Influence of cAMP and Calcium on [^{3}H]Inositol Efflux, Inositol Phosphate Accumulation, and Insulin Release From Isolated Rat Islets

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The influence of cyclic AMP (cAMP) and extracellular calcium on phosphoinositide (PI) hydrolysis in isolated islets was assessed and related to insulin output. Three stimulants were chosen to activate the β-cell: sulfated cholecystokinin (CCK-8S, 200 nM), high-level glucose (20 mM), and the sulfonylurea tolbutamide (200 μM). The insulin secretory response to all three agonists was amplified by forskolin (which increases cAMP levels) and reduced by nitrendipine (which decreases calcium influx). All three stimulants increased the hydrolysis of inositol-containing phospholipids, an event monitored by an increase in [^{3}H]inositol efflux from [^{3}H]inositol-prelabeled islets and by the accumulation of labeled inositol phosphates. Forskolin, despite its positive impact on insulin secretion, reduced [^{3}H]inositol efflux and inositol phosphate accumulation in response to all agonists. A similar inhibitory effect on these parameters was noted with nitrendipine; however, nitrendipine abolished secretion in response to all agonists. These findings support the following conclusions: *1*) an increase in cellular cAMP levels reduces the quantitative impact of various agonists on these indices of PI hydrolysis; *2*) despite this inhibitory effect, cAMP amplifies the insulin secretory response to these agonists; and *3*) extracellular calcium is a crucial determinant of both PI hydrolysis and the ensuing insulin secretory response. *Diabetes* 37:1478–83, 1988

Second-messenger molecules generated during phosphoinositide (PI) hydrolysis in β-cells are thought to participate in the insulin secretory response to various agonists (1–4). Particularly cogent in this regard are studies with the possible incretin- and satiety-factor cholecystokinin (CCK). This peptide induces a glucose-dependent insulin secretory response (5–7), an event accompanied by the hydrolysis of islet inositol-containing lipids, the efflux of ^{3}H from [^{3}H]inositol-labeled islets, and the accumulation of inositol phosphates (8,9). We recently reported that forskolin treatment of perifused islets dramatically amplified their insulin secretory response to sulfated cholecystokinin (CCK-8S) stimulation (8). It was not determined in these studies whether forskolin influenced PI hydrolysis. In our study we employed three secretagogues, CCK-8S, high glucose (20 mM), and the sulfonylurea tolbutamide to stimulate β-cells. Their effects on insulin output and PI hydrolysis, monitored by [^{3}H]inositol efflux and inositol phosphate accumulation in [^{3}H]inositol prelabeled islets, were assessed in the presence of elevated cyclic AMP (cAMP) levels (achieved with forskolin treatment) and in the absence of calcium influx (achieved with nitrendipine treatment). The results demonstrate that while forskolin dramatically amplifies the insulin secretory response to all three agonists, it reduces their impact on [^{3}H]inositol efflux and inositol phosphate accumulation. Nitrendipine, on the other hand, reduces agonist-induced insulin release, [^{3}H]inositol efflux, and inositol phosphate levels. These results emphasize that both the cellular levels of cAMP and extracellular calcium determine the extent of PI hydrolysis and the β-cell response, in terms of insulin output, to the second-messenger molecules generated by this pathway.

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MATERIALS AND METHODS

The detailed methodologies employed to assess insulin output from collagenase-isolated islets have been previously described (7–9). Male Sprague-Dawley rats purchased from Charles River were used in all studies. The animals were fed ad lib and weighed 300–400 g. After Nembutal (pentobarbital sodium, 50 mg/kg; Abbott, North Chicago, IL)-induced anesthesia, islets were isolated by collagenase digestion (10). Some islets were immediately perifused. To monitor PI hydrolysis, other groups of islets were subjected to a 2-h incubation period in *myo*-[2-^{3}H]inositol before perifusion. In

these labeling experiments, 10 μCi of *myo*-[2-^{3}H]inositol (initial sp act 16.6–19.9 Ci/mmol) were added to 250 μl of media (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 35–70 islets were loaded onto nylon filters and then placed in small glass vials. The radioactive incubation medium (200 μl) was gently added, the vials stoppered, and the atmosphere above the islets 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. Some of these islets were statically incubated to measure inositol phosphate accumulation. Other islets were perifused to assess the kinetics of ^{3}H efflux. Islets were perifused (flow rate 1 ml/min) with 2.75 or 5.5 mM glucose for 30 min to establish stable basal insulin secretory rates. After this stabilization period, the islets were subjected to various protocols indicated in the figure legends. Effluent samples were collected and analyzed for insulin content (11) with rat insulin as standard (lot 615-D63-12-3, Lilly, Indianapolis, IN) and, when appropriate, ^{3}H content. In the theophylline experiments, labeled inositol phosphate accumulation was assessed immediately after the perifusion. Labeled inositol phosphates were measured as previously described (8,12). After extraction with 400 μl 10% perchloric acid, neutralization with 0.25–0.28 ml 6 N KOH, further addition of 5 ml water, and centrifugation, the supernatant was applied to columns. These columns were prepared by adding anion-exchange resin (AG1-X8 formate form, Bio-Rad, Richmond, CA) to Pasteur pipettes to achieve a length of 3 cm. Further additions to the column included 10 ml water and 5 ml 5 mM Borax/60 mM sodium formate. Elution of the inositol phosphates was accomplished by the sequential addition of 10 ml 0.1M formic acid/0.2 M ammonium formate (IP_1), 0.1 M formic acid/0.4 M ammonium formate (IP_2), and 0.1 M formic acid/1M ammonium formate (IP_3). This methodology does not distinguish between the various inositol trisphosphate isomers, inositol 1,4,5-trisphosphate and inositol 1,3,4-trisphosphate. Aliquots (0.4 ml) of the eluate were then analyzed for radioactive contents.

Reagents. Hank's 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-labeled insulin used for the insulin assay was purchased from New England Nuclear (Boston, MA) and the *myo*-[2-^{3}H]inositol from Amersham (Arlington Heights, IL). cAMP was determined by radioimmunoassay with kits purchased from Amersham. CCK-8S (COOH-terminal, 8 amino acid fragment 26–33 amide, sulfated on the tyrosine residue, lot 124F-0445), bovine serum albumin (RIA grade), theophylline, formic acid, lithium chloride, as well as the salts used to make the Hank's solution and perifusion medium were purchased from Sigma (St. Louis, MO). Forskolin (dissolved in DMSO) was purchased from Calbiochem (La Jolla, CA). Sodium tolbutamide was the gift of Upjohn (Kalamazoo, MI), and nitrendipine (dissolved in DMSO) was the gift of A. Scriabine of the Miles Institute for Preclinical Pharmacology (Elkhart, IN). The amount of DMSO used in these studies (never >0.1%) was without any adverse impact on islet function (unpublished observations).

Statistics. Statistical significance was determined using the Student's *t* test for unpaired data or analysis of variance in conjunction with the Newman-Keuls multiple comparison test. $P < .05$ was taken as significant. Values presented in the figures and RESULTS represent means ± SE of the specified number of observations.

RESULTS

Exposure of freshly isolated perifused islets to 200 nM CCK-8S in the presence of 5.5 mM glucose resulted in a small delayed insulin secretory response (Fig. 1). This response was dramatically amplified, however, when forskolin (1 μM) was included in the perifusion medium. Although this level (1 μM) of forskolin did not significantly increase basal insulin secretory rates with 5.5 mM glucose alone, its inclusion amplified both the initial and sustained insulin responses to CCK-8S. Peak first-phase insulin release averaged 44 ± 5 and 510 ± 48 pg · islet^{-1} · min^{-1} (n = 5; mean ± SE) in the control and forskolin-treated islets, respectively. Although release measured after 40 min of stimulation in the presence of forskolin (165 ± 14 pg · islet^{-1} · min^{-1}) was greater than control release rates (122 ± 19), these values were not significantly different. At 200 nM, the calcium-channel blocker nitrendipine (13) abolished the weak insulin secretory response to 200 nM CCK-8S. When PI hydrolysis

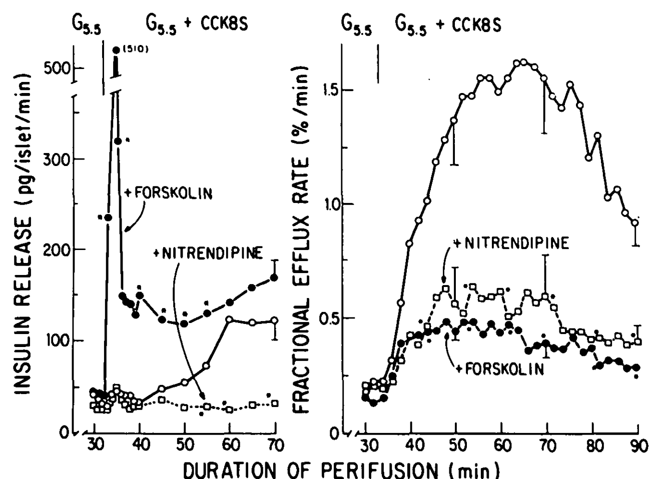

FIG. 1. Impact of cholecystokinin (CCK-8S) on insulin output from freshly isolated islets and ^{3}H efflux from [^{3}H]inositol-labeled islets. Figure has been corrected for dead space in perifusion system (2.5 ml, ~2.5 min with 1-ml/min flow rate). Freshly isolated islets were used to monitor insulin secretion, and islets preincubated with [2-^{3}H]inositol were used for ^{3}H efflux studies. Insulin output from these incubated islets is reduced from that observed in freshly isolated islets (see responses to CCK-8S + 5.5 mM glucose; Fig. 4). However, qualitative relationship between these islet responses in presence of forskolin or nitrendipine was same as that noted from freshly isolated islets. *n* > 4 for each condition. ○, Controls; ●, forskolin (1 μM) treated; □, nitrendipine (200 nM) treated. *Significant differences vs. controls. *Left*: groups of 12–15 freshly isolated islets were perifused for 30 min with 5.5 mM glucose to establish basal secretory rates and for additional 40 min in medium supplemented with 200 nM CCK-8S. In indicated groups, forskolin was present during entire 70-min perifusion, or nitrendipine was added 10 min before addition of CCK-8S for duration of perifusion. *Right*: after 2-h labeling period with *myo*-[2-^{3}H]inositol, groups of islets were perifused under conditions described above. In these experiments the islets were stimulated with CCK-8S for 60 min. Fractional efflux rates were calculated as described previously (38).

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TABLE 1
Effects of forskolin and nitrendipine on inositol phosphate accumulation in cholecystokinin (CCK-8S)-, tolbutamide-, and glucose-stimulated islets

Group	Glucose (mM)	Treatment	Inositol phosphate accumulation (cpm/40 islets)		
			IP_1	IP_2	IP_3
1	5.5		3094 ± 635	329 ± 58	309 ± 65
2	5.5	Forskolin	2610 ± 263	264 ± 17	253 ± 36
3	5.5	Nitrendipine	2458 ± 303	320 ± 108	344 ± 79
4	5.5	CCK-8S	20,471 ± 1900	2036 ± 185	961 ± 137
5	5.5	CCK-8S + forskolin	14,853 ± 1649	1691 ± 208	697 ± 144
6	5.5	CCK-8S + nitrendipine	12,163 ± 2851	1349 ± 208	662 ± 70
7	5.5	Tolbutamide	6873 ± 912	962 ± 73	702 ± 60
8	5.5	Tolbutamide + forskolin	6670 ± 292	808 ± 29	500 ± 19
9	5.5	Tolbutamide + nitrendipine	3260 ± 183	450 ± 12	287 ± 48
10	2.75		1458 ± 183	167 ± 32	178 ± 34
11	2.75	Forskolin	1720 ± 213	194 ± 18	203 ± 31
12	2.75	Nitrendipine	1688 ± 193	225 ± 34	218 ± 40
13	20		14,807 ± 2037	1418 ± 181	734 ± 82
14	20	Forskolin	8717 ± 902	882 ± 130	463 ± 36
15	20	Nitrendipine	8068 ± 1012	1061 ± 88	549 ± 51

Batches of islets were incubated for 2 h in *myo*-[2-^{3}H]inositol, washed with 5 ml of fresh medium, and incubated in 200 μl of fresh medium containing 2.75 or 5.5 mM glucose and forskolin (1 μM) or nitrendipine (200 nM) for 10-min stabilization period followed by 30-min stimulation period with CCK-8S (200 nM), tolbutamide (200 μM), or glucose (20 mM). In forskolin- and nitrendipine-treated islets, compounds were present for initial 10-min period and subsequent 30-min stimulation. Values are means ± SE of ≥3 experiments. For statistical analysis of the data, see MATERIALS AND METHODS.

was monitored in islets prelabeled for 2 h in *myo*-[2-^{3}H]inositol, a markedly different picture emerged. Under experimental conditions where insulin output from freshly isolated islets was amplified by forskolin, this compound significantly reduced CCK-8S–induced [^{3}H]inositol efflux at virtually all time points after agonist addition. Nitrendipine also diminished the impact of CCK-8S on ^{3}H efflux, an effect not as dramatic, however, as its complete inhibition of insulin output. Measurements of labeled inositol phosphate accumulation corroborated these efflux results (Table 1). Both forskolin and nitrendipine reduced the stimulatory effect of CCK-8S.

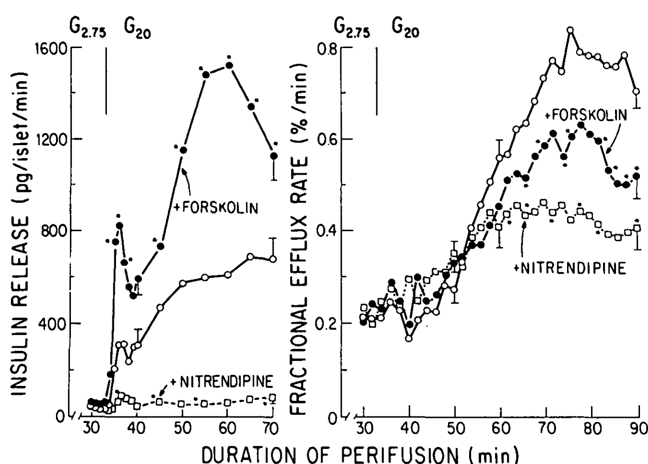

FIG. 2. Impact of 20 mM glucose on insulin output and ^{3}H efflux from [^{3}H]inositol-labeled islets treated with forskolin (●) or nitrendipine (□). ○, Controls. $n \geq 4$ for each condition. Figure has been corrected for dead space in perifusion system (2.5 ml, ~2.5 min with flow rate of 1 ml/min). *Left*: groups of 10–15 freshly isolated islets were perifused for 30 min with 2.75 mM glucose and for additional 40 min with 20 mM glucose. Forskolin was present during entire 70-min perifusion. Nitrendipine (200 nM) was added to medium 10 min before 20 mM glucose addition and maintained at this level for the duration of perifusion. *Right*: after 2-h labeling period, fractional efflux rates of ^{3}H were monitored in response to 20 mM glucose alone, 20 mM glucose plus forskolin (present during entire perifusion) and 20 mM glucose plus 200 nM nitrendipine (present during final 10 min with 2.75 mM glucose and entire stimulatory phase with 20 mM glucose).

Similar conditions were employed to assess the impact of forskolin on insulin output and [^{3}H]inositol efflux in response to 20 mM glucose (Fig. 2). Similar to the results obtained with CCK-8S stimulation, forskolin (1 μM) treatment of freshly isolated and subsequently perifused islets amplified both the first- and second-phase insulin secretory responses to glucose. Forskolin significantly reduced 20 mM glucose–induced ^{3}H efflux (Fig. 2) and its ability to increase inositol phosphate levels (Table 1). Nitrendipine virtually abolished the effect of 20 mM glucose on insulin output. It had a similar, although less decisive, inhibitory effect on inositol phosphate accumulation (Table 1).

In the presence of 5.5 mM glucose, the sulfonylurea tolbutamide (200 μM) evoked a brisk secretory response with peak first-phase release rates averaging 180 ± 24 pg · islet^{-1} · min^{-1} (n = 5; Fig. 3). Release rates after 35–40 min of stimulation averaged 159 ± 22 pg · islet^{-1} · min^{-1}. The presence of forskolin significantly amplified both phases of secretion. Peak first-phase release rates then averaged 1529 ± 55 pg · islet^{-1} · min^{-1}. Release rates after 35–40 min of stimulation averaged 270 ± 23 pg · islet^{-1} · min^{-1}. Nitrendipine abolished the impact of tolbutamide on insulin output. Tolbutamide at this level (200 μM) slightly, but significantly, increased [^{3}H]inositol efflux and inositol phosphate levels, and the addition of forskolin or nitrendipine reduced this stimulatory action (Fig. 3; Table 1).

Two additional groups of experiments were conducted. First, islet levels of cAMP in response to 1 μM forskolin were measured. In islets perifused for 60 min with 2.75 mM glucose alone, levels of the nucleotide averaged 10.3 ± 1.4

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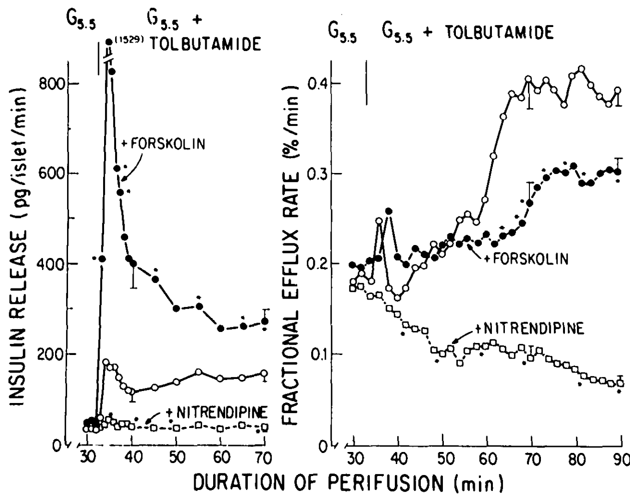

FIG. 3. Impact of tolbutamide on insulin output and ^{3}H efflux. Figure has been corrected for dead space in perifusion system (2.5 ml, ~2.5 min with flow rate of 1 ml/min). *Left*: groups of 12–15 freshly isolated islets were perifused with 5.5 mM glucose for 30 min before tolbutamide (200 μM) addition. Forskolin (●) was present in some experiments during entire 70-min perifusion period. In other experiments, calcium-channel blocker nitrendipine (200 nM, □) was present for final 50 min of perifusion. *Right*: after labeling, phosphoinositide hydrolysis was measured in response to tolbutamide alone or with further addition of forskolin or nitrendipine as detailed above.

fmol/islet (mean ± SE, n = 5). The presence of 1 μM forskolin increased this value to 33.8 ± 6.9 fmol/islet (n = 5), a value significantly different ($P < .05$) from that found in control islets. Second, experiments were conducted with theophylline, a phosphodiesterase inhibitor known to increase islet cAMP levels (14). In these studies, both the insulin secretory profile and ^{3}H efflux were monitored in the same islets. Similar to forskolin, theophylline (2 mM) increased the secretory response to 200 nM CCK-8S, and again similar to forskolin, reduced ^{3}H efflux in response to CCK-8S (Fig. 4). After the perifusion, islet levels of IP_1, IP_2, and IP_3 in these same islets were measured after extraction with 10% perchloric acid. Theophylline significantly reduced islet levels of IP_1 and IP_3 in the presence of 5.5 mM glucose alone (Table 2). Whereas CCK-8S addition increased inositol phosphate levels, theophylline also attenuated this response (Table 2).

DISCUSSION

Several interrelated issues were addressed in this study. First we examined whether forskolin, a compound that elevates islet cAMP levels and amplifies insulin output (15), has any effect on PI hydrolysis induced by either CCK-8S, glucose, or tolbutamide. PI hydrolysis was assessed by several different methodologies that yielded essentially the same qualitative and quantitative results. We took advantage of the fact that during an appropriate prelabeling period, [^{3}H]inositol is exclusively incorporated into islet PIs (1,16). We have previously established that the pool of free [^{3}H]inositol in these prelabeled unstimulated islets is small (~1400 cpm/40 islets), and this pool, like the labeled inositol phosphates, increases dramatically in response to high glucose stimulation (17). Furthermore, a 60-min perifusion of [^{3}H]inositol-prelabeled islets with 20 mM glucose re sults in the efflux of ~3500 cpm of ^{3}H/40 islets (W.S.Z., unpublished observations). Because 90% of this radioactivity is [^{3}H]inositol (17), and because the only significant source, of effluent [^{3}H]inositol is [^{3}H]inositol phosphates derived from ^{3}H-PI, the conclusion is inescapable that changes in [^{3}H]inositol efflux reflect to a large extent changes in PI hydrolysis. Of course, it cannot be excluded in this study that cAMP or calcium affects the intraconversion of the various inositol phosphates generated. The complexity of these intraconversions promises to be a most difficult issue to address (18). We also investigated the role of extracellular calcium in the responses to these various agonists. We reasoned in the latter studies that if intracellular calcium mobilization plays a major role in the insulin secretory and PI responses to CCK-8S, glucose, or tolbutamide, then reducing calcium influx with nitrendipine (13) might have only a minimal impact on these parameters. An interesting relationship between cAMP and PI hydrolysis was observed in these studies, and futher comment on them is appropriate.

The gut hormone CCK, a postulated incretin and satiety factor (19), evokes a glucose- and calcium-dependent insulin secretory response (5–7). The impact of CCK-8S on insulin output is initiated by the interaction of CCK-8S with its membrane receptor, some of whose characteristics have been established (6). Linked to receptor occupancy is the activation of phospholipase C. This enzyme catalyzes the conversion of membrane PIs into a variety of second-messenger molecules including a family of inositol phosphates and diacylglycerol (20). Of the phosphates formed, inositol-

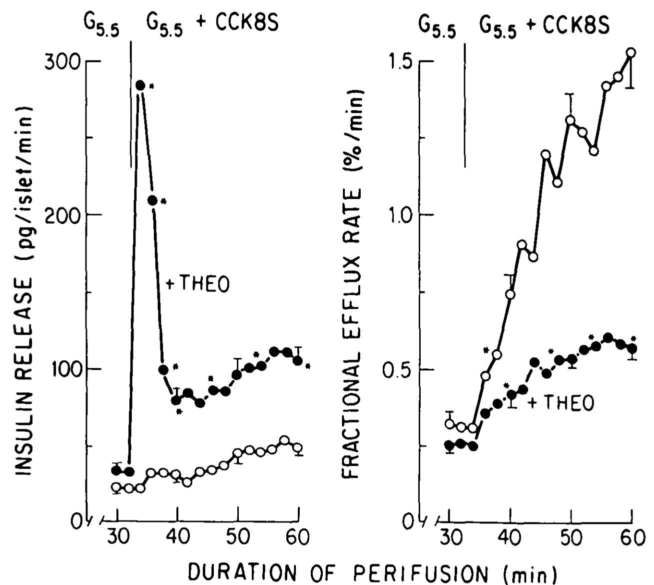

FIG. 4. Impact of theophylline on insulin output and ^{3}H efflux in response to CCK-8S. Groups of islets were incubated for 2 h to label their inositol-containing lipids and then perifused. Insulin release (*left*) in response to cholecystokinin (CCK-8S; 200 nM) addition and ^{3}H efflux (*right*) was monitored in presence (for entire perifusion, ●) and absence (○) of theophylline (2 mM). Same islets were then analyzed for inositol phosphate accumulation after extraction with 10% perchloric acid (Table 2). Insulin output, ^{3}H efflux, and inositol phosphate accumulation were monitored in same islets. $n \geq 4$ with each protocol.

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TABLE 2
Influence of theophylline on inositol phosphate accumulation in isolated perifused islets

Group	Glucose	Addition	Inositol phosphate accumulation (cpm/40 islets)		
			IP_1	IP_2	IP_3
1	5.5		517 ± 22	116 ± 13	95 ± 6
2	5.5	Theophylline	429 ± 30	98 ± 10	69 ± 5
3	5.5	CCK-8S	1429 ± 53	626 ± 30	281 ± 19
4	5.5	Theophylline + CCK-8S	902 ± 48	382 ± 42	141 ± 14

Groups of 40–50 islets were incubated for 2 h in *myo*-[2-^{3}H]inositol to label their phosphoinositides. After washing to remove unincorporated label, they were perifused for 60 min. Group 1 islets were perifused for entire 60 min with 5.5 mM glucose alone, whereas group 2 islets were perifused for the entire 60 min with 5.5 mM glucose plus 2 mM theophylline. Group 3 islets, after a 30-min perifusion with 5.5 mM glucose, were exposed to cholecystokinin (CCK-8S, 200 nM) plus 5.5 mM glucose for 30 min. Group 4 islets were treated similarly to group 3 except that 2 mM theophylline was present for the entire 60-min perifusion. $n = \geq 4$ for each protocol. Statistical analysis: group 1 vs. group 2, $P < .05$ for IP_1 and IP_3; group 3 vs. group 4, $P < .05$ for IP_1, IP_2, and IP_3.

1,4,5-trisphosphate (IP_3) has been implicated in the mobilization of intracellular calcium (21). In the presence of 5.5 mM glucose, CCK-8S addition to prelabeled islets is accompanied by a dramatic increase in ^{3}H efflux and inositol phosphate accumulation but only a small rise in insulin output from freshly isolated islets (Fig. 1; Table 1). If forskolin is included in the medium, an amplified insulin secretory response results, an event accompanied by a reduction in ^{3}H efflux and inositol phosphate accumulation. From a quantitative standpoint, the direct contribution of PI-derived second-messenger molecules to an insulin secretory response appears minimal unless accompanied by other metabolic alterations, a conclusion reached by others as well (8,22). A modest increase (from 5 to 7 mM) in the glucose level bathing the islet produces the necessary metabolic alterations to convert weak or impotent PI-derived second-messenger molecules into effective secretory signals. Part of this so-called permissive effect of glucose may reside in its ability to elevate islet cAMP levels (23). Our studies with forskolin support this contention. It is also equally clear that an increase in islet cAMP levels actually reduces the quantitative impact of CCK-8S on PI hydrolysis, at least as assessed by the present methodologies. That cAMP negatively affects PI turnover has been documented in other tissues as well, although the basis for this action has yet to be elucidated (24,25). Our suggestion that cAMP is responsible for this reduction in CCK-8S–induced ^{3}H efflux and inositol phosphate levels is further supported by the data presented in Fig. 4 and Table 2. In these experiments, theophylline was used to increase cAMP levels. Results virtually identical to these noted with forskolin were obtained. Our previous report with the gut hormone gastric inhibitory polypeptide (GIP) is also consistent with these findings (26). GIP increases islet cAMP levels, reduces the stimulatory effect of CCK-8S on PI hydrolysis, but amplifies insulin secretion. The physiological significance of this cAMP-induced reduction in PI metabolism is not clear, but this area warrants further study.

We previously reported that the calcium-influx inhibitor nitrendipine slightly reduced the impact of CCK-8S (in the presence of 7 mM glucose) on PI hydrolysis but completely abolished its insulin stimulatory efficacy (8). Our study reinforces this notion and also leaves little doubt that reducing calcium influx negatively affects the quantitative impact of CCK-8S on ^{3}H efflux and inositol phosphate accumulation (Fig. 1; Table 1). It is difficult to precisely define the actual sites of calcium action in these studies. It is clear from this type of analysis, however, that extracellular calcium is playing an important role in both the phospholipase C–mediated hydrolysis of PI and ensuing insulin secretory response. This conclusion is supported by the finding that nitrendipine partially but significantly reduces CCK-8S–induced ^{3}H efflux and inositol phosphate accumulation but completely abolishes the impact of these changes on insulin output. Incidentally, [^{3}H]inositol efflux and inositol phosphate levels in these islets (CCK-8S plus nitrendipine) are comparable to those observed with CCK-8S plus forskolin, yet the insulin secretory performance of the islets is dramatically different.

There can be little doubt that glucose increases the hydrolysis of inositol-containing phospholipids (1–4,8,16,17, 27,28). This is also supported by this study, which uses both the efflux of ^{3}H from [^{3}H]inositol-prelabeled islets and labeled inositol phosphate production as indices of PI hydrolysis in response to 20 mM glucose. We also demonstrated that both forskolin and nitrendipine reduce the impact of glucose on these parameters.

The sulfonylureas are widely employed in the therapy of non-insulin-dependent diabetes (29). Their impact on insulin release is regulated to a large extent by the ambient glucose level, and most studies support the concept that their secretory action is mediated by their ability to directly or indirectly increase calcium influx (30–32). In islets treated with forskolin, the signal generated by the sulfonylurea tolbutamide is dramatically amplified (Fig. 3). Not surprisingly, considering its postulated mechanism of action, the calcium-channel blocker nitrendipine completely blocked the impact of the sulfonylurea on insulin output. We also monitored the effects of tolbutamide on PI hydrolysis, an issue addressed previously with different methodologies. Laychock (3) reported that 1 mM tolbutamide increased PI metabolism, whereas a small but nonsignificant effect with the sulfonylurea glyburide was reported by Best and Malaisse (33). We previously reported that 25 μM tolbutamide (in the presence of 5.5 mM glucose) was without any discernible effect on ^{3}H efflux from [2-^{3}H]inositol-prelabeled islets (34). In more comprehensive studies, we further established that the impact of tolbutamide on PI hydrolysis is tightly regulated not only by the level of the sulfonylurea but also by the glucose concentration bathing the islet (31). The latter finding would seem to explain some of the discrepant results in the literature (33,35), because these studies were performed at different glucose levels. In this study, we observed a small but significant effect of 200 μM tolbutamide (with 5.5 mM glu-

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cose) on ^3H efflux and labeled inositol phosphate levels, effects that were reduced by forskolin and abolished by nitrendipine. If the primary action of tolbutamide is the elevation of intracellular calcium by its ionophoretic action, these findings would then imply that an increase in intracellular calcium alone may suffice to initiate PI hydrolysis via phospholipase C activation. A similar conclusion has been reached by others as well (3,36). Note that, whereas ^3H efflux and inositol phosphate production are both reduced in the presence of forskolin, this effect is much more pronounced for ^3H efflux. However, the duration of the efflux studies was considerably greater than the batch incubation studies, and the inhibitory effect of forskolin on ^3H efflux was most evident during final 30 min of the perifusion. A similar type of inhibitory action of cAMP on PI metabolism has been reported with adrenal glomerulosa cells (25).

Note that both CCK-8S and tolbutamide induce a significant insulin secretory response only in the presence of a moderate glucose level. The precise nature of the signal contributed by glucose in the insulin secretory response to these agonists and, in the case of tolbutamide at least, the nature of the glucose-derived signal that permits the sulfonylurea to influence PI metabolism is not known. The possibility that forskolin or nitrendipine influence this potentiating effect of glucose on the responses noted here must be considered also.

When taken in their entirety, these results demonstrate a remarkable relationship between cAMP levels in the islet and efficacy of PI-derived second-messenger molecules to stimulate secretion. That this interaction functions in vivo is a reasonable speculation (26,37). Future studies will have to investigate the possible involvement of PI hydrolysis in situations characterized by altered patterns of insulin secretion.

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