Interactions between lithium, inositol and mono-oleoylglycerol in the regulation of insulin secretion from isolated perfused rat islets

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In response to stimulation by 20 mM-glucose, 15 mM-4-methyl-2-oxopentanoate or 10 mM-glyceraldehyde, isolated perfused rat islets respond with brisk biphasic insulin-secretory responses. The inclusion of 10 mM-LiCl significantly decreased second-phase insulin release in response to all agonists. Inositol, at a concentration (10 mM) which has no effect on secretion in the presence of 2.75 mM-glucose, restored significantly glucose-, 4-methyl-2-oxopentanoate- or glyceraldehyde-induced second-phase release from Li⁺-treated islets. The addition of the diacylglycerol kinase inhibitor mono-oleoylglycerol, at a concentration (25 μ M) which has no stimulatory effect on insulin secretion in the presence of 2.75 mM-glucose, significantly amplified both the first- and second-phase insulin responses to 20 mM-glucose. This amplifying effect of mono-oleoylglycerol was readily reversible and dependent on Ca²⁺ influx into the β -cell. Li⁺ decreased the amplified insulin response to 20 mM-glucose plus mono-oleoylglycerol. Inositol restored release under this condition. These findings suggest that Li⁺ inhibits release by sequestering inositol into biosynthetically ineffective inositol phosphates. By limiting phosphoinositide resynthesis, the continued hydrolysis of phosphoinositides is diminished. Our results with mono-oleoylglycerol suggest further that diacylglycerol content may play a critically important role in the regulation of both the first and second phases of insulin secretion.

INTRODUCTION

Li⁺ inhibits the dephosphorylation of several inositol phosphates generated during cellular increases in phosphoinositide (PI) hydrolysis [1,2]. Because of this action, it is often employed to facilitate measurements of these PI-derived water-soluble compounds during stimulation. In islets at least, an additional effect on insulin secretion has been observed. At concentrations of Li⁺ which cause exorbitant increases in inositol phosphate accumulation in islets [3,4] as well as other tissues [5], it inhibits insulin release, particularly the second phase, in response to glucose stimulation [6–8].

In the present series of experiments we explored the influence of inositol supplementation on Li⁺-inhibited insulin release. We reasoned that Li⁺, by trapping inositol into biosynthetically ineffective (in regard to PI resynthesis) inositol phosphates, inhibits the resynthesis of islet PI. By decreasing PI availability, this action diminishes the supply of PI-derived second-messenger molecules, particularly diacylglycerol (DAG), and attenuates stimulated insulin release. If this suggestion is correct, then the inclusion of inositol might be expected to circumvent this insulin-secretion-inhibitory effect of Li⁺ and restore insulin secretory rates. The present findings emphasize the importance of both inositol and DAG availability in maintaining insulin release from isolated perfused rat islets.

MATERIALS AND METHODS

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-induced (50 mg/kg) anaesthesia, islets were isolated by collagenase digestion [9] 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 μ l portions were analysed for insulin [10] with rat insulin (Eli Lilly and Co., Indianapolis, IN, U.S.A.; batch no. 615-D63-12-3) as standard. The radioisotope used to measure insulin release (¹²⁵I-insulin) was purchased from New England Nuclear, Boston, MA, U.S.A. Inositol, LiCl, 4-methyl-2-oxopentanoate, D-glyceraldehyde, mono-oleoylglycerol (mono-olein, MOG, dissolved in dimethyl sulphoxide), bovine serum albumin, as well as the salts used to make the perfusion medium, were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. In the MOG experiments, the dimethyl sulphoxide level was maintained at 0.1% (v/v).

Statistics

Where appropriate, statistical significance was determined by Student's *t* test for unpaired data. A *P* value less than 0.05 was taken as significant. Values shown in the Figures represent means $\pm$ S.E.M. of the specified numbers of observations.

RESULTS

Stimulation of islets with a concentration of 20 mM-glucose plus 10 mM-LiCl resulted in a prompt insulin

Abbreviations used: PI, phosphoinositide; MOG, mono-oleoylglycerol; DAG, diacylglycerol.

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Fig. 1. Influence of LiCl (10 mM) on chronic glucose stimulation of isolated islets

After 30 min with 2.75 mM-glucose ($G_{2.75}$), groups of 12–15 islets were perfused under three different sets of conditions. One group was stimulated with 20 mM-glucose (G_{20}) in the presence of 10 mM-LiCl (●). A second group was similarly treated except for the added presence of 10 mM-inositol (○). The final group was stimulated with 10 mM-inositol in the continued presence of 2.75 mM-glucose (■----■). In the LiCl experiments, this compound was added to the medium 10 min before the 20 mM-glucose stimulation. At least four experiments were performed under each condition. Means $\pm$ selected S.E.M. values are given. This and all subsequent Figures have been corrected for the dead space in the perfusion system, 2.5 ml or 2.5 min with a flow rate of 1 ml/min. Asterisks indicate significant ($P < 0.05$) differences between the two 20 mM-glucose-stimulated groups.

response characterized by three phases (Fig. 1). After an initial first phase, a slowly rising second phase was evident. Peak release was reached approx. 30 min after the onset of stimulation and averaged 1023 ± 187 pg/min per islet. Release rates fell continuously as the perfusion progressed, and averaged 435 ± 108 pg/min per islet in samples collected 75–80 min after the onset of stimulation. The inclusion of 10 mM-inositol, a concentration with no insulin-stimulatory effect in the presence of 2.75 mM-glucose, altered significantly the response of islets to the combination of 20 mM-glucose plus 10 mM-Li⁺ (Fig. 1). Most dramatic is the maintenance of second-phase insulin release. For example, 75–80 min after the onset of stimulation, insulin-release rates in response to the combination of 20 mM-glucose, 10 mM-lithium and 10 mM-inositol averaged 1042 ± 162 pg/min per islet. The insulin-secretory response at this time to this combination of compounds was significantly ($P < 0.05$) greater than that from islets stimulated with 20 mM-glucose alone (539 ± 58 pg/min per islet; see Fig. 4).

Similar results were obtained when islets were stimu-

Fig. 2. Influence of LiCl (10 mM) on chronic 4-methyl-2-oxo-pentanoate (KIC) stimulation of isolated islets

After 30 min with 2.75 mM-glucose ($G_{2.75}$), groups of 12–15 islets were stimulated under three different sets of conditions. One group was stimulated with 15 mM-KIC alone (Δ), another group with 15 mM-KIC plus 10 mM-LiCl (●), and the final group with 15 mM-KIC plus 10 mM-LiCl plus 10 mM-inositol (○). In the Li⁺-treated islets this compound was added to the medium 10 min before KIC stimulation. At least four experiments were performed under each condition.

Fig. 3. Influence of LiCl (10 mM) on chronic D-glyceraldehyde stimulation of isolated islets

After 30 min with 2.75 mM-glucose ($G_{2.75}$), groups of 12–15 islets were stimulated for 80 min with 10 mM-glyceraldehyde. One group (Δ) was stimulated with glyceraldehyde alone. Another group was stimulated with glyceraldehyde plus Li⁺ (●). The final group was stimulated with 10 mM-glyceraldehyde, 10 mM-LiCl plus 10 mM-inositol (○). In the Li⁺-treated islets the compound was added to the medium 10 min before glyceraldehyde stimulation. At least four experiments were performed under each condition.

Fig. 4. MOG (25 μM) prevents β -cell desensitization to stimulation by 20 mM-glucose

Groups of 12–15 islets were perfused for 30 min with 2.75 mM-glucose ($G_{2.75}$). One group was maintained with this glucose concentration plus 25 μM -MOG ($\bullet$) for 80 min ($n = 4$). Another group ($n = 8$) was stimulated with 20 mM-glucose (G_{20}) alone ($\circ$), and the third group ($n = 9$) was stimulated with 20 mM-glucose plus 25 μM -MOG ($\square$). The asterisks indicate significant ($P < 0.05$) differences between the groups stimulated with 20 mM-glucose or 20 mM-glucose plus 25 μM -MOG.

lated with 15 mM-4-methyl-2-oxopentanoate. Alone, this compound caused a brisk biphasic insulin-secretory response (Fig. 2). The presence of Li^+ attenuated this stimulatory effect (Fig. 2). Inositol (10 mM) significantly restored 4-methyl-2-oxopentanoate-induced second-phase release from Li^+ -treated islets.

Li^+ also attenuated insulin release in response to 10 mM-D-glyceraldehyde (Fig. 3). In the continued presence of 2.75 mM-glucose, the addition of glyceraldehyde initially evoked a distinct biphasic insulin-secretory response. With time, however, release rates decreased dramatically. The inclusion of 10 mM- Li^+ altered both the kinetics and the amplitude of first-phase release in response to glyceraldehyde and diminished significantly second-phase release. The inclusion of 10 mM-inositol circumvented to a large extent the inhibitory effects of Li^+ on sustained insulin release. Its inclusion, however, did not maintain second-phase insulin rates (Fig. 3).

We recently reported [11] that the DAG kinase in-

Fig. 5. Influence of LiCl on chronic glucose stimulation in the presence of MOG and inositol

Groups of 12–15 islets were perfused with 2.75 mM-glucose ($G_{2.75}$). One group ($n = 4$) was stimulated for 80 min with 20 mM-glucose (G_{20}) plus MOG (25 μM) plus LiCl ($\bullet$). The other group ($n = 4$) was stimulated with these same compounds plus the further addition of 10 mM-inositol ($\circ$). Asterisks indicate significant ($P < 0.05$) differences.

hibitor [12] MOG restored second-phase insulin release from islets desensitized by chronic glucose stimulation. Additional experiments were conducted with this compound. At 25 μM , MOG has no significant insulin-stimulatory effect in the presence of 2.75 mM-glucose alone (Fig. 4). However, in the presence of 20 mM-glucose, MOG restored significantly the waning pattern of insulin secretion usually noted during chronic stimulation with 20 mM-glucose (Fig. 4). Most dramatic is the maintenance of second-phase insulin release in response to 25 μM -MOG plus 20 mM-glucose. In additional studies the influence of 10 mM- Li^+ on MOG-potentiated insulin release was explored. Li^+ significantly decreased the dramatic secretory response of islets perfused with 25 μM -MOG plus 20 mM-glucose (Fig. 5). The pattern of secretion now resembled that noted from islets stimulated with 20 mM-glucose plus Li^+ (cf. Figs. 1 and 5). Most interestingly, 10 mM-inositol circumvented this inhibitory effect of Li^+ (Fig. 5).

Because of the dramatic effect of MOG on glucose-stimulated insulin release, its mechanism of action was explored further. First, removal of MOG along with decreasing glucose to 2.75 mM resulted in a rapid decline in insulin-secretory rates (Fig. 6). Upon re-stimulation with 20 mM-glucose insulin release was readily evoked. Second, decreasing Ca^{2+} influx with 0.5 μM -nitrendipine abolished the dramatic insulin response usually observed with 20 mM-glucose plus 25 μM -MOG. These islets were able, however, to respond to a subsequent glucose stimulus.

Fig. 6. Characteristics of MOG-potentiated insulin release: reversibility and blockade by nitrendipine

Three groups of perfused islets were used. One group ($n = 4$) was perfused for 30 min with 20 mM-glucose (G_{20}) plus 25 μ M-MOG after a 30 min stabilization period with 2.75 mM-glucose ($G_{2.75}$) (○). For an additional 20 min these islets were again perfused with 2.75 mM-glucose. A 30 min stimulatory period with 20 mM-glucose alone followed. The second group of islets (●; $n = 4$) was first perfused for 30 min with 2.75 mM-glucose, and for 30 min with 0.5 μ M-nitrendipine, 20 mM-glucose plus 25 μ M-MOG. After a 20 min period with 2.75 mM-glucose alone, these islets were re-stimulated with 20 mM-glucose alone. The third group of islets (○—○; $n = 4$) was perfused for 80 min with 2.75 mM-glucose and for an additional 30 min with 20 mM-glucose. Only the final 30 min of perfusion is depicted in this group.

DISCUSSION

The initial aim of the present studies was to explore whether the inclusion of inositol has any mitigating influence on Li^+ -inhibited insulin secretion [6–8]. We reasoned that the decline in second-phase release rates in Li^+ -treated islets might be the result of impaired PI resynthesis. By trapping inositol into membrane-impermeant inositol phosphates (thus facilitating their measurement), Li^+ may prevent the re-incorporation of inositol back into PI [13]. This inositol-sequestering effect of Li^+ decreases the supply of PI available for hydrolysis and the subsequent generation of PI-derived second-messenger molecules. Our previous studies [14,15], using unlabelled inositol to facilitate the analysis of fractional efflux rates of [3H]inositol, support our position that reuptake of inositol into PI is a most dynamic process. As the present studies progressed, however, it became evident that these experiments may also be pertinent to our understanding of how chronic exposure to a β -cell agonist may lead to a desensitization of the insulin-secretory apparatus, a process referred to as the third phase of

insulin release by Bolaffi, Grodsky and co-workers [16]. Particularly pertinent in this regard are our studies with the DAG kinase inhibitor MOG [12].

Chronic stimulation of isolated perfused islets with 20 mM-glucose plus 10 mM- Li^+ is accompanied by three phases of insulin release (Fig. 1). A rapid, transient, first-phase secretion is followed by a slowly rising output of the hormone. With time, however, release rates during this sustained phase subside. This decline in release rates is not caused by insulin depletion. Inclusion of inositol has little or no effect on the initial phases of secretion, but has a significant restorative effect on the waning insulin-secretory response (Fig. 1). It is important to emphasize that this concentration of inositol has absolutely no stimulatory effect on insulin secretion during prolonged perfusion of islets with 2.75 mM-glucose (Fig. 1). Thus inositol appears to be supplying a component which has become rate-limiting for stimulus-response coupling when islets are stimulated for long periods *in vitro* with 20 mM-glucose plus 10 mM- Li^+ . Since inositol is not converted by the islet into metabolically useful fuels (e.g. lactate or pyruvate), and since its only fate in islets appears to be its incorporation into PI [17,18], we suggest that the normal fall in insulin release rates during prolonged exposure to high glucose plus Li^+ may be, in part, the result of a depletion of free inositol, an essential component for PI resynthesis during prolonged perfusion.

Similar to the results obtained with glucose stimulation, Li^+ also significantly attenuated secretion in response to both 4-methyl-2-oxopentanoate and glyceraldehyde. Both of these compounds increase PI hydrolysis in islets, an event paralleled by significant increases in insulin release [8,15]. With either agonist, inositol supplementation circumvented the inhibitory effect of Li^+ on the pattern of insulin release. With glyceraldehyde at least, the presence of inositol did not restore the usual dramatic decline in insulin release rates observed in the presence of glyceraldehyde alone. The precise reason for this marked decline in release during chronic glyceraldehyde stimulation may reflect impaired ATP generation in islets [19].

Our results with MOG, a recently described DAG kinase inhibitor [12], support the conclusion that DAG availability also plays a major role in the capacity of the β -cell to sustain an insulin-secretory response during chronic stimulation. Limited tissue availability has precluded the direct analysis of DAG levels in glucose-stimulated islets with or without MOG. However, MOG has been shown to increase DAG content in a variety of cells [12,20]. Our results demonstrate that MOG (25 μ M) by itself has no significant effect on insulin release in the presence of 2.75 mM-glucose, that it potentiates insulin release dramatically when PI hydrolysis has been initiated by 20 mM-glucose, that the effect of MOG is reversible, that the potentiating effect of MOG is blocked by Li^+ , and, finally, that the inhibitory effect of Li^+ in the presence of MOG is reversed by inositol. Moreover, the effect of MOG, like glucose-induced insulin release and PI hydrolysis, is blocked by eliminating Ca^{2+} influx into the β -cell with the Ca^{2+} -channel antagonist nitrendipine. It seems reasonable to suggest that the effect of MOG is related, at least in part, to its ability to maintain agonist-induced increases in islet DAG content.

Our results with $LiCl$ in the presence of 20 mM-glucose plus 25 μ M-MOG further emphasize the importance of

PI resynthesis in the ability of islets to maintain an insulin-secretory response. Whereas the presence of MOG converts a diminishing insulin-secretory response to 20 mM-glucose into a robust and sustained one (Fig. 4), the presence of Li^+ blocks this potentiating effect of MOG (Fig. 5). Tentatively, at least, this effect of Li^+ may be attributed to the capacity of this cation to prevent the hydrolysis of inositol phosphates, so that inositol is sequestered into biosynthetically ineffective (with regard to PI resynthesis) inositol phosphates. This action diminishes PI resynthesis and its subsequent hydrolysis, and limits DAG availability. Hence the potentiating effect of MOG wanes because PI hydrolysis is decreased, i.e., there is no continued production of DAG. Most dramatic is the finding that inositol, at a concentration which has no effect on insulin release in islets perfused with 2.75 mM-glucose (Fig. 1), restores the secretory response of LiCl -treated islets to glucose and MOG (Fig. 5). This result emphasizes the need for both DAG production from phosphoinositides and the inhibition of DAG phosphorylation in sustaining insulin secretion under these conditions.

Our results obtained by using Li^+ to inhibit second-phase release add further credence to the concept that both inositol and DAG availability can influence stimulus-response coupling in islets. The usual dramatic decrease in second-phase insulin output in response to 20 mM-glucose plus 10 mM- LiCl is effectively overcome by the simultaneous inclusion of 10 mM-inositol in the LiCl -containing perfusate (Fig. 4). Thus, in the presence of Li^+ , insulin release rates decline more rapidly because of effective sequestering of inositol into membrane-impermeant and biosynthetically ineffective inositol phosphates. Normally inhibited as a result of this process, PI resynthesis (and presumably its subsequent hydrolysis) is allowed to proceed if inositol is included in the medium. As a result of this, insulin release rates are partially restored.

When taken in their entirety, the present results imply that the continued turnover (hydrolysis plus resynthesis) of islet PI is an essential component of the islet's ability to sustain an insulin-secretory response during chronic stimulation with glucose. We suggest further that the process of β -cell desensitization [16,21,22] may be related to altered islet PI metabolism. Perhaps experimental emphasis should now be placed on studying the factors responsible for PI resynthesis, and not just on the generation of the second messengers derived from PI hydrolysis.

These studies were supported by N.I.D.D.K. Grant 19813. The expert secretarial assistance of Nancy Canetti is gratefully acknowledged.

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Received 10 March 1989/10 May 1989; accepted 16 May 1989