treated rats. The GBHA-Ca content of the islets in calcium-containing buffer (2.5 mM $CaCl_2$ + 2.5 mM glucose) is shown at time −30 min. Then the islets are washed with and preincubated in buffer (2.5 mM glucose) without added calcium; islets shown at time zero. At time zero 2.5 mM $CaCl_2$ is added to the incubation medium and the degranulated islets are incubated at 2.5 mM (▽) or 15 mM glucose (□). Mean ± SEM of four separate experiments.

creases to a low value (see Figures 4 and 6). Reintroduction of $CaCl_2$ into the incubation medium of calcium-depleted normal islets causes a large increase of GBHA-Ca, followed by a decrease and a secondary rise after 30 min. However, reintroduction of $CaCl_2$ into the incubation medium of calcium-depleted degranulated islets has no significant effect on their GBHA-Ca content (Figure 8).

DISCUSSION

The mobile calcium content of fed and fasted islets in situ and the effect of isolation. Previously we have found that GBHA stains only ionized or readily ionizable calcium[15] called mobile calcium. The mobile calcium content of islets in situ is strongly decreased by fasting. Since neither the total calcium content of islets in situ[17] nor in vitro[18] is significantly altered by fasting, the reduction of the mobile calcium content must be due to conversion of GBHA-detectable to GBHA-undetectable calcium. During the isolation procedure the mobile calcium content of fasted islets increases. In view of the aberrant behavior of GBHA-Ca in the islets on subsequent incubation it does not represent a simple restoration of the fasting-induced depletion of GBHA-Ca.

Properties and localization of GBHA-Ca. GBHA-Ca does not represent extracellular membrane-bound calcium, but only calcium localized inside the B-cells, since washing with La^{3+} does not alter the GBHA-Ca content. The GBHA-Ca content of the B-cells can increase or decrease, e.g., as a result of glucose stimulation or fasting, without significant alteration of the total islet calcium content.[17,18] On the other hand, the GBHA-Ca content can be decreased by 70% by washing and incubation in Ca-free buffer (2.5 mM glucose) and by 80% on degranulation of the islets, while under both conditions the total calcium content decreased only by 35%.[17,18] Thus, GBHA-Ca is a fraction of the total calcium content, which can change independently of the total calcium content. Gradual degranulation of islets in vivo (by tolbutamide) is attended by gradual reduction of the GBHA-Ca content and regranulation by reappearance of GBHA-Ca. The GBHA-Ca content correlates very well with the degree of granulation. The equation of linear regression is GBHA-Ca = 14.0 + 1.0 AF and the correlation coefficient 0.92; staining intensities of GBHA-Ca and aldehyde fuchsin are expressed as percentage (unpublished observation). Furthermore, the low GBHA-Ca content of isolated degranulated islets is not influenced by glucose stimulation, calcium depletion, or reintroduction of extracellular calcium to the Ca-depleted degranulated islets. These results suggest that the GBHA-Ca fraction is mainly localized in the secretory granules of the B-cells. However, it cannot be excluded that a minor part is present in other cell compartments. The question why the granules contain GBHA-stainable calcium might be related to prevailing conditions in the B-granules. The B-granules contain a relatively large amount of calcium.[21–23] Furthermore, a low pH has been demonstrated in several kinds of granules[24–26] and recently also in B-granules,[27] which favors ionization of calcium and consequently the staining by GBHA.

Effect of fasting on calcium handling by islets in vitro. Although the low GBHA-Ca content of islets of fasted rats increases on isolation, different levels are maintained during incubation at 2.5 mM glucose in the presence of $CaCl_2$. The difference between fed and fasted islets disappears when extracellular Ca^{2+} is omitted and reappears when Ca^{2+} is introduced to islets largely depleted of GBHA-Ca. Thus, fasting causes an abnormality in the handling of Ca^{2+} that manifests itself even at 2.5 mM glucose, which is nonstimulatory for insulin secretion. No effect of the duration of fasting is seen in all three experimental conditions used.

The effect of 15 mM glucose in the presence of 2.5 mM $CaCl_2$ on GBHA-Ca of fed islets shows two phases, a rapid depletion and a subsequent replenishment of the GBHA-Ca fraction. However, the total islet calcium content does not change under these conditions.[18] Therefore, it seems likely that the alteration of the amount of Ca^{2+} detected by GBHA might reflect changes in binding of calcium to cytoplasmic constituents, sequestration in organelles, or a shift of the pH. Assuming that GBHA-Ca is mainly localized in the granules, a decrease might be caused by a shift of the intragranular pH or an efflux of Ca^{2+} from the granules into the cytosol. In fasted islets the first phase of the GBHA-Ca response and the accumulation of GBHA-Ca after 30 min are inhibited.

The response of GBHA-Ca of fed and fasted islets to glucose stimulation depends on the presence of extracellular calcium. In the absence of calcium the response of GBHA-Ca to 15 mM glucose seems to be blocked, since identical time courses are seen as at 2.5 mM glucose in the presence of calcium (compare Figure 5B with 4A). Moreover, the time courses of GBHA-Ca of fed and fasted islets at 15 mM glucose in the absence of calcium are similar now; only the levels are different. The rapid decrease of GBHA-Ca, seen in fed and fasted islets on removal of calcium at 2.5 mM glucose, is inhibited by 15 mM glucose. The inhibitory effect (fall GBHA-Ca at 15 mM glucose minus fall at 2.5 mM) in fed islets is higher than in fasted islets. Fasting also has an effect on the recovery of GBHA-Ca in Ca-depleted islets, when $CaCl_2$ plus 15 mM glucose is added to the incubation medium. In fed islets the normal GBHA-Ca level is restored within 5 min, but in fasted islets lower levels are obtained than in nondepleted islets.

Thus, the response of GBHA-Ca of fed and fasted islets to glucose stimulation and their different time kinetics depends on the presence of extracellular Ca^{2+}. This would indicate that GBHA-Ca is a glucose-sensitive Ca^{2+} fraction, which is decreased on fasting. Furthermore, fasting seems to impair the ability to decrease and increase the Ca^{2+} content of the GBHA-Ca-containing compartment(s) during glucose stimulation. The disturbance in 24-h-fasted islets cannot be due to a diminished Ca^{2+} uptake since we recently found that the uptake is unimpaired after 24 h of fasting but decreased after 72 h of fasting.[28]

Relationship between GBHA-Ca and insulin secretion of islets of fed and fasted rats. Comparison of the time course of insulin secretion and GBHA-Ca of fed islets suggests that the fall of GBHA-Ca at 15 mM glucose is associated with a rapid increase of secretion, whereas the subsequent rise of GBHA-Ca is associated with a decrease of secretion. Removal of extracellular Ca^{2+} blocks the effect of glucose on GBHA-Ca as well as on insulin secretion. Reintroduction restores the secretory response concomitantly with the normal GBHA-Ca response. GBHA-Ca in fasted islets, which is lower than in fed islets, does not show a glucose-induced decrease and shows reduced or no increase after 30 min. This is

attended by a slow and gradual increase of the secretion, which is followed by a slower decrease in 24-h-fasted islets and no decrease in 72-h-fasted islets. The different secretory responses of 24-h- and 72-h-fasted islets cannot be fully explained on the basis of their GBHA-Ca time pattern.

Although the results suggest a role of GBHA-Ca in the regulation of the insulin secretory response, the relationship appears to be complex. As has been argued above, GBHA-Ca is probably mainly localized in the granules. However, it should be stressed that Ca^{2+} from several compartments can be expected to contribute to the increase of the cytosolic Ca^{2+} concentration, which is generally thought to regulate the secretion rate. Furthermore, the distribution of calcium over different compartments is affected by the cAMP level in the B-cells of islets.[22] Conditions with increased cAMP levels have been shown to increase the efflux of ^{45}Ca from the secretory granules and to reduce the uptake by mitochondria.[10] Previously we have shown that the cAMP level in islets of fed rats rapidly increases on glucose stimulation but decreases again after 30 min,[9] which thus shows a remarkable inverse relationship with the GBHA-Ca response. Removal of extracellular Ca^{2+} blocks the cAMP response, the secretory response, and the response of GBHA-Ca to glucose stimulation. Therefore, it seems likely that the glucose-induced fall in GBHA-Ca is related to the glucose-induced increase of the cAMP level. Since fasting decreases the cAMP response,[8,9] (and the longer the period of fasting the greater the decrease) it may consequently decrease the response of GBHA-Ca in fasted islets. In 72-h-fasted islets, moreover, the glucose metabolism is impaired.[8,21] Presumably, the simultaneous presence of various differences between both categories of fasted islets may be responsible for their different secretory response. The effect of fasting on the GBHA-Ca content of islets at low glucose cannot be accounted for by the differences in cAMP levels since fasting does not affect the basal cAMP level.[8]

The results of the present study suggest that rat pancreatic islets maintain in the secretory granules a large amount of ionic calcium, which can be rapidly released or converted to nonionic calcium on glucose stimulation. Fasting decreases the Ca^{2+} content of the granules and diminishes the ability to lower the Ca^{2+} content of this compartment, which is associated with a delayed secretory response. Such a disturbance could also be involved in the impaired insulin release in the diabetic state.[14,29]

ACKNOWLEDGMENTS

We thank H. Moes for technical assistance and J. Pleiter for preparing the figures.

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