Cholecystokinin-Induced Alterations in β-Cell Sensitivity

Duration, Specificity, and Involvement of Phosphoinositide Metabolism

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SUMMARY

Prior exposure of isolated perifused rat islets to the sulfated gut hormone cholecystokinin-8 (CCK-8S) dramatically increased their insulin secretory response to 7.5 mM glucose, 10 mM arginine, and 10 mM α-ketoisocaproate. In the case of glucose, the heightened secretory response was still apparent 60–80 min after CCK-8S removal from the perifusion medium. Prior exposure of perifused islets to arginine (10 mM), tolbutamide (25 μM), or forskolin (1.0 μM) did not sensitize them to 7.5 mM glucose. CCK-8S exposure increased ^{3}H efflux from islets prelabeled with [^{3}H]inositol, and the increase in ^{3}H efflux was sustained after CCK-8S removal from the perifusion medium. The duration of this increase in ^{3}H efflux paralleled the temporal characteristics of this sensitization process and was significantly attenuated by 25 μM asperlicin, a competitive antagonist of CCK binding to its membrane receptor. Arginine, tolbutamide, or forskolin treatment of islets did not increase ^{3}H efflux from [^{3}H]inositol-prelabeled islets. The results suggest that the turnover of membrane phosphoinositides induced by CCK-8S is largely responsible for this heightened state of secretory responsiveness to various stimulants. Second-messenger molecules generated during phosphoinositide turnover may be responsible for the phenomenon of sensitization displayed by islet tissue to CCK-8S addition. *Diabetes* 36:1420–24, 1987

Previous studies clearly established that pancreatic β-cell response characteristics can be influenced by prior glucose exposure (1–4). In these reports, it was demonstrated that islets previously stimulated with glucose dramatically increase insulin output during a subsequent glucose stimulation. Neither stimulated insulin secretion per se nor islet cAMP levels appeared to be involved in this process, although glucose metabolism did appear essential. We previously reported that sulfated cholecystokinin (CCK-8S) sensitized islets to a moderate elevation in the ambient glucose concentration (5). An established effect of both CCK-8S and glucose is an increase in the turnover of membrane phosphoinositides (PI), including phosphatidylinositol, phosphatidylinositol 4-phosphate (PIP), and phosphatidylinositol 4,5-bisphosphate (PIP_2) (6–9). We decided to examine the involvement of this metabolic pathway in the sensitization to glucose induced by CCK-8S. Our results demonstrate that 200 nM CCK-8S induces a sustained increase in PI turnover, an effect still evident long after removal of CCK-8S from the medium, and that the temporal characteristics of this response parallel the sensitization process. On the other hand, arginine, tolbutamide, or forskolin neither increased PI turnover nor sensitized islets to glucose stimulation. These results suggest that the events in the inositol cycle are intimately involved in this sensitization process. Therefore, CCK-8S–induced PI turnover may play an important role in the postulated "incretin" effect of the peptide (10,11), sensitizing the β-cells to small postprandial increases in the plasma glucose concentration.

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Received for publication 11 February 1987 and accepted in revised form 21 April 1987.

MATERIALS AND METHODS

The detailed methodologies employed to assess insulin output from collagenase-isolated islets have been previously described (5,9). Male Sprague-Dawley rats purchased from Charles River (Kingston, NY) were used in all studies. The animals were fed ad libitum and at death weighed between 300 and 400 g. After Nembutal-induced (50 mg/kg) anesthesia, islets were isolated by collegenase digestion and either perifused to assess secretory responsiveness or subjected to a 2-h incubation period in *myo*-[2-^{3}H]inositol. In these labeling experiments, 10 μCi of *myo*-[2-^{3}H]inositol (initial sp act 16.6 Ci/mmol) were added to 250 μl of medium (gassed with 95% O_2/5% CO_2 and maintained at 37°C) similar to that used for the perifusion studies. Glucose (2.75 mM) was also present. Groups of 50–70 islets were loaded onto

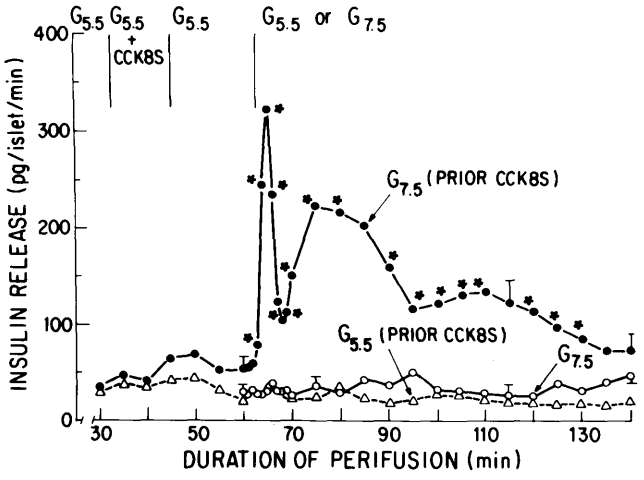

FIG. 1. CCK-8S–induced sensitization to glucose stimulation. Groups of islets were collagenase isolated and perifused with 5.5 mM glucose (G) for 30 min to establish basal, stable rates of release. Medium was then supplemented with 200 nM CCK-8S for 10 min. After a further 20-min perifusion in CCK-8S–free medium, these islets were then stimulated for 80 min with 7.5 mM glucose. Secretory response of control islets, not previously exposed to CCK-8S, is depicted as well as the response of islets exposed to CCK-8S for 10 min but maintained with 5.5 mM glucose for rest of perifusion (n = at least 4 for each condition). *Significant differences ($P < .05$) in release rates between control islet response to 7.5 mM glucose versus those previously exposed to CCK-8S.

nylon filters and then placed in small glass vials. Two hundred microliters of the radioactive incubation medium were gently added, the vials were stoppered, and the atmosphere above the islets was aerated with 95% O_2/5% CO_2. After 60 min, the islets were again gassed with 95% O_2/5% CO_2. After 2 h the islets were washed with 5 ml of nonradioactive medium and perifused. Islets were usually perifused (at a flow rate of 1 ml/min) with 5.5 mM glucose for 30 min to establish stable, basal secretory rates. After this stabilization period, the islets were then subjected to various protocols indicated in the figure legends. Effluent samples were collected and analyzed for insulin content with rat insulin (lot 615-D63-12-3; Lilly, Indianapolis, IN) as standard and, when appropriate, ^3H content.

Hanks' solution was used for the islet isolation. The perifusion medium consisted of 115 mM NaCl, 5 mM KCl, 2.2 mM $CaCl_2$, 1 mM $MgCl_2$, 24 mM $NaHCO_3$, and 0.17 g/100 L bovine serum albumin. Other compounds were added where indicated, and the solution was gassed with a mixture of 95% O_2/5% CO_2. The ^{125}I-labeled insulin used for the insulin assay was purchased from New England Nuclear (Boston, MA) and the *myo*-[2-^3H]inositol from Amersham (Arlington Heights, IL). CCK-8S (COOH-terminal, 8–amino acid fragment 26-33 amide, sulfated on the tyrosine residue, lot 124F-0445), arginine, α-ketoisocaproate (KIC; sodium salt), bovine serum albumin (RIA grade), and the salts used to make the Hanks' solution and perifusion medium were purchased from Sigma (St. Louis, MO). Asperlicin was the generous gift of V. Lotti (Merck, Sharp, and Dohme, West Point, PA). It was dissolved in dimethyl sulfoxide (DMSO) before use. The amount of DMSO used in these studies (never >0.1%) was without an adverse impact on islet function (unpublished observations). Forskolin (dissolved in DMSO) was purchased from Calbiochem (La Jolla, CA), and the sodium tolbutamide was the generous gift of Upjohn (Kalamazoo, MI).

Statistics. Statistical significance was determined via Student's t test for unpaired data with $P < .05$ taken as significant. Values presented in the figures or RESULTS represent means ± SE of the specified number of observations.

RESULTS

The process of sensitization and the duration of this effect are illustrated in Fig. 1. In this group of experiments, islets were perifused for 30 min with 5.5 mM glucose to establish basal, stable secretory rates. For an additional 10 min, islets were exposed to 200 nM CCK-8S. A small insulin secretory response was observed in response to CCK-8S, but release rates returned to basal values within 10 min after CCK-8S removal from the medium. Twenty minutes after CCK-8S removal, these islets were stimulated with 7.5 mM (135 mg/dl) glucose. When compared with the response observed from untreated islets, CCK-8S–treated islets showed a dramatic first phase of insulin output accompanied by a sustained second phase. Considering that CCK-8S was omitted from the medium 20 min before 7.5 mM glucose addition and that the stimulation period with 7.5 mM glucose lasted another 80 min, the impact of CCK-8S on the islet persists for ~80 min. When the interval between CCK-8S exposure and 7.5 mM glucose stimulation was extended to 40–60 min, the sensitization process was not observed (results not shown).

We next examined whether CCK-8S exposure heightened the secretory response to either 10 mM arginine or 10 mM KIC. In these experiments, islets were perifused for 30 min

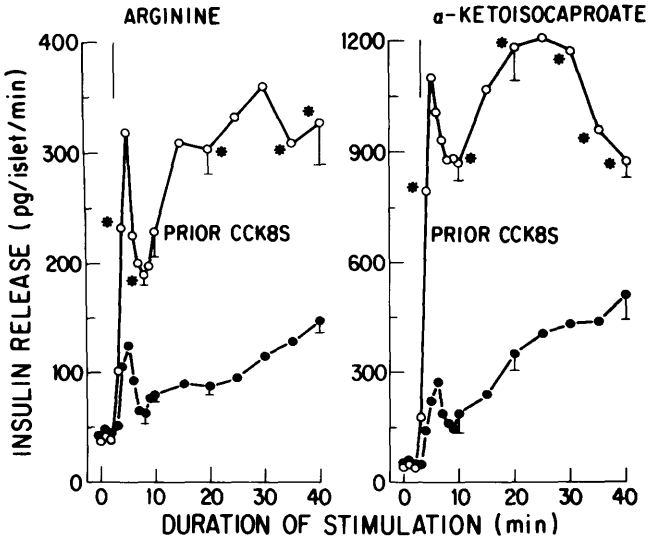

FIG. 2. Prior CCK-8S exposure sensitizes islets to arginine and α-ketoisocaproate. *Left panel*: groups of islets were perifused for 30 min with 5.5 mM glucose. For an additional 10 min, 200 nM CCK-8S was included in medium. After 10 min perifusion in CCK-8S–free medium, islets were provoked with 10 mM arginine for 40 min in continued presence of 5.5 mM glucose. Response to control islets (untreated with CCK-8S) is also shown (n = 3 for each condition). *Right panel*: similar to protocol described above, islets were provoked with 10 mM α-ketoisocaproate for 40 min (in continued presence of 5.5 mM glucose) after prior exposure to 200 nM CCK-8S for 10 min. Control response from untreated islets is also shown (n = 3 for each condition). *Selected time points were analyzed for significance ($P < .05$).

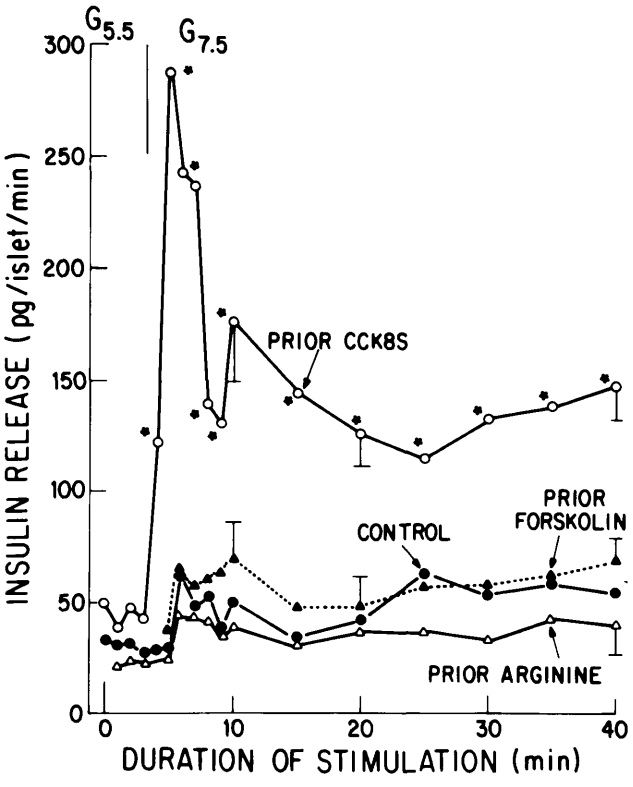

FIG. 3. Prior exposure to arginine or forskolin does not sensitize islets to 7.5 mM glucose (G). Similar to protocol described in Fig. 2, islets were exposed to 10 mM arginine, 1.0 μM forskolin, or 200 nM CCK-8S for 10 min. Although not depicted, release rates (in continued presence of 5.5 mM glucose) in response to arginine, forskolin, tolbutamide, or CCK-8S averaged 96 ± 7, 132 ± 23, 127 ± 15, and 57 ± 6 pg · islet^{-1} · min^{-1} (means ± SE), respectively, during the final 5 min of exposure to these agents. This compares with control release rates of 31 ± 4 pg · islet^{-1} · min^{-1} during same period. After a further 10-min perifusion with 5.5 mM glucose alone, islets were stimulated for 40 min with 7.5 mM glucose. Response of control islets is also shown. Arginine, forskolin, and tolbutamide did not sensitize islets to 7.5 mM glucose (n = at least 4 for each; not shown). *Significant difference ($P < .05$) from control untreated islet response.

with 5.5 mM glucose and then exposed to 200 nM CCK-8S for 10 min. After an additional 10-min perifusion with 5.5 mM glucose alone, these islets were stimulated with 10 mM arginine or 10 mM KIC in the continued presence of 5.5 mM glucose. The results are depicted in Fig. 2 and demonstrate that prior CCK-8S exposure augmented the islet secretory response to both compounds. Both the first and second phases of secretion were significantly amplified.

We next determined whether prior exposure to 10 mM arginine, 1.0 μM forskolin, or 25 μM tolbutamide (results not shown) sensitizes islets to 7.5 mM glucose. After a 30-min perifusion with 5.5 mM glucose alone, the medium was supplemented with these agents for 10 min. After a further 10-min perifusion with 5.5 mM glucose alone, the islets were provoked with 7.5 mM glucose. None of these compounds increased the response to 7.5 mM glucose above that noted in control, nontreated islets, even though they, like CCK-8S, induced a small insulin secretory response (Fig. 3).

CCK-8S increases the turnover of islet inositol-containing lipids, an effect independent of the glucose concentration (9). We next determined the effect of 200 nM CCK-8S, 10 mM arginine, 25 μM arginine, 25 μM tolbutamide, or 1.0 μM forskolin on ^{3}H efflux from [^{3}H]inositol-prelabeled islets (Fig. 4). CCK-8S addition to islets perifused with 5.5 mM glucose was accompanied by a dramatic and sustained efflux of ^{3}H. This effect was not reproduced by arginine, tolbutamide, or forskolin.

The possibility that CCK-8S–induced sensitization to various stimulants was the result of a sustained activation of PI turnover was investigated next. In these experiments, islets prelabeled with [^{3}H]inositol were perifused for 30 min with 5.5 mM glucose and then exposed to 200 nM CCK-8S for 10 min. The efflux of ^{3}H in the perifusate was monitored before, during, and for 40 min after CCK-8S exposure (Fig. 5). Under these conditions, the influence of CCK-8S on ^{3}H efflux [plotted as the fractional efflux rate (12)] was sustained for ~30 min after its removal from the perifusion medium. In other words, a brief 10-min exposure to CCK-8S resulted in the sustained turnover of inositol-containing phospholipids, and the duration of this effect parallels the duration of CCK-induced sensitization. The inclusion of asperlicin, a

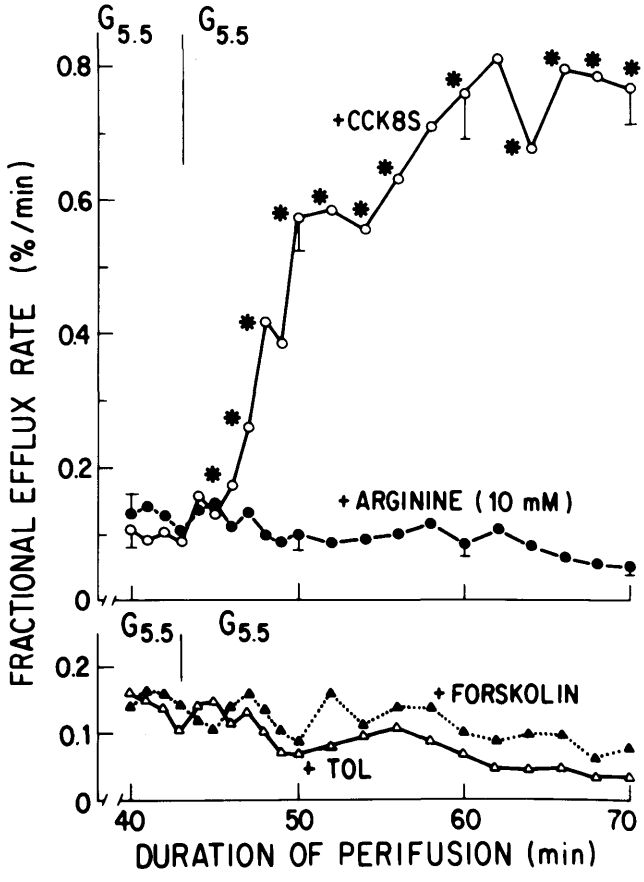

FIG. 4. Fractional efflux rates of ^{3}H from islets prelabeled with [^{3}H]inositol. Islets were incubated for 2 h in [^{3}H]inositol to label inositol-containing lipids. After being washed with 5 ml of nonradioactive medium, islets were perifused for 40 min with 5.5 mM glucose (G) alone. They were then stimulated with 200 nM CCK-8S, 10 mM arginine, 25 μM tolbutamide (TOL), or 1.0 μM forskolin (n = at least 3 for each condition). Efflux rates of ^{3}H were calculated according to Borle et al. (12). Only addition of CCK-8S to these islets significantly increased (*$P < .05$) efflux of [^{3}H]inositol, an index of accelerated phosphoinositide turnover, above prestimulatory values.

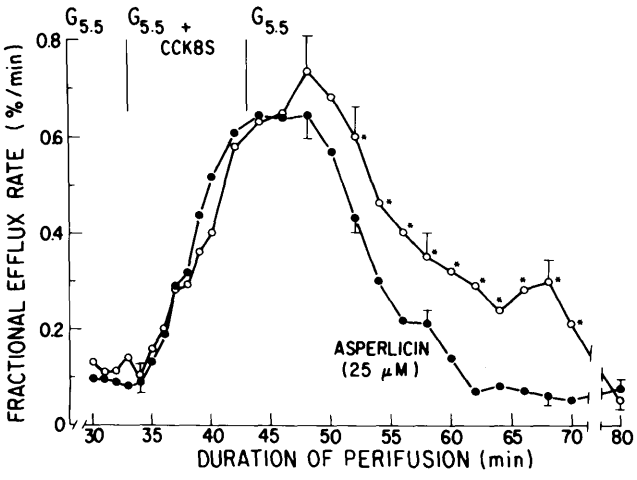

FIG. 5. Sustained activation of ^3H efflux induced by brief CCK-8S exposure. After 2-h labeling period in [^3H]inositol, islets were perifused for 30 min with 5.5 mM glucose (G) alone. They were then stimulated with 200 nM CCK-8S for 10 min followed by 40 min perifusion with 5.5 mM glucose ± 25 μM asperlicin. CCK-8S–induced efflux of ^3H from these islets remained significantly ($P < .05$) elevated above prestimulatory rates for ~30 min after CCK-8S removal from medium ($n = 6$ for each condition). Inclusion of asperlicin during final 40-min period significantly (*$P < .05$) reduced this persistent increase in ^3H efflux.

specific antagonist of CCK binding to its membrane receptor (13), significantly reduced the persistent stimulatory effect of CCK-8S on ^3H efflux. This level of asperlicin completely blocks the stimulatory impact of CCK-8S on insulin output (14).

DISCUSSION

Several salient points emerge from this series of experiments. First, the addition of the gut hormone and possible incretin and satiety factor CCK-8S to islets whose inositol phospholipids are prelabeled with [^3H]inositol results in a dramatic increase in perifusate ^3H as previously reported (9). Whereas the source of ^3H was not directly investigated in this report, previous studies indicate that it results from an increased turnover of inositol-containing phospholipids (8,9). Second, despite its removal from the perifusion medium, the impact of CCK-8S on phosphoinositide metabolism is sustained, and this heightened turnover of membrane phosphoinositides seems to contribute, in a poorly defined manner, to CCK-8S–induced sensitization of stimulated secretion. Third, the persistent effect of CCK-8S on ^3H efflux is reduced by the competitive antagonist asperlicin (13), indicating that sustained agonist-receptor interaction participates in this process. We previously demonstrated that asperlicin also attenuates the increased secretory response to glucose usually observed after CCK-8S exposure (5). Fourth, although arginine, tolbutamide, and forskolin are able to directly augment secretion from islets in the presence of 5.5 mM glucose, they neither induce PI turnover nor sensitize islets to a subsequent glucose stimulus. These particular stimulants were chosen because at the concentrations used, they, like CCK-8S, evoke a glucose-dependent insulin secretory response. Of course, the notion that higher levels or more prolonged exposure times to these agents similarly sensitize islets to various stimulants cannot be excluded (15).

We conclude that under the conditions employed in these studies, cellular events activated by phosphoinositide turnover somehow contribute to this sensitization process, although the exact nature of the cellular changes remains to be completely defined. Considering the events coupled to PI turnover in excitable tissues, possible mediators of this phenomenon exist. PI hydrolysis generates at least two separate but interrelated second messengers: the water-soluble inositol 1,4,5-trisphosphate (IP_3) and the lipid-soluble diacylglycerol (DG) (16–18). IP_3 seems to be involved in mobilization of intracellular calcium (16), which along with DG participates in the activation of the calcium phospholipid-dependent kinase, C-kinase (18). Changes in the cellular concentrations of one or both of these PI-derived products may account for this sensitization effect. Recently, Imai and Gershengorn (19) reported a similar type of sustained PI turnover in hormonally stimulated pituitary cells. Their data indicated that the turnover of PIP_2 is only transiently stimulated, whereas the turnover of phosphatidylinositol is persistently stimulated. Their findings are similar to previously reported studies in activated platelets (20). If a similar situation occurs in islets, a transient increase in IP_3 (generated from PIP_2) but a more sustained increase in DG formation (generated from PIP_2, PIP, and phosphatidylinositol) would be anticipated. The transient nature of IP_3 generation might explain why CCK-8S addition to islets, sensitized to its action by forskolin, results in a large but transient increase in the insulin secretory response (9). Furthermore, if these findings in pituitary cells are applicable to islets, the transient nature of IP_3 generation would seem to relegate it to a minor role in this sensitization process. Rather, DG (or a metabolic product) generated during PI turnover may be playing a pivotal role in this phenomenon. Of course, the notion that inositol 1-phosphate generated from phosphatidylinositol or inositol 1,4-bisphosphate, generated from PIP, may contribute to this process also has to be considered. Finally, because the phorbol ester TPA produces a similar state of sensitization (21), a possible role for enzyme C-kinase or one of its phosphorylated protein products has to be entertained. Studies designed to analyze the nature of this sensitizing compound seem appropriate.

We previously suggested that the biphasic pattern of insulin release from islet tissue noted with high glucose was the result of activation of events in the calmodulin and C-kinase branches of the calcium-messenger system (22). The observation that a synthetic activator of C-kinase, the phorbol ester TPA, caused a slowly developing but sustained output of insulin (second-phase release) from islets supported this contention (23). The generation of DG, the endogenous activator of C-kinase, in response to high glucose might be the result of glucose-induced PI turnover (8,9) and/or the de novo synthesis of DG (24). However, the obligatory participation of PI turnover with the subsequent activation of C-kinase in the sustained second phase of insulin release under all stimulatory conditions has yet to be established. For example, significant rates of second-phase insulin secretion can be obtained with the combination of 7 mM glucose plus 10 mM arginine or 7 mM glucose plus 200 μM of the sulfonylurea tolbutamide (unpublished observations). PI hy-

drolysis does not seem to play a major role in either of these responses (25,26), indicating perhaps that the C-kinase branch of the calcium-messenger system can be bypassed under certain circumstances. In addition, it is possible to dissociate PI turnover from stimulated release. This is best demonstrated with CCK-8S stimulation in the presence of different glucose levels. CCK-8S causes a similar activation of PI turnover in the presence of 2.75 or 7 mM glucose but augments release only at the higher glucose level (9). It would seem, therefore, that second-messenger molecules generated during PI turnover are themselves insufficient to initiate and sustain an insulin secretory response. Moderate glycemia, however, permits their conversion into effective secretory signals.

The results of these experiments suggest that under certain conditions, PI turnover may represent an important futile cycle of sorts, activated by various ligands but not necessarily accompanied by insulin secretion. PI turnover is increased but is not translated into a secretory response until the glucose level (or levels of other fuels that supply a similar permissive signal) bathing the islet increases above some critical threshold value. Under circumstances where PI turnover has been augmented (by cholinergic stimulation during the cephalic phase of digestion or by protein- or fat-induced increases in enteric CCK secretion; 27,28), a small elevation in the glucose level bathing the islet is accompanied by a dramatic augmentation of insulin output, an effect clearly beneficial to fuel homeostasis. The existence of such an amplification mechanism would be physiologically advantageous.

Finally, how might this sensitizing effect of CCK contribute to the possible incretin effect of the peptide (10). Glucose does not directly induce enteric CCK secretion (10). However, the fat or protein components of a mixed meal are clearly effective in this regard (27,28) and serve to elevate circulating CCK levels. The peptide, presumably by activating β-cell PI hydrolysis, sensitizes islets to small subsequent elevation in either postprandial glucose or amino acids. Most important, because the impact of CCK on the β-cell persists for some time after its removal from the medium, the circulating levels of the peptide do not have to remain elevated to influence agonist-induced secretion. A transient increase in the plasma CCK concentration exerts a sustained secretory effect. Although uncertainty about the nature of the biochemical changes that underlie this sensitizing effect exists, its benefit to insulin-regulated fuel homeostasis seems obvious even to the casual observer. More important, the possible contribution of this process to the hyperinsulinemia of obesity and type II (non-insulin-dependent) diabetes should be investigated.

ACKNOWLEDGMENTS

The expert secretarial assistance of Patricia Cross and Joan Fettes is gratefully acknowledged.

This work was supported by NIH Grant AM-34381 and the American Heart Association (84-709).

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