The conditions under which rat islets are labelled with [³H]inositol alter the subsequent responses of these islets to a high glucose concentration

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Isolated rat islets were incubated with *myo*-[2-³H]inositol for 2 h to label their phosphoinositide (PI) pools. Labelling was carried out under three separate conditions: in media containing low (2.75 mM) glucose, high (13.75 mM) glucose, or low (2.75 mM) glucose plus sulphated cholecystokinin (CCK-8S; 200 nM). After labelling, the islets were perfused and the insulin-secretory response to 20 mM-glucose was measured. PI hydrolysis in these same islets was assessed by measurements of both [³H]inositol efflux and the accumulation of labelled inositol phosphates. The following major observations were made. After prelabelling for 2 h in low glucose, perfusion with 20 mM-glucose resulted in a biphasic insulin-secretory response, an increase in [³H]inositol efflux and a parallel increase in the accumulation of labelled inositol phosphates. After prelabelling in high (13.75 mM) glucose, peak first-phase insulin secretion induced by 20 mM-glucose increased 2–2.5-fold, whereas the second phase of insulin release, as well as [³H]inositol efflux and inositol phosphate accumulation, were significantly decreased. The simultaneous infusion of the diacylglycerol kinase inhibitor 1-mono-oleoylglycerol (50 μM), along with 20 mM-glucose, restored the second-phase insulin-secretory response from these islets. After labelling in low (2.75 mM) glucose plus CCK-8S, the initial phases of the insulin-secretory and [³H]inositol-efflux responses to 20 mM-glucose were blunted and the sustained phases of both responses were markedly decreased. Inositol phosphate accumulation was also impaired. Labelling islets in high (13.75 mM) glucose or low (2.75 mM) glucose plus CCK-8S suppresses, in a parallel fashion, glucose-induced increases in PI hydrolysis and in second-phase insulin release. These findings suggest that desensitization of the insulin-secretory response is a consequence of impaired information flow in the inositol lipid cycle.

INTRODUCTION

Glucose stimulates the hydrolysis of inositol phospholipids in isolated rat islets of Langerhans [1–6]. On the basis of their studies, Rana *et al.* [7,8] concluded that, in order to demonstrate this effect, it is necessary to prelabel islets with [³H]inositol in the presence of a high ambient glucose concentration (16.7 mM). They found that, even though [³H]inositol is incorporated into islets incubated in low ambient glucose (3.3 mM), subsequent challenge with 16.7 mM-glucose does not lead to a significant loss of radioactivity from the total phosphoinositide (PI) pool. From these results, they proposed that pancreatic islets contain two pools of PIs, one glucose-responsive and the other glucose-unresponsive. However, insulin release was not examined in these studies. Hence, no correlation was made between PI hydrolysis and insulin secretion.

The results of Rana *et al.* [7,8] stand in contrast with a large number of studies [1–4,9], including ones from our laboratory [5,10–12], in which islets incubated in low ambient glucose and labelled with [³H]inositol display significant increases in PI hydrolysis when subsequently stimulated with high glucose. We have recently demonstrated that the measurement of [³H]inositol efflux from perfused prelabelled islets is a valid and convenient way (when combined with measurements of tissue inositol

phosphate contents at particular times) to assess the rates of PI hydrolysis and to correlate these changes with insulin secretion [10]. Using this approach, we decided to re-explore the relationship between the labelling conditions under which [³H]inositol is incorporated into tissue PIs and the subsequent behaviour of these islets, in terms of changes in both PI hydrolysis and insulin secretion, when stimulated with 20 mM-glucose. Because of our interest in the relationship between PI hydrolysis and insulin secretion, three specific labelling conditions were chosen: 2.75 mM-glucose, 13.75 mM-glucose, and 2.75 mM-glucose plus 200 nM-cholecystokinin 8-sulphate (CCK-8S). The changes in inositol phosphate accumulation, [³H]inositol efflux and insulin secretion induced by 20 mM-glucose are different in islets prelabelled under these different conditions.

EXPERIMENTAL

Male Sprague–Dawley rats purchased from Charles River were used in all studies. The animals were fed *ad libitum* and weighed 275–350 g. After Nembutal-induced (50 mg/kg) anaesthesia, islets were isolated by collagenase digestion [13]. Groups of 30–50 islets were loaded on nylon filters and placed in small glass vials. They were incubated for 90–120 min in 200 μl of a *myo*-

Abbreviations used: CCK-8S, cholecystokinin-8 sulphate; PI, phosphoinositide; MOG, mono-oleoylglycerol.

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[2-³H]inositol-containing solution prepared by adding 10 μ Ci of *myo*-[2-³H]inositol (initial sp. radioactivity 19 Ci/mmol) to 250 μ l of incubation medium. Three separate conditions were used during this labelling period. Islets were labelled in (a) low (2.75 mM) glucose, (b) high (13.75 mM) glucose, or (c) low (2.75 mM) glucose plus CCK-8S (200 nM). The medium used for this incubation procedure was similar to that employed during the islet perfusion and consisted of 115 mM-NaCl, 5 mM-KCl, 2.2 mM-CaCl₂, 1 mM-MgCl₂, 24 mM-NaHCO₃ and 0.17 g of bovine serum albumin/dl. The solution was gassed with O₂/CO₂ (19:1).

After termination of the incubation, the islets, still attached to the nylon filters, were washed with 5 ml of non-radioactive medium 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 perfused for 30 min to establish stable insulin-secretory rates and then exposed to various agonists indicated in the Figure legends. Perfusate samples were collected at time intervals indicated in the Figures, and 200 μ l portions were analysed for [³H]inositol content. Our previous report using the exact same methodologies [10] has demonstrated that approx. 90% of the effluent radioactivity from stimulated islets is free [³H]inositol. Insulin was measured by using rat insulin (Eli Lilly Co., Indianapolis, IN, U.S.A.; batch no. 615-D63-12-3) as standard. After retrieval from the perfusion apparatus, amounts of labelled inositol phosphate in islets still attached to the nylon filters were measured after extraction with 10% (w/v) HClO₄ [10,14]. The radiolabel used to measure insulin (¹²⁵I-insulin) was purchased from New England Nuclear, Boston, MA, U.S.A., and *myo*-[2-³H]inositol was from Amersham, Arlington Heights, IL, U.S.A. Formic acid, borax, 1-mono-oleoyl-*rac*-glycerol (MOG), bovine serum albumin, cholecystokinin (CCK-8S, the C-terminal 8-amino-acid derivative, fragment 26–33-amide, sulphated on the tyrosine residue; lot no

123F-04451), as well as the salts used to make the perfusion medium, were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Ammonium formate was purchased from Fisher Scientific, Fair Lawn, NJ, U.S.A.

Statistics

Statistical differences were analysed by ANOVA in conjunction with the Newman–Keuls test for multiple comparisons or by Student's *t* test. A *P* value less than 0.05 was taken as significant. Values presented in the Figures represent means $\pm$ S.E.M. of at least four experiments.

RESULTS

Effect of glucose concentration on total [³H]inositol incorporation

As shown in Table 1, the conditions under which the labelling of islets with [³H]inositol is performed have an effect on the total incorporation of label. It is noteworthy that islets labelled in 2.75 mM-glucose for 90 min incorporate the same amount of [³H]inositol as do islets labelled for 120 min in the presence of either 13.75 mM-glucose or 2.75 mM-glucose plus 200 nM-CCK-8S (compare line 4 with lines 2 and 3 in Table 1).

Effects of 20 mM-glucose on insulin secretion and [³H]inositol efflux from islets prelabelled in 2.75 mM-glucose

Islets prelabelled in 2.75 mM-glucose for 120 min and perfused with 2.75 mM-glucose have very low and stable rates of both insulin release (Fig. 1a) and [³H]inositol efflux rate (Fig. 1b). Challenge with 20 mM-glucose, however, leads to a marked stimulation of insulin secretion and [³H]inositol efflux (Fig. 1). In response to 20 mM-glucose, a brisk biphasic insulin-secretory response occurs (Fig. 1a). Under this stimulatory condition, a significant increase in [³H]inositol efflux rate is also observed from these islets (Fig. 1b).

Effects of 20 mM-glucose on insulin secretion and [³H]inositol efflux from islets prelabelled in 13.75 mM-glucose

In islets prelabelled for 120 min in 13.75 mM-glucose, a subsequent challenge with 20 mM-glucose leads to an enhanced first phase of insulin secretion (compared with that seen in islets prelabelled in 2.75 mM-glucose), but a considerably suppressed second phase of secretion (Fig. 2a). The rate of [³H]inositol efflux from unstimulated islets was slightly lower than that seen in islets prelabelled in the presence of 2.75 mM-glucose. In these islets, a subsequent challenge with 20 mM-glucose has only a small and transient effect on the efflux of [³H]inositol (Fig. 2b).

It might be argued that the differences in total incorporation of [³H]inositol between islets labelled in 2.75 mM versus 13.75 mM-glucose account for these dramatic differences in [³H]inositol-efflux rates. Similar studies were carried out with islets incubated in 2.75 mM-glucose for only 90 min. Under this condition, total incorporation of [³H]inositol into islets prelabelled in 2.75 mM-glucose for 90 min is similar to that found in islets prelabelled in 13.75 mM-glucose for 120 min (Table 1, cf. lines 4 and 2). In islets prelabelled in 2.75 mM-glucose for 90 min, a subsequent challenge with 20 mM-glucose induced

Table 1. Influence of labelling conditions on total [³H]inositol after short-term stimulation incorporation in isolated islets

Groups of islets were incubated for 90–120 min with *myo*-[2-³H]inositol in the presence of 2.75 mM-glucose (G_{2.75}), 13.75 mM-glucose (G_{13.75}) or 2.75 mM-glucose plus 200 nM-CCK-8S. After this they were perfused for 28 min to wash out unincorporated label, and then perfused for an additional 32 min. Total incorporation refers to the amount of effluent radioactivity collected from 28 to 60 min of perfusion, total radioactivity recovered in HClO₄ extract as InsP₁, InsP₂ and InsP₃, and the amount of radioactivity remaining in the islets after the perfusion and HClO₄ extraction. At least eight experiments were performed under each condition; results are given as means $\pm$ S.E.M. Statistical analysis: line 1 versus line 2, line 1 versus line 3, *P* < 0.05; line 2 versus line 3, line 4 versus line 2, line 4 versus line 3, not significant.

Labelling condition		Total ³ H incorporation (c.p.m./40 islets)
1. G _{2.75} , 120 min	(<i>n</i> = 12)	16242 $\pm$ 706
2. G _{13.75} , 120 min	(<i>n</i> = 13)	10693 $\pm$ 441
3. G _{2.75} + CCK-8S, 120 min	(<i>n</i> = 10)	11739 $\pm$ 478
4. G _{2.75} , 90 min	(<i>n</i> = 8)	11572 $\pm$ 1005

Fig. 1. Insulin secretion and [³H]inositol efflux from islets labelled in 2.75 mM-glucose

Groups of 30–50 islets were incubated for 90–120 min in *myo*-[2-³H]inositol to label their PIs. Glucose (2.75 mM) was also present. They were then perfused for 30 min with 2.75 mM-glucose (G_{2.75}) to establish basal stable insulin and [³H]inositol-efflux rates. One group of islets (labelled for 120 min) was then maintained with 2.75 mM-glucose for an additional 30 min (●), and the other groups were stimulated for 30 min with 20 mM-glucose (G₂₀) (O, 90 min labelling; Δ, 120 min labelling). Insulin secreted into the perfusate (a) and [³H]inositol released by the islets (b) were measured. The fractional efflux rate of [³H]inositol was calculated as described previously [10,11]. Mean values ± selected S.E.M. values are indicated for six similar experiments. This and all subsequent Figures have been corrected for the dead space in the perfusion apparatus, approx. 2.5 ml or 2.5 min with a flow rate of 1 ml/min.

changes in rates of insulin release and [³H]inositol efflux nearly identical with those seen in islets prelabelled for 120 min in 2.75 mM-glucose (see Fig. 1).

Effects of 20 mM-glucose on insulin secretion and [³H]inositol efflux from islets prelabelled in 2.75 mM-glucose plus 200 nM-CCK-8S

In islets prelabelled for 120 min in 2.75 mM-glucose plus 200 nM-CCK-8S, a subsequent challenge with 20 mM-glucose causes practically no first phase of insulin secretion and induces a slow, progressive, but submaximal increase in the rate of second-phase insulin secretion (Fig. 3a). In the same islets, the challenge with 20 mM-glucose resulted in no immediate change in [³H]inositol efflux, but as the perfusion progressed a submaximal rise in [³H]inositol efflux was observed (Fig. 3b).

Effects of mono-oleoylglycerol on patterns of insulin secretion in islets prelabelled in 13.75 mM-glucose

One possible explanation for the suppression of insulin secretion found in islets preincubated for 120 min in 13.75 mM-glucose (Fig. 2a) is a depletion of insulin stores during the period of exposure to 13.75 mM-glucose, a concentration of glucose known to induce high rates of insulin secretion. To examine this possibility, we attempted to restore the impaired second phase of insulin secretion by using an agent, MOG, which is a known inhibitor of the enzyme diacylglycerol kinase [15] and has

been shown to cause an elevation of the diacylglycerol content of several tissues [15,16].

The addition of 50 μM-MOG to a medium containing 2.75 mM-glucose was without any significant effect on insulin-secretory rates from islets prelabelled in 13.75 mM-glucose (Fig. 4). However, if similar islets were challenged with 20 mM-glucose plus 50 μM-MOG, second-phase insulin secretion was considerably greater than that seen after a challenge with 20 mM-glucose alone (Fig. 4). Even though addition of MOG restored the insulin-secretory response induced by 20 mM-glucose, it did not change the pattern of [³H]inositol efflux seen when islets preincubated in 13.75 mM-glucose are challenged with 20 mM-glucose (results not shown). Finally, it should be noted that MOG significantly increased second-phase insulin secretion from islets prelabelled in 2.75 mM-glucose alone. In the absence of MOG, insulin content of perfusate samples collected 28–30 min after the onset of stimulation with 20 mM-glucose averaged 727 ± 86 pg/min per islet. The further addition of 50 μM-MOG increased this value at this time to 977 ± 75 pg/min per islet (mean ± S.E.M.).

Glucose-induced changes in the production of inositol phosphates by islets prelabelled under different conditions

In addition to measurements of [³H]inositol efflux, measurements of the labelled inositol phosphate pools were carried out after a 6 or 30 min perfusion with

Fig. 2. Insulin secretion and [3 H]-inositol efflux from islets labelled in 13.75 mM-glucose

Groups of islets were labelled for 120 min. The glucose concentration used during the prelabelling period with *myo*-[2- 3 H]inositol was 13.75 mM ($G_{13.75}$). Islets were subsequently perfused, and insulin-secretory rates (a) and fractional rates of [3 H]inositol efflux (b) were monitored in islets maintained with 2.75 mM-glucose ($G_{2.75}$) (●) or stimulated with 20 mM-glucose (G_{20}) (○). For comparative purposes, the insulin-secretory and [3 H]inositol efflux responses from islets labelled in the presence of 2.75 mM-glucose and subsequently stimulated during the perfusion with 20 mM-glucose (△) are also depicted.

Fig. 3. Insulin secretion and [3 H]inositol efflux from islets labelled in 2.75 mM-glucose plus CCK-8S

Islets were treated as indicated in Fig. 1 except that, in addition to 2.75 mM-glucose ($G_{2.75}$), 200 nM-CCK-8S was also present during the 120 min labelling period with *myo*-[2- 3 H]inositol. After this, islets were perfused, and both insulin output (a) and the fractional rates of 3 H efflux (b) were monitored in response to 2.75 mM-glucose (●) or 20 mM-glucose (G_{20}) (○). For comparative purposes, the insulin-secretory and [3 H]inositol-efflux responses from islets labelled in the presence of 2.75 mM-glucose alone and subsequently stimulated during the perfusion with 20 mM-glucose (△) are also depicted.

Fig. 4. Effect of mono-oleoylglycerol (MOG) on the insulin-secretory responses of islets prelabelled in 13.75 mM-glucose and challenged with 20 mM-glucose

Three groups of islets were labelled in the presence of 13.75 mM-glucose for 2 h. After a subsequent 30 min perfusion with 2.75 mM-glucose ($G_{2.75}$), one group was stimulated with 20 mM-glucose (G_{20}) (○), a second with 20 mM-glucose plus 50 μ M-MOG (●), and the third with 50 μ M-MOG in the presence of 2.75 mM-glucose (□).

20 mM-glucose in islets prelabelled in 2.75 mM-glucose, 13.75 mM-glucose, or 2.75 mM-glucose plus 200 nM-CCK-8S. The results found after a 6 min exposure to 20 mM-glucose are shown in Table 2. Islets prelabelled in 2.75 mM-glucose display early increases in $InsP_1$, $InsP_2$ and $InsP_3$ in response to 20 mM-glucose (cf. line 2 with line 1). In islets prelabelled in 13.75 mM-glucose and then perfused for 36 min with 2.75 mM-glucose, the contents of labelled $InsP_1$, $InsP_2$ and $InsP_3$ are higher than those found in islets prelabelled in 2.75 mM-glucose (cf. line 3 with line 1, Table 2). In these islets (prelabelled in 13.75 mM-glucose), subsequent challenge with 20 mM-glucose leads to significant early increases in the size of the $InsP_1$, $InsP_2$ and $InsP_3$ pools (cf. line 4 with line 3). After prelabelling in 2.75 mM-glucose plus 200 nM-CCK-8S, no immediate increase in the size of any of these inositol phosphate pools was observed in response to stimulation with 20 mM-glucose (cf. line 6 with line 5, Table 2).

As recorded in Table 3, similar experiments were conducted in which the contents of the inositol phosphates were measured after 30 min of perfusion with 20 mM-glucose. Islets prelabelled in 2.75 mM-glucose for 90 or 120 min show an increase in the size of all pools in response to 20 mM-glucose (line 1 versus line 2, line 5 versus line 6, Table 3). In islets prelabelled for 2 h in 13.75 mM-glucose, there is no significant increase in $InsP_1$, $InsP_2$ or $InsP_3$ (line 7 versus line 8) after a 30 min exposure to 20 mM-glucose. However, in islets labelled for only 90 min in 13.75 mM-glucose, a significant increase in $InsP_1$ was observed (line 3 versus line 4). In islets prelabelled in 2.75 mM-glucose plus 200 nM-CCK-8S, exposure to 20 mM-glucose for 30 min leads to a significant increase in the size of all the pools (line 9 versus line 10, Table 3).

DISCUSSION

On the basis of their studies, Rana *et al.* [7,8] suggested that glucose-responsive and -unresponsive pools of PIs exist in islets. Only when islets were labelled with [3 H]inositol plus 16.7 mM-glucose were they able to demonstrate subsequent increases in glucose-induced PI breakdown. Those studies [7,8] stand in sharp contrast with the studies by Axen *et al.* [3], Clements & Rhoten [1], Best *et al.* [4,9] and Zawulich *et al.* [5,10–12]. Because of these conflicting data, we re-investigated the influence of the glucose content in the medium used during the labelling period with [3 H]inositol on glucose-induced PI hydrolysis and insulin secretion. In contrast with the findings of Rana *et al.* [7,8], we found that there was significant [3 H]inositol incorporation into the PI pool in islets incubated in the presence of 2.75 mM-glucose, and that a subsequent challenge with 20 mM-glucose led to the rapid accumulation of cellular inositol phosphates (Tables 2 and 3) and a rapid increase in rate of [3 H]inositol efflux (Fig. 1). Surprisingly, and again in sharp contrast with the earlier report by Rana *et al.* [7], the incorporation of [3 H]inositol into the PI pool was less in islets incubated in high (13.75 mM) glucose as compared with low (2.75 mM) glucose. Of particular interest was our unexpected discovery that the glucose concentration present during the labelling period (with [3 H]inositol) had a profound effect on the subsequent ability of high glucose to stimulate insulin secretion, [3 H]inositol efflux and the accumulation of inositol phosphates. At least under the experimental conditions employed here, these results do not support the concept of unique and exclusive glucose-dependent and glucose-independent pools of inositol-containing phospholipids.

Pretreatment with 13.75 mM-glucose (Fig. 2) alters the responses of these islets to a subsequent challenge with 20 mM-glucose. In addition, labelling in CCK-8S also impaired islet sensitivity (Fig. 3). In both cases, increases in second-phase insulin secretion, sustained increases in [3 H]inositol efflux and the accumulation of inositol phosphates were decreased as compared with the same responses from islets labelled in 2.75 mM-glucose alone. However, the patterns of first-phase insulin secretion and the initial phase of [3 H]inositol efflux seen after preincubation with 13.75 mM-glucose (Fig. 2) differed from those induced by preincubation with CCK-8S (Fig. 3). In islets preincubated with 13.75 mM-glucose, the first phase of insulin secretion was enhanced, but the second phase was markedly suppressed. This change in insulin-

Table 2. Inositol phosphate accumulation in isolated perfused islets after short-term stimulation

Groups of 30–50 islets were incubated for 2 h with *myo*-[2-³H]inositol. Labelling was carried out in the presence of 2.75 mM-glucose ($G_{2.75}$), 13.75 mM-glucose ($G_{13.75}$), or 2.75 mM-glucose plus 200 nM-CCK-8S. After this, the islets were washed and perfused with 2.75 mM-glucose for 30–36 min and with 20 mM-glucose (G_{20}) for 6 min in some cases. The duration of these perfusion periods (min) is indicated in parentheses below each protocol. After the perfusion, labelled inositol phosphates were analysed. It should be emphasized that, although the islets were perfused for 6 min with 20 mM-glucose, it takes 1.5 min for the high-glucose solution to reach the islets. Consequently, islets are actually exposed to 20 mM-glucose for 4.5 min before analysis of inositol phosphates. At least four experiments were performed for each protocol; results are given as means $\pm$ S.E.M. Statistical analysis: line 1 versus line 2, line 3 versus line 4, $P < 0.05$ for all inositol phosphates; line 5 versus line 6, no significant difference.

Labelling condition	Perfusion protocol	Accumulation (c.p.m./40 islets)		
		Ins P_1	Ins P_2	Ins P_3
1. $G_{2.75}$, 120 min	$G_{2.75}$ (36)	357 $\pm$ 21	114 $\pm$ 12	70 $\pm$ 13
2. $G_{2.75}$, 120 min	$G_{2.75} \rightarrow G_{20}$ (30) (6)	559 $\pm$ 36	199 $\pm$ 15	105 $\pm$ 9
3. $G_{13.75}$, 120 min	$G_{2.75}$ (36)	467 $\pm$ 23	125 $\pm$ 14	106 $\pm$ 10
4. $G_{13.75}$, 120 min	$G_{2.75} \rightarrow G_{20}$ (30) (6)	643 $\pm$ 35	210 $\pm$ 11	165 $\pm$ 12
5. $G_{2.75}$ + CCK-8S, 120 min	$G_{2.75}$ (36)	453 $\pm$ 44	117 $\pm$ 19	75 $\pm$ 8
6. $G_{2.75}$ + CCK-8S, 120 min	$G_{2.75} \rightarrow G_{20}$ (30) (6)	506 $\pm$ 51	119 $\pm$ 16	71 $\pm$ 6

Table 3. Inositol phosphate accumulation in isolated perfused islets

Groups of 30–50 islets were incubated for 90–120 min with *myo*-[2-³H]inositol. Labelling was carried out in the presence of 2.75 mM-glucose ($G_{2.75}$), 13.75 mM-glucose ($G_{13.75}$) or 2.75 mM-glucose plus 200 nM-CCK-8S. After this, the islets were washed and perfused for 60 min. Secretory integrity was assessed by stimulation with 20 mM-glucose (G_{20}) for 30 min (actually 28.5 min, because the dead-time of the system is 1.5 min). The duration of perfusion (min) is indicated in parentheses below each protocol condition. After the perfusion, labelled inositol phosphates were analysed as described in the Experimental section. At least four experiments were performed for each protocol; results are given as means $\pm$ S.E.M. Statistical analysis: line 1 versus line 2, $P < 0.05$ for all inositol phosphates; line 3 versus line 4, $P < 0.05$ for Ins P_1 ; line 5 versus line 6, $P < 0.05$ for all inositol phosphates; line 7 versus line 8, no significant differences; line 9 versus line 10, $P < 0.05$ for all inositol phosphates.

Labelling condition	Perfusion protocol	Accumulation (c.p.m./40 islets)		
		Ins P_1	Ins P_2	Ins P_3
1. $G_{2.75}$, 90 min	$G_{2.75}$ (60)	370 $\pm$ 39	85 $\pm$ 10	51 $\pm$ 4
2. $G_{2.75}$, 90 min	$G_{2.75} \rightarrow G_{20}$ (30) (30)	685 $\pm$ 56	229 $\pm$ 24	107 $\pm$ 9
3. $G_{13.75}$, 90 min	$G_{2.75}$ (60)	342 $\pm$ 12	71 $\pm$ 10	54 $\pm$ 18
4. $G_{13.75}$, 90 min	$G_{2.75} \rightarrow G_{20}$ (30) (30)	463 $\pm$ 28	91 $\pm$ 9	72 $\pm$ 13
5. $G_{2.75}$, 120 min	$G_{2.75}$ (60)	388 $\pm$ 25	101 $\pm$ 11	74 $\pm$ 6
6. $G_{2.75}$, 120 min	$G_{2.75} \rightarrow G_{20}$ (30) (30)	983 $\pm$ 71	392 $\pm$ 71	229 $\pm$ 16
7. $G_{13.75}$, 120 min	$G_{2.75}$ (60)	336 $\pm$ 17	98 $\pm$ 5	102 $\pm$ 12
8. $G_{13.75}$, 120 min	$G_{2.75} \rightarrow G_{20}$ (30) (30)	382 $\pm$ 22	106 $\pm$ 17	121 $\pm$ 15
9. $G_{2.75}$ + CCK-8S, 120 min	$G_{2.75}$ (60)	426 $\pm$ 38	124 $\pm$ 12	68 $\pm$ 8
10. $G_{2.75}$ + CCK-8S, 120 min	$G_{2.75} \rightarrow G_{20}$ (30) (30)	805 $\pm$ 68	325 $\pm$ 34	158 $\pm$ 19

secretory pattern was accompanied by transient increases in the accumulation of labelled inositol phosphates (Table 2) and by a small initial increase in [³H]inositol efflux, followed by a progressive decline in

the efflux rate (Fig. 2) and in the inositol phosphate pools (Table 3). Preincubation of islets with CCK-8S resulted in a decreased first phase of insulin secretion in response to 20 mM-glucose. There was no immediate increase in

[³H]inositol efflux (Fig. 3) or inositol phosphate production (Table 2). However, second-phase insulin secretion, [³H]inositol efflux and the accumulation of inositol phosphates increased slowly as the perfusion progressed (Fig. 3 and Table 3).

It is noteworthy that 200 nM-CCK-8S stimulates PI hydrolysis, but not insulin secretion, in the presence of 2.75 mM-glucose [5,17]. Thus the suppression of glucose-induced insulin secretion caused by a prior 2 h exposure to CCK-8S cannot be due to a depletion of the insulin stores of these islets. Furthermore, CCK-8S does not influence either glucose metabolism [17] or cyclic AMP content of islets [18,19]. On the other hand, since 13.75 mM-glucose does stimulate insulin secretion, it could be argued that the failure of 20 mM-glucose to induce a sustained second phase of insulin release (Fig. 2) is due to a depletion of insulin stores. Arguing against this conclusion are our results with MOG. Perfusion of these islets with MOG plus 20 mM-glucose restores a high and sustained second phase of insulin secretion (Fig. 4). Further exploration of the effects of MOG on diacylglycerol metabolism is necessary before the mechanism of its effect is clear. However, this result suggests that impaired PI hydrolysis, accompanied by decreased β -cell diacylglycerol content, may impair the activation of protein kinase C. This biochemical defect could result in a deficient second-phase insulin-secretory response [20,21].

The present studies provide further evidence for a very close link between glucose-induced changes in PI hydrolysis and glucose-induced insulin secretion [10]. The present data imply that prolonged stimulation of PI hydrolysis (during the 2 h labelling period with 13.75 mM-glucose or CCK-8S) renders this metabolic pathway refractory to subsequent stimulation, perhaps by inducing some type of negative-feedback signal. The nature of this inhibitory signal is not known. However, other agents such as the phorbol ester 12-*O*-tetradecanoylphorbol 13-acetate [22], the monokine interleukin 1 [23] and 4-methyl-2-oxopentanoate [22] also cause a desensitization or suppression of the insulin-secretory response. All these agents share in common an ability either to stimulate the hydrolysis of PI and/or to activate protein kinase C. If a single mechanism underlies the suppression of glucose-induced insulin secretion, then the involvement of protein kinase C is suggested. Finally, it is not immediately evident why the pattern of suppression induced by exposure to high glucose should differ from that induced by cholecystokinin. However, understanding the mechanisms which underlie this difference may be quite important. The altered pattern of insulin secretion induced by an exposure to high glucose *in vitro* (Fig. 2) is the same as that induced by glucose intoxication *in vivo* (W. S. Zawulich, K. C. Zawulich, G. Shulman & L. Rossetti, unpublished work), and the

altered insulin-secretory pattern induced by CCK-8S *in vitro* (Fig. 3) is quite similar to that seen in type II diabetes [24].

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REFERENCES

1. Clements, R. S. & Rhoten, W. B. (1976) *J. Clin. Invest.* **57**, 684–691
2. Laychock, S. G. (1983) *Biochem. J.* **216**, 101–106
3. Axen, K. V., Schubart, U. K., Blake, A. D. & Fleischer, N. (1983) *J. Clin. Invest.* **72**, 13–21
4. Best, L. & Malaisse, W. J. (1984) *Endocrinology* (Baltimore) **115**, 1814–1820
5. Zawulich, W. S., Takuwa, N., Takuwa, Y., Diaz, V. A. & Rasmussen, H. (1987) *Diabetes* **36**, 426–433
6. Biden, T. J., Peter-Riesch, B., Schlegel, W. & Wollheim, C. B. (1987) *J. Biol. Chem.* **262**, 3567–3571
7. Rana, R. S., Mertz, R. J., Kowluru, A., Dixon, J. F., Hokin, L. E. & MacDonald, M. J. (1985) *J. Biol. Chem.* **260**, 7861–7867
8. Rana, R. J., Kowluru, A. & MacDonald, M. J. (1986) *Arch. Biochem. Biophys.* **245**, 411–416
9. Best, L., Tomlinson, S., Hawkins, P. J. & Downes, C. P. (1987) *Biochim. Biophys. Acta* **927**, 112–116
10. Zawulich, W. S. & Zawulich, K. C. (1988) *Diabetes* **37**, 1294–1300
11. Zawulich, W. S., Diaz, V. A. & Zawulich, K. C. (1988) *Am. J. Physiol.* **254**, E609–E616
12. Zawulich, W. S. (1988) *Diabetes* **37**, 137–141
13. Lacy, P. E. & Kostianovsky, M. (1967) *Diabetes* **16**, 35–39
14. Berridge, M. J., Dawson, R. M. C., Downes, C. P., Heslop, J. P. & Irvine, R. F. (1983) *Biochem. J.* **212**, 473–482
15. Bishop, W. R., Ganong, B. R. & Bell, R. M. (1986) *J. Biol. Chem.* **261**, 6993–7000
16. Takuwa, N., Takuwa, Y. & Rasmussen, H. (1988) *J. Biol. Chem.* **263**, 9738–9745
17. Zawulich, W. S., Cote, S. B. & Diaz, V. A. (1986) *Endocrinology* (Baltimore) **119**, 616–621
18. Verspohl, E. J., Breuning, I., Ammon, H. P. T. & Mark, M. (1987) *Regul. Peptides* **17**, 229–241
19. Zawulich, W. S. (1988) *Metab. Clin. Exp.* **37**, 778–781
20. Zawulich, W. S., Brown, C. & Rasmussen, H. (1983) *Biochem. Biophys. Res. Commun.* **117**, 448–452
21. Zawulich, W., Zawulich, K. & Rasmussen, H. (1984) *Cell Calcium* **5**, 551–558
22. Bolaffi, J. L., Bruno, Z., Heldt, A. & Grodsky, G. M. (1988) *Endocrinology* (Baltimore) **122**, 1801–1809
23. Zawulich, W. S., Zawulich, K. C. & Rasmussen, H. (1989) *Endocrinology* (Baltimore), in the press
24. Kahn, S. E. & Porte, D. (1988) *Am. J. Med.* **85**, Suppl. 5A, 4–8