Prior Cholecystokinin Exposure Sensitizes Islets of Langerhans to Glucose Stimulation

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SUMMARY

Prior, short-term exposure of isolated perifused islets to cholecystokinin (CCK8S) sensitizes them to subsequent glucose stimulation. This sensitization effect develops quickly and persists long after the removal of CCK8S from the perifusion medium. Continued binding of CCK8S to its receptor on the β-cell and the increase in glucose metabolism noted with glucose stimulation are essential for the full expression of this response. This sensitization process may play an integral role in the postulated incretin effect of the peptide. *Diabetes* 36:118–22, 1987

Insulin release from the pancreatic β-cell after food ingestion is thought to be facilitated by the release of various gut hormones termed incretins (1,2). In this capacity, these gut hormones sensitize the β-cell to the stimulant action of glucose, amplify insulin release, and thus ensure adequate insulinization of liver and peripheral tissues. Although some uncertainty still exists regarding the nature of the main incretin factors, evidence continues to accumulate that cholecystokinin (CCK) may be playing an essential role. The description of CCK receptors on β-cells (3), the observation that CCK-containing nerve terminals innervate the islet (4), and the exquisite glucose dependency of CCK-induced insulin release support this contention (5–7). The secretory effect of CCK on the β-cell is presumably mediated by the peptide's interaction with a specific membrane receptor and the subsequent hydrolysis of polyphosphoinositides (PPI) (8–10). We document the existence of a hitherto undescribed action of CCK on the β-cell: prior, short-term exposure to CCK induces a heightened state of secretory responsiveness to a moderate physiologic elevation in the ambient glucose level. This sensitizing effect of CCK develops within minutes, persists long after CCK removal from the medium, and may play an integral role in the postulated incretin effect of the hormone.

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MATERIALS AND METHODS

Male Sprague-Dawley rats purchased from Charles River (Wilmington, MA) were used in all studies. The animals were fed ad lib and weighed 300–400 g when killed. After Nembutal-induced (50 mg/kg) anesthesia, islets were isolated by collagenase digestion (11) and perifused (12). Usually 6 groups of 12 islets were studied from each animal. The islets, supported on a nylon filter, were placed in Millipore chambers and perifused at a flow rate of 1 ml/min. The pH of the perifusion medium was maintained at 7.4 and the temperature at 37°C. Islets were perifused with 5.5 mM glucose for 30 min to establish stable basal secretory rates. After this stabilization period, some islets were exposed to CCK8S for various times. The perifusion was continued in 5.5 mM glucose, again for various lengths of time, and then all islets were stimulated with 7.5 mM glucose for 40 min. Effluent samples were collected and analyzed for insulin content with rat insulin as standard (Lilly, Indianapolis, IN, lot 615-D63-12-3) (13).

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/dl 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-insulin used for the insulin assay was purchased from New England Nuclear (Boston, MA). CCK8S (lot 124F-04451), mannoheptulose, bovine serum albumin (RIA grade), and salts used to make the Hanks' solution and perifusion medium were purchased from Sigma (St. Louis, MO). Asperlicin was the generous gift of Dr. V. Lotti (Merck, Sharp, and Dohme, West Point, PA). It was dissolved in DMSO before use. The amount of DMSO used in these studies (0.1%) had no adverse impact on islet function (unpublished observations).

Statistical significance was determined with Student's *t*

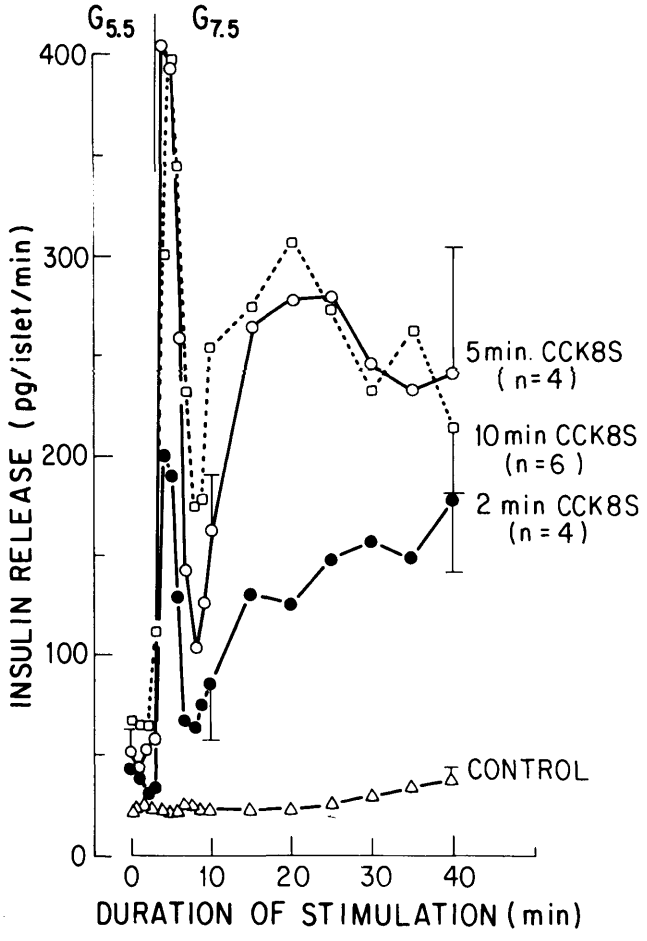

FIG. 1. Influence of prior CCK8S exposure on glucose-induced insulin output. All groups of islets were perifused for 30 min with 5.5 mM glucose to achieve basal, stable rates of hormone output. Three groups were then exposed to 200 nM CCK8S in continued presence of 5.5 mM glucose for 2, 5, or 10 min, perifused for 10 min more with 5.5 mM glucose alone, and then stimulated with 7.5 mM glucose. Control islets were perifused for 50 min with 5.5 mM glucose before provocation with 7.5 mM glucose. This and subsequent figures have been corrected for dead space in perifusion system of 3 ml (equivalent to 3 min with flow rate of 1 ml/min). Although increase in insulin secretory rate to 7.5 mM glucose is only apparent after ~25–30 min of stimulatory period, islets previously exposed to CCK8S for various times displayed dramatic first phase of release and sustained second-phase output. Insulin response from all CCK8S-pretreated islets was significantly greater ($P < .05$) than that noted from control islets at all time points past 1st min of stimulant presentation.

test for unpaired data, and $P < .05$ was taken as significant. Values presented in the figures or RESULTS represent means ± SE.

RESULTS

Perifusion of isolated islets in a medium containing 5.5 mM (100 mg/dl) glucose for 50 min results in stable, basal rates of insulin release that average ~20 pg · islet^{-1} · min^{-1} before stimulation. Exposure of these islets to 7.5 mM (135 mg/dl) glucose is accompanied by a delayed rise in insulin output that, after 40 min stimulation, is about twofold greater than prestimulatory rates (Fig. 1). A markedly different secretory response to 7.5 mM glucose is evoked if islets are previously exposed to 200 nM CCK8S for various times, and the effect is apparent even after removal of the peptide from the perifusion medium. In the experiments shown in Fig. 1, after a 30-min perifusion with 5.5 mM glucose islets were exposed to 200 nM CCK8S for 2, 5, or 10 min followed by a 10-min perifusion in 5.5 mM glucose alone. They were then provoked with 7.5 mM glucose and the secretory response monitored (Fig. 1). Particularly dramatic is the increase in first-phase release seen in islets previously exposed to 200 nM CCK8S. Peak secretion during this phase averages 200 ± 34, 408 ± 109, and 399 ± 43 (means ± SE, $N \leq 4$ for all groups) in islets previously perifused for 2, 5, or 10 min with 200 nM CCK8S. Control islets, not previously exposed to CCK8S, release insulin at a rate of 22 ± 3 ($N = 6$) pg · islet^{-1} · min^{-1} at the same time point. Moreover, the augmented sensitivity to 7.5 mM glucose induced by CCK8S persisted during the 40-min perifusion with this level of the hexose. Release averaged 180 ± 34, 240 ± 67, and 212 ± 32 pg · islet^{-1} · min^{-1} in effluent samples collected 35–40 min after the onset of stimulation in the groups exposed for 2, 5, and 10 min, respectively, to CCK8S. Control islets released insulin at a rate of 36 ± 2 pg · islet^{-1} · min^{-1} at this time. Considering that the stimulatory phase with 7.5 mM glucose lasted 40 min, the effect of CCK8S on secretion was clearly still evident ~50 min after its removal from the medium.

It proved possible to sensitize islets to glucose (7.5 mM) stimulation with lower levels of CCK8S. Although a 20-min

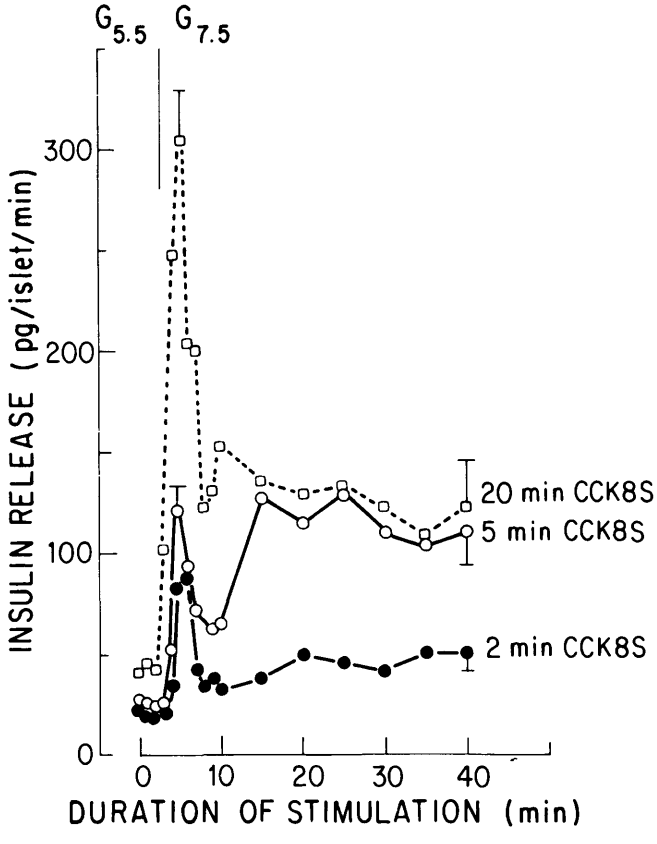

FIG. 2. Capacity of CCK8S to sensitize islet is related to duration of exposure to CCK8S. Islets were perifused for 30 min with 5.5 mM glucose and for 2, 5, or 20 min more with 200 nM CCK8S plus 5.5 mM glucose. After 20 min perifusion with 5.5 mM glucose alone, all groups were provoked with 7.5 mM glucose, and secretory response was monitored. When analyzed statistically, peak first-phase and second-phase secretory responses were greater in 20-min CCK8S group than in 2-min CCK8S group.

exposure to 2 nM CCK8S, followed by a 10-min washout with 5.5 mM glucose alone, did not sensitize the islet to 7.5 mM glucose, perifusion with 10, 25, or 50 nM CCK8S for 20 min was accompanied by a significant augmented secretory response to the hexose. Peak first-phase release after exposure to these three levels of CCK8S averaged 68 ± 8, 201 ± 11, and 386 ± 38 pg · islet^{-1} · min^{-1}, and the second-phase response noted 35–40 min after the onset of stimulation averaged 53 ± 6, 73 ± 3, and 232 ± 21 pg · islet^{-1} · min^{-1} in the 10, 25, and 50 nM CCK8S-pretreated groups, respectively (N = 4 for all groups). In contrast, control islets, not previously exposed to CCK8S, released insulin at the rates of 20 ± 3 and 27 ± 2 pg · islet^{-1} · min^{-1} during the first and second phases of the response to 7.5 mM glucose (N = 3).

The capacity of CCK8S to sensitize the islets to glucose stimulation persisted for some time, and the duration of this "memory" effect was related to the duration of CCK8S exposure. Islets were exposed to CCK8S for 2, 5, or 20 min and then perifused for an additional 20 min with 5.5 mM glucose alone. They were then stimulated for 40 min with 7.5 mM glucose. As shown in Fig. 2, the shorter exposure times to CCK8S were accompanied by smaller secretory responses to the addition of 7.5 mM glucose, even though the duration of perifusion in CCK8S-free medium was similar (20 min). Moreover, a prior 20-min CCK8S exposure, even after a 20-min washout period with 5.5 mM glucose alone, resulted in a significantly greater insulin secretory response to 7.5 mM glucose, and both the first and second phases of release were significantly augmented above control release rates (Fig. 2), indicating again that the impact of CCK8S on the islet is sustained long after its removal from the medium.

In pancreatic exocrine tissue, persistent binding of CCK to its membrane receptor occurs despite a prolonged washout period in CCK-free medium (14). This continued interaction of the peptide and its receptors seems responsible for the persistant stimulation of exocrine secretion noted in this tissue, and the existence of several CCK-binding sites per receptor has been proposed to explain this phenomenon (14,15). A similar situation may occur in pancreatic β-cells, and experiments were designed to test this possibility. Islets were perifused for 30 min with 5.5 mM glucose and then stimulated with 200 nM CCK8S for 10 min. In the continued presence of 5.5 mM glucose the perifusion was continued for an additional 10 min in a medium supplemented with 10 uM asperlicin, a membrane-active competitive inhibitor of CCK binding to its receptor (16). We have previously demonstrated that this level of asperlicin does not influence release when islets are stimulated with glucose alone (17). Islets were then exposed to 7.5 mM glucose (in the absence of 10 μM asperlicin) for 40 min and the secretory response assessed. Under this condition, asperlicin virtually abolished the CCK8S-induced sensitization to 7.5 mM glucose (Fig. 3). The ability of CCK8S to augment glucose-induced insulin secretion depends on the catabolism of the hexose (7). To test whether a similar dependency on glucose metabolism also characterizes this sensitization process, islets were exposed to 200 nM CCK8S for 10 min, perifused for 10 min with 5.5 mM glucose, and then stimulated with the combination of 7.5 mM glucose plus 7.5 mM mannoheptulose. The

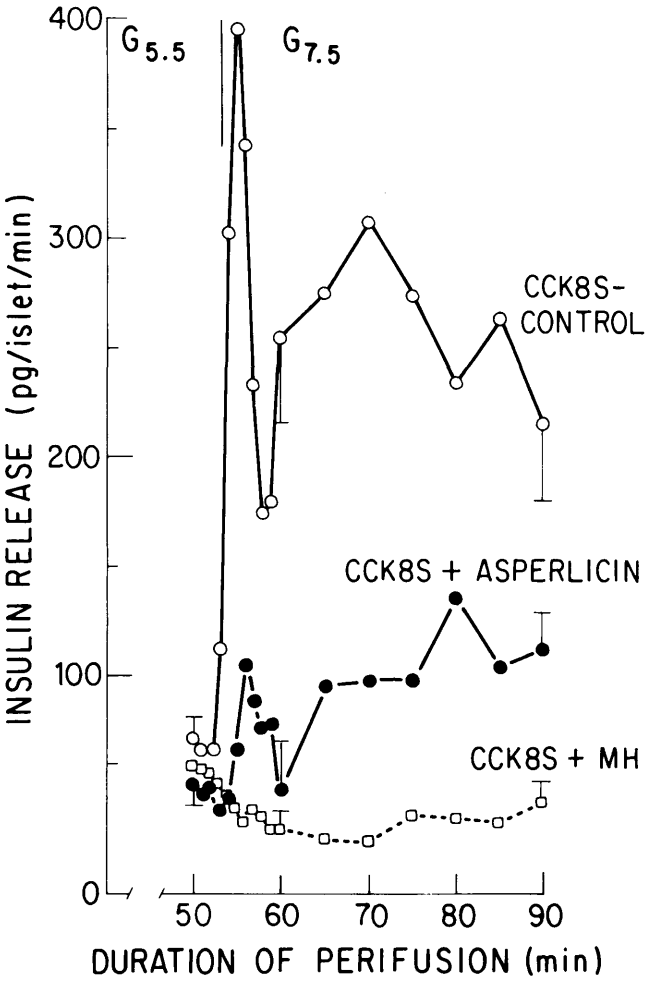

FIG. 3. Effect of asperlicin and mannoheptulose (MH) on CCK8S-induced sensitization. Islets were perifused for 30 min with 5.5 mM glucose alone and for 10 min more with 200 nM CCK8S. In asperlicin group, perifusion was continued for 10 min more in 5.5 mM glucose plus 10 μM asperlicin. Then they were provoked with 7.5 mM glucose without further addition of asperlicin. In MH group, this 7-carbon sugar (at 7.5 mM) was included during stimulation period with 7.5 mM glucose. When compared statistically with CCK8S control group, asperlicin and MH significantly ($P < .05$) reduced insulin output at all time points during stimulatory period with 7.5 mM glucose.

latter compound dose dependently blocks glucose phosphorylation and further catabolism by the islet (18). It also effectively abolishes the heightened secretory response to 7.5 mM glucose usually noted after sensitization with 200 nM CCK8S (Fig. 3).

DISCUSSION

The results of this study reveal a previously unreported effect of CCK8S on the β-cells of the islets of Langerhans: a short-term increase in the level of the peptide bathing these cells results in a persistent sensitization to glucose stimulation even after removal of the peptide from the medium. This effect of CCK8S results in a marked sensitization of the receptor systems that regulate secretion to moderate physiologic elevations in the ambient glucose level and dramatically potentiates glucose-induced release. However, this memory effect of CCK8S on secretion surfaces only if

the level of glucose increases into the range usually noted postprandially. Of particular physiologic importance, perhaps, is the finding that the memory effect appears greatest for the initial or first phase of secretion. The first phase or acute response of the β-cell to glucose stimulation is thought to be most important in the maintenance of glucose homeostasis (19).

If this sensitizing action of CCK functions in vivo, it represents an elegant homeostatic mechanism, ensuring that appropriate amounts of insulin are secreted, particularly during the initial minutes of glucose stimulation, to promote fuel disposal. Moreover, and considering that this sensitization process persists for some time, the elevation of CCK8S secretion thought to be initiated during mixed-meal digestion by protein and fat products (20,21) prepares the β-cell for activation by glucose or perhaps even other nutrient secretagogues. The advantages of this homeostatic mechanism seem obvious and would serve to facilitate the disposition of fuels dependent on insulin's action. Whether this process of CCK-induced sensitization to glucose stimulation plays a pathophysiologic role in the initiation and/or maintenance of the hyperinsulinemia noted in obese normoglycemic or obese hyperglycemic individuals is unknown (22,23). However, evidence suggesting that CCK may play an important physiologic role in the regulation of plasma insulin levels continues to accumulate: CCK is released during mixed-meal digestion (20,21), the same levels of CCK that activate pancreatic enzyme secretion augment insulin output (5), CCK receptors have been localized on β-cells (3), CCK-containing nerve terminals innervate the islet (4), and CCK potentiates glucose-dependent insulin release (5–7). Our results with CCK further emphasize the evolving complexity of CCK actions on the β-cell. The possible involvement of CCK in various hyperinsulinemic syndromes warrants further investigation.

It is difficult to precisely define, in biochemical terms, how CCK8S induces this state of heightened sensitivity to glucose. Because asperlicin, which inhibits CCK binding to its receptor (16) and abolishes CCK8S- but not glucose-induced insulin release (17), significantly attenuated this sensitization process, the continued association of CCK with its membrane receptor seems to be important for this response. Similar to the situation noted in exocrine tissue (14,15), each CCK receptor may have several binding sites, differing in affinity for the peptide, and one or both of these sites may regulate this process. Further experiments examining CCK receptor sites on the islets seem appropriate. In addition to the continued association of CCK8S with its membrane receptor, the metabolism of glucose is also essential to the emergence of this heightened secretory response, because mannoheptulose effectively abolished it.

There is little doubt that CCK8S has a major effect on islet polyphosphatidylinositol (PPI) metabolism, and events in the PPI cycle are implicated (8,10). If PPI hydrolysis does play an important role here, the various second messengers (IP_3 and diacylglycerol) generated during PPI turnover may mediate this process (24,25). In addition, other agents that activate the hydrolysis of membrane PPI might produce a similar degree of sensitization. Particularly relevant in this regard might be the action of acetylcholine on the β-cell. Similar to CCK8S, it induces a glucose-dependent increase in insulin output (26), an effect thought to be at least partly mediated by alterations in PPI metabolism (8). Consequently, the cephalic phase of meal ingestion, accompanied by neuronally derived increases in the level of acetylcholine bathing the islet, may serve to sensitize these cells to glucose, much like CCK8S does. Whether other gut factors (e.g., gastric inhibitory polypeptide and secretin) implicated in the enteroinsular axis produce a similar state of persistent sensitization to glucose is not known and should be studied.

Note that the concentration of CCK8S used in this study to sensitize the β-cell to glucose are considerably greater than circulating levels of the peptide (21). However, considering the possible contribution of neuronally derived CCK to the islet extracellular space (4), it is difficult to precisely estimate the level of the peptide bathing the islet. In the intact pancreas, the levels of CCK that activate enzyme release from exocrine cells are equivalent to those that activate secretion from endocrine cells (5). Consequently, as previously pointed out (6), the process of collagenase isolating islets diminishes the sensitivity of the β-cell to CCK stimulation, thus necessitating the use of higher peptide levels.

Although the nature of the intracellular signals involved in this sensitization process needs to be more clearly defined, our results underscore the complexity of CCK8S actions on the islet. However, the physiologic and clinical significance of these findings remain a fertile area for speculation.

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