Interactions between cholinergic agonists and enteric factors in the regulation of insulin secretion from isolated perfused rat islets

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Abstract. The ability of the cholinergic agonist carbachol to sensitize islets to the action of combined glucose, cholecystokinin and gastric inhibitory polypeptide was determined in isolated rat islets. In response to this combination, peak first phase insulin secretion from control islets averages 85 ± 5 pg·islet⁻¹·min⁻¹ (mean ± SEM) and the insulin secretory rates measured 35–40 min after the onset of stimulation averages 127 ± 34 pg·islet⁻¹·min⁻¹. A prior 20 min exposure to 1 mmol/l carbachol potentiates the modest insulin stimulatory response to this combination of stimulants; peak first phase release is 354 ± 61 pg·islet⁻¹·min⁻¹, and release measured 35–40 min after the onset of stimulation is 179 ± 34 pg·islet⁻¹·min⁻¹. This sensitizing effect of carbachol lasts for at least 40 min and can be duplicated by the natural in vivo agonist acetylcholine. These results demonstrate that cholinergic stimulation of isolated islets primes them to the subsequent stimulatory effect of a moderate increase in the circulating glucose level and to several postulated incretin factors. If operative in vivo, this communications network between cephalic and enteric factors represents a remarkable control system to ensure the release of insulin in amounts commensurate to meet the anticipated and actual insulin requirements for insulin-mediated fuel disposition.

Thought to be mediated by vagally-derived acetylcholine, the existence of the cephalic phase of insulin secretion has long been recognized (Louis-Sylvestre 1976, 1978: Trimble et al. 1981; Berthoud et al. 1984). We recently reported that the cholinergic agonist carbachol, in addition to acutely stimulating insulin secretion in the presence of 5.5 or 7.0 mmol/l glucose, sensitized the beta-cell to the weak insulin stimulatory effect of 7.5 mmol/l glucose (unpublished observations). This sensitization process appears to be mediated by phosphoinositide-derived second messengers and lasts for at least 40 min after carbachol removal.

In addition to acetylcholine, the release of various enteric factors, particularly gastric inhibitory polypeptide and cholecystokinin, is thought to participate in the regulation of insulin secretion from the beta-cell (Creutzfeldt 1979; Creutzfeldt & Ebert 1985; McCullough et al. 1985; Rushakoff et al. 1987). Termed the entero-insular axis (Unger & Eisentraut 1969), this network is thought to play an important role in insulin-mediated fuel homeostasis.

In the present report we have examined the possible effect of prior cholinergic stimulation of the beta-cell on the capacity of several gut factors to influence the release of insulin from the beta-cell.

Materials and Methods

Male Sprague-Dawley rats purchased from Charles River (Kingston, NY) were used in all studies. The animals were fed ad libitum and weighed between 300–400 g. After Nembutal-induced (50 mg/kg) anesthesia, islets were isolated by collagenase digestion (Lacy & Kostianovsky 1967) and perfused. The pH of the perfusion medium was maintained at 7.4, the temperature at 37°C, and the flow rate at 1 ml/min. Islets were usually perfused for 30 min to establish stable insulin secretory rates and then
exposed to various agonists indicated in the figure legends. Perifusate samples were collected at time intervals indicated in the figures and 200-µl aliquots analyzed for insulin (Alban et al. 1972) using rat insulin (Eli Lilly and Co, Indianapolis, IN, #615-D63-12-1-2) as standard. The radioisotope used to measure insulin release (\(^{125}\text{I-}\)insulin) was purchased from New England Nuclear, Boston, MA. Acetylcholine, carbachol, cholecystokinin (the C-terminal 8-amino acid derivative sulfated on the tyrosine residue) bovine serum albumin, as well as the salts used to make the perifusion medium, were purchased from Sigma Chemical Co, St. Louis, MO. Gastric inhibitory polypeptide was obtained from Peninsula Labs, Belmont, CA.

Statistical differences were analyzed by ANOVA in conjunction with the Newman-Keuls test for multiple comparisons or by the Student's \(t\)-test. A \(P\) value less than 0.05 was taken as significant. Values in the figures represent means±SEM of the specified number of observations.

**Results**

The insulin secretory responses of three groups of islets are depicted in Fig. 1. Perifusion of isolated islets with 5.5 mmol/l glucose alone for 60 min resulted in low stable rates of insulin secretion (open circles). In response to 6.5 mmol/l glucose stimulation, insulin release rates increased from pre-stimulatory values of 33±5 pg·islet\(^{-1}\)·min\(^{-1}\) to 51±7 pg·islet\(^{-1}\)·min\(^{-1}\) 3 min after 6.5 mmol/l glucose presentation. After 40 min release rates averaged only 32±3 pg·islet\(^{-1}\)·min\(^{-1}\).

In response to the combination of 6.5 mmol/l glucose, 5 nmol/l cholecystokinin 8-sulfate (CCK-8S) and gastric inhibitory polypeptide (GIP, 50 µg/l), control islets release insulin in a biphasic pattern (Fig. 1, closed circles). Peak first phase release averages 85±5 pg·islet\(^{-1}\)·min\(^{-1}\) (N = 5), while release after 40 min of stimulation averages 127±34 pg·islet\(^{-1}\)·min\(^{-1}\). This modest response was significantly amplified if islets were primed with 1 mmol/l carbachol. In the presence of 5.5 mmol/l glucose, this level (1 mmol/l) of the cholinergic agonist significantly increased the secretion of insulin from pre-stimulatory rates of 42±7 pg·islet\(^{-1}\)·min\(^{-1}\) to 83±6 pg·islet\(^{-1}\)·min\(^{-1}\) after 10 min of stimulation (Fig. 1, triangles). Release rates fell to 49±8 pg·islet\(^{-1}\)·min\(^{-1}\) 10 min after withdrawal of the compound. In response to subsequent stimulation with 6.5 mmol/l glucose, 5 nmol/l CCK-8S and 50 µg/l GIP, a significantly amplified first phase insulin secretory response was noted. Peak first phase release now averaged 354±61 pg·islet\(^{-1}\)·min\(^{-1}\) (vs 85±5 pg·islet\(^{-1}\)·min\(^{-1}\)). Release 35–40 min after the onset of stimulation averaged 179±34 pg·islet\(^{-1}\)·min\(^{-1}\), a value not significantly different (\(P > 0.05\), unpaired Student's \(t\)-test) from control non-carbachol-treated islets (127±34 pg·islet\(^{-1}\)·min\(^{-1}\)). Extending the
Time-dependent potentiation induced by carbachol lasts at least 45 min. Islets were treated similar to the protocol given in Fig. 1. In this case, however, the interval between carbachol priming and stimulation with 6.5 mmol/l glucose, 5 nmol/l cholecystokinin-8-sulfate (CCK-8S) and 50 µg/l gastric inhibitory peptide (GIP) was extended to 40 min. Four experiments were conducted with each protocol.

interval (to 40 min) between carbachol exposure and stimulation of islets with the combination of 6.5 mmol/l glucose, 5 nmol/l CCK-8S and 50 µg/l GIP was not sufficient to abolish the effect of previous exposure to carbachol on insulin output (Fig. 2). Significantly amplified is the first phase of insulin secretion in response to this agonist combination. Second phase release rates were comparable to those noted from control islets.

It might be suggested that the sensitizing effect of carbachol noted here is a unique property of this compound and that the in vivo cholinergic agonist acetylcholine may not be able to duplicate this action. To counter this possible criticism, studies were conducted with acetylcholine (1 mmol/l)-primed islets. Similar to carbachol, acetylcholine induced a sensitization of perfused islets. In these studies (Fig. 3), islets were perfused for 20 min with 1 mmol/l acetylcholine. Similar to carbachol, acetylcholine in the presence of 5.5 mmol/l glucose stimulated the release of insulin. After perfusion for 10 min with 5.5 mmol/l glucose alone, these islets were then stimulated for 40 min with the combination of 6.5 mmol/l glucose, CCK-8S (5 nmol/l) and GIP (50 µg/l). Similar to the results obtained with carbachol, first phase release (206 ± 31 pg · islet⁻¹ · min⁻¹) to this agonist combination is significantly (P < 0.05, Student's t-test) amplified above that noted from control, non-primed islets. A similar, albeit less dramatic and not quite significant, increase was also observed during the second phase response.

Discussion

The release of insulin from the pancreatic beta-cell depends upon the appropriate integration of metabolic, neurotransmitter and enteric signals. In the present report, we have investigated the interaction of components thought to be involved in the control of the cephalic and enteric phases of insulin secretion. Our results demonstrate that the temporal presentation of various signals perceived by the beta-cell dramatically influences in a positive fashion the secretion of insulin by these cells. That the in vitro findings reported here reflect events occurring in vivo is a reasonable speculation.

Fig. 2.

Discussion
Acetylcholine induces time-dependent potentiation. Groups of islets were perfused as indicated in the legend of Fig. 1. In the case of one group (open triangles), these islets were exposed to 1 mmol/l acetylcholine for 20 min followed by a 10-min washout with 5.5 mmol/l glucose alone. Subsequently these islets were stimulated with 6.5 mmol/l glucose, 5 nmol/l cholecystokinin 8-sulfate (CCK-8S) and 50 µg/l gastric inhibitory peptide (GIP). Four experiments were performed under each experimental setting.

Fig. 3.

at this time and further comment on these results seems appropriate.

The prompt release of the neurotransmitter acetylcholine in response to food ingestion is thought to regulate the cephalic phase of insulin secretion (Louis-Sylvestre 1976; Trimble et al. 1981). Recent work with the cholinergic agonist carbachol or acetylcholine has demonstrated that these compounds induce a glucose-independent increase in phosphoinoside (PI) hydrolysis but a glucose-dependent insulin secretory response (unpublished observations; Morgan et al. 1985; Garcia et al. 1988). In other words, while cholinergic stimulation results in the breakdown of these strategically-situated phospholipids, and the subsequent generation of second messenger molecules, an insulin secretory response ensues only if the level of glucose bathing the islet is in the postprandial range. This elegant homeostatic mechanism provides a reasonable safeguard against inappropriate insulin secretion followed by hypoglycemia. We have suggested previously that while increases in PI hydrolysis may not be an event sufficient in and of itself to activate insulin secretion, these increases in PI hydrolysis seem to sensitize the beta-cell to subsequent stimulation by a variety of compounds (Zawalich et al. 1987a; Zawalich 1988). Our results with different agonists including glucose, glycertdehyde, carbachol, tolbutamide, leucine, alpha-ketoisocaprate and the monokine interleukin-1 have demonstrated what appears to be the obligatory participation of events associated with PI hydrolysis in the induction of this sensitization process (Zawalich 1988a,b; Zawalich et al. 1987a, 1988, 1989; Zawalich & Zawalich 1988), and unpublished observations.

The present results with carbachol and acetylcholine indicate that cholinergic stimuli act as proemial messengers to sensitize the beta-cell to subsequently imposed, physiologically relevant stimuli. Thus, while the responses of control non-primed islets to 6.5 mmol/l glucose alone or to the combination of 6.5 mmol/l glucose plus CCK-8S plus GIP are modest at best, a significantly amplified response to this agonist combination occurs after the proemial priming with carbachol or acetylcholine. Most interestingly, the duration of this priming effect is compatible with the concept that this temporal arrangement functions in vivo to support the release of insulin in amounts commensurate with both the anticipated and actual needs of the organism. The data in Fig. 2 demonstrate that islets primed with carbachol display heightened insulin secretory responses for at least 45 min after perfusion in a carbachol-free medium.

In the present series of experiments we employed a combination of three physiologically relevant stimuli to induce insulin release from naïve or cholinergically-primed islets. GIP was employed to elevate cAMP levels (Siegel & Creutzfeldt 1985), a biochemical alteration which also amplifies the beta-cells’ response to PI-derived second messenger molecules (Zawalich et al. 1987b). CCK-8S was used further to activate PI hydrolysis and to simulate in vivo conditions. A moderate elevation in glucose (6.5 mmol/l) was also employed since this hexose level permits activation of the secretory apparatus by mechanisms yet to be defined. Finally, it should be pointed out that the levels of enteric hormones employed are pharmacologic,
rather than physiologic. However, the process of collagenase isolating islets appears to diminish their sensitivity to extracellular messengers, thus necessitating the use of higher concentrations of these hormones.

It is not yet possible to define in precise molecular detail how carbachol, or for that matter any other compound, primes islets and how PI hydrolysis might regulate this process. Our previous studies indicate that in the case of CCK-8S, sustained hormone-receptor activation appears important (Zawalich et al. 1987a; Zawalich & Díaz 1987). For heuristic purposes at least, it is attractive to suggest that cholinergically-induced sustained increases in PI hydrolysis supply amounts of diacylglycerol sufficient to keep the enzyme protein kinase C anchored to the plasma membrane. Alternatively, the C-kinase may be anchored to the membrane by some covalent modification of its structure (Alkon & Rasmussen 1988). In this anchored state, the enzyme appears particularly sensitive to calcium. Consequently, any agonist which increases calcium influx in primed islets will evoke a C-kinase-dependent amplified response. The fact that the phorbol ester tetradecanoylphorbol ester, an established activator of the enzyme C-kinase, induces time-dependent potentiation in islets is consistent with this suggestion (Sorensen 1986). Other published observations also support this concept (Niki et al. 1988).

The present results, when taken in conjunction with previous studies (Zawalich et al. 1987a, unpublished observations), indicate that vagally-derived acetylcholine and enteric-derived CCK-8S participate in the regulation of glucose homeostasis, at least in part by priming or sensitizing the beta-cell to small, physiologically relevant increases in plasma glucose. That sensitization involves agonist-induced increases in PI hydrolysis is also indicated. Because priming has such a disproportionate effect on first phase insulin secretion and since first phase insulin release appears to be both physiologically significant and most susceptible to pathological alterations, it might be suggested further that the amplitude of first phase release depends in part on the amount of C-kinase associated with the membrane at any given time. This association may play a major role in glucose homeostasis (Seltzer et al. 1967). The fact that so many different compounds induce a PI-dependent increase in first phase insulin secretion emphasizes, perhaps, the importance of this phase in glucose homeostasis. Future experiments designed to explore in detail the factors which influence protein C-kinase translocation and activation in islets seem appropriate.

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References


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