The effect of monooleoylglycerol on insulin secretion from isolated perfused rat islets

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Summary. The effect of monooleoylglycerol on cholecystokinin- and tolbutamide-induced insulin secretion was examined in isolated perfused rat islets. In the presence of 5.5 mmol/l glucose, addition of 10 nmol/l cholecystokinin or 50 μ mol/l tolbutamide had practically no effect on insulin secretion. Combined tolbutamide and cholecystokinin led to a biphasic insulin secretory response which was significantly enhanced by addition of 50 μ mol/l monooleoylglycerol, an inhibitor of diacylglycerol kinase. Monooleoylglycerol (50 μ mol/l) alone had a minimal stimulatory effect on insulin release in the presence of 5.5 mmol/l glucose. Perfusion of islets with 1 μ mol/l forskolin had no significant effect on basal insulin secretion in the presence of 5.5 mmol/l glucose, but

markedly enhanced the responses to both cholecystokinin plus tolbutamide, and to the combination of cholecystokinin, tolbutamide and monooleoylglycerol. Lowering the glucose level to 2.75 mmol/l abolished the profound stimulatory effect to these agonist combinations on insulin release. Finally, monooleoylglycerol also enhanced the first and second phase insulin secretory responses induced by 20 mmol/l glucose. These results are discussed in relationship to the possible role of protein kinase C in mediating insulin secretion.

Key words: Insulin secretion, diacylglycerol, isolated islets, second messengers, phosphoinositides.

There is considerable evidence which supports the view that protein kinase C plays a role in mediating insulin secretion from the B cell of the pancreas [1–3]. However, there is no consensus as to its precise and obligatory involvement in this process [1–5]. In previous studies in perfused rat islets, we showed that tolbutamide and forskolin acted synergistically with 12-*o*-tetradecanoyl phorbol-13-acetate (TPA) to stimulate insulin secretion [6, 7] and proposed that protein kinase C plays a key role in mediating the sustained or second phase of glucose-induced insulin secretion [6–8]. However, this combination of pharmacologic agents did not, even at optimal concentrations, induce as great an insulin secretory response as did 16.7 mmol/l glucose, the pre-eminent physiological agonist for stimulated insulin secretion.

Our recent studies [9–12] of the complex relationships between the actions of cholecystokinin 8-sulfate (CCK-8S) and of glucose on insulin secretion have led to further insights into the mechanisms by which insulin secretion is controlled. In these studies, it was shown that 100–200 nmol/l CCK-8S induces a rapid hydrolysis of polyphosphoinositides, the production of inositol phosphates, and a sustained increase in efflux of

[³H]-inositol from islets in which the phosphoinositide pool was prelabelled. The effect of CCK-8S on these parameters of cellular activation is nearly identical whether islets are perfused with 2.75 or 7.0 mmol/l glucose, but the accompanying insulin secretory response is quite different; CCK-8S induces a biphasic insulin secretory response from islets incubated in media containing 7 mmol/l glucose, but there is no significant secretory response from islets incubated in 2.75 mmol/l glucose. Pharmacologic agents were used to determine whether or not either Ca²⁺ influx and/or an increase in cAMP concentration were sufficient to restore the insulin secretory responsiveness of islets (incubated in 2.75 mmol/l glucose) to CCK-8S. Forskolin, an activator of adenylate cyclase, restores partial responsiveness to CCK-8S and tolbutamide, a stimulator of Ca²⁺ influx, further enhances the restorative effect of forskolin [11]. However, addition of CCK-8S to islets incubated in 2.75 mmol/l glucose and treated with either 1 μ mol/l forskolin and/or 200 μ mol/l tolbutamide, leads to a marked first phase insulin secretory response, but no sustained second phase [11]. These results imply that glucose has an additional effect on islet cell metabolism related to the second or sustained phase of insulin se-

cretion. We postulated that this is on the metabolism of diacylglycerol (DAG) by a pathway other than PI hydrolysis (see also [13]). This unique effect of glucose on DG production has recently been demonstrated [14].

Recently Bishop et al. [15] have reported that monooleoylglycerol (MOG) is a relatively specific inhibitor of the enzyme DAG kinase which converts DAG to phosphatidic acid. We have shown that MOG causes an increase in the DAG content of cultured 3T3 cells and that this leads to an activation of protein kinase C in these cells [16]. The present study reports the effect of monooleoylglycerol on glucose- and CCK-8S-induced insulin secretion under several circumstances. We reasoned that if glucose and CCK-8S have their postulated effects on DAG metabolism then application of MOG might significantly enhance both CCK-8S- and glucose-induced insulin secretion.

Materials and methods

The detailed methodology employed to assess insulin output from collagenase isolated islets has been previously described [9–12]. Male Sprague-Dawley rats purchased from Charles River (Kingston, NY, USA) were used in all studies. The animals were fed ad lib and at the time killed weighed between 300–400 g. After Nembutal-induced (50 mg/kg) anesthesia, islets were isolated by collagenase digestion and perfused to assess secretory responsiveness. Groups of 12–15 islets were loaded onto nylon filters and perfused at a flow rate of 1 ml/min for 30 min to establish stable, basal secretory rates. After this stabilisation period, the islets were then subjected to various protocols indicated in the Figures. Effluent samples were collected and analysed for insulin content using rat insulin as standard (Lilly, Indianapolis, Ind., USA, Lot # 615-D63-12-3).

Reagents

Hank's solution was used for the islet isolation. The perfusion medium consisted of 115 mmol/l NaCl, 5 mmol/l KCl, 2.2 mmol/l CaCl₂, 1 mmol/l MgCl₂, 24 mmol/l NaHCO₃, and 0.17 g/dl bovine serum albumin. Other compounds were added as indicated and the solution was gassed with a mixture of 95% O₂/5% CO₂. The ¹²⁵I-insulin used for the insulin assay was purchased from New England Nuclear, Boston, Mass., USA. Bovine serum albumin (RIA grade), monooleoylglycerol (monoolein), sulfated cholecystokinin (fragment 26–33 amide), as well as the salts used to make the Hank's solution and perfusion medium were purchased from Sigma Chemical Company, St. Louis, Mo., USA. Forskolin was purchased from Calbiochem, La Jolla, Calif., USA. Tolbutamide (sodium salt) was the generous gift of Upjohn (Kalamazoo, Mich., USA). In the experiments involving MOG, DMSO was used as the solvent. The level of DMSO used (never greater than 0.2%) had no effect on insulin secretory patterns.

Statistical Analysis

Statistical significance was determined using the Student's *t*-test for unpaired data and where appropriate, analysis of variance. A *p* value less than 0.05 was taken as significant. Values presented in the figures or results sections represent means $\pm$ SEM of the specified number of observations.

Results

CCK-8S (10 nmol/l) had a minimal and transient effect on insulin secretion from islets incubated in media containing 5.5 mmol/l glucose (Fig. 1, left). Likewise, addition of 50 μ mol/l tolbutamide causes only a very small and transient increase in insulin secretion (Fig. 1, center). No sustained insulin stimulatory effect of either compound is observed if the glucose level is 2.75 mmol/l. When CCK-8S and tolbutamide are combined, however, they induce a modest biphasic insulin secretory response from islets incubated in 5.5 but not 2.75 mmol/l glucose (Fig. 1, right). If islets (incubated in 5.5 mmol/l glucose) are exposed to 50 μ mol/l MOG, along with CCK-8S and tolbutamide, there is a dramatic biphasic insulin secretory response (Fig. 2, left) which is markedly enhanced when compared to CCK-8S and tolbutamide alone (see Fig. 1, right). This insulin secretory response to the combination of MOG, CCK-8S, and tolbutamide is similar to that induced by 20 mmol/l glucose alone (Fig. 2, right). It is noteworthy that 50 μ mol/l MOG alone has only a minimal effect on insulin secretion in either the absence (Fig. 2, left) or presence of forskolin (Fig. 2, center).

The most striking result is that found when islets are perfused with 5.5 mmol/l glucose plus 1 μ mol/l forskolin and then exposed to either 10 nmol/l CCK-8S and 50 μ mol/l tolbutamide, or to CCK-8S, tolbutamide and 50 μ mol/l MOG (Fig. 2, center). The major effect of forskolin on the response induced by CCK-8S and tolbutamide is on the first phase of insulin secretion. Peak first phase release is 145 ± 12 pg $\cdot$ islet⁻¹ $\cdot$ min⁻¹ in the absence of forskolin and 1466 ± 133 pg $\cdot$ islet⁻¹ $\cdot$ min⁻¹ in the presence of forskolin. The rate of secretion at min 60 is 504 ± 73 pg $\cdot$ islet $\cdot$ min (mean $\pm$ SEM, *n* = 4) without forskolin and 600 ± 39 pg $\cdot$ islet⁻¹ $\cdot$ min⁻¹ with forskolin. The further addition of MOG (50 μ mol/l) along with CCK-8S, tolbutamide and forskolin (Fig. 2, center) leads to a marked enhancement of second phase secretion (2306 ± 98 vs 1238 ± 119 pg $\cdot$ islet⁻¹ $\cdot$ min⁻¹ in the presence and absence of forskolin, respectively). A small additional effect on the peak of the first phase secretion (1825 ± 191 vs 1466 ± 133 pg $\cdot$ islet⁻¹ $\cdot$ min⁻¹ in the presence and absence of forskolin, respectively) was also observed. Under these conditions (CCK-8S, forskolin, tolbutamide, and MOG) both phases of the insulin secretory response are considerably greater than that induced by 20 mmol/l glucose alone—peak first phase to this combination averaged 1825 ± 191 vs 445 ± 51 pg $\cdot$ islet⁻¹ $\cdot$ min⁻¹ for 20 mmol/l glucose alone and peak second phase release to this combination averaged 2306 ± 98 pg $\cdot$ islet⁻¹ $\cdot$ min⁻¹ vs 1283 ± 116 pg $\cdot$ islet⁻¹ $\cdot$ min⁻¹ for 20 mmol/l glucose alone. However, in the simultaneous presence of 50 μ mol/l MOG, 20 mmol/l glucose does induce a second phase secretory response (1843 ± 78 pg $\cdot$ islet⁻¹ $\cdot$ min⁻¹) similar to that noted with the combination of 5.5 mmol/l glucose, 50 μ mol/l tolbutamide, 1 μ mol/l

Fig. 1. Influence of cholecystikinin (CCK) and tolbutamide (TOL) on insulin secretory patterns in the presence of different glucose (G) concentrations. Groups of 12–15 islets were perfused for 60 min with 2.75 (●—●) or 5.5 mmol/l (○—○) glucose. For the final 30 min, **A**) 10 nmol/l CCK-8S (left panel) or **B**) 50 μmol/l tolbutamide alone (middle panel) or **C**) in combination (right panel) were included in the medium. At least 4 experiments were performed under each experimental setting. Mean values plus or minus selected SEM are given. This and all subsequent figures have been corrected for the dead space in the perfusion system, approximately 2.5 ml or 2.5 min at a flow rate of 1 ml/min

Fig. 2. Influence of forskolin (1 μmol/l) on stimulated insulin secretion. Groups of 12–15 islets were perfused for 60 min in the presence (middle panel) or absence (left and right panels) of 1 μmol/l forskolin. Left panel. **A**) Insulin output in the presence of 5.5 mmol/l glucose plus the further addition of 50 μmol/l monooleoylglycerol (MOG) alone (●—●), or the combination of 10 nmol/l CCK-8S plus 50 μmol/l tolbutamide (●—●), or the combination of all 3 agonists (MOG, CCK-8S plus tolbutamide ○—○) is shown. Note that the line labelled ○—○ in Figure 1C and ●—● in Figure 2A are the same data. Middle panel. **B**) Experimental protocol was identical to that outlined in the left panel except that 1 μmol/l forskolin was present for the entire 60 min. Right panel. **C**) Insulin output in response to a maximally effective glucose stimulus (20 mmol/l) plus or minus 50 μmol/l MOG is shown. At least 4 experiments were performed under each experimental setting. Mean values ± selected SEM are given. * = MOG; **CCK-8S plus tolbutamide; *** MOG, CCK-8S, and tolbutamide

forskolin, 10 nmol/l CCK, and 50 μmol/l MOG (Fig. 2, center). Peak first phase insulin secretion rates in the presence of 50 μmol/l MOG plus 20 mmol/l glucose (953 ± 98 pg·islet⁻¹·min⁻¹) were significantly greater than those noted with 20 mmol/l glucose alone (445 ± 51 pg·islet⁻¹·min⁻¹).

Although not shown, the response to the agonist combination of 1 μmol/l forskolin, 50 μmol/l tolbut-

Fig. 3. The effects of MOG are reversible and dependent on calcium influx. Results from 2 sets of experiments are depicted. One group (○—○) of islets was perfused for 30 min with 2.75 mmol/l glucose (G) and for an additional 30 min with 20 mmol/l glucose (G), 0.5 μmol/l of the calcium channel blocker nitrendipine (NITR), plus 50 μmol/l monooleoylglycerol (MOG). After a 20 min perfusion with 2.75 mmol/l glucose alone, these islets were stimulated with 20 mmol/l glucose. The second group (●—●) of islets was perfused for 80 min with 2.75 mmol/l glucose and for an additional 30 min with 20 mmol/l glucose alone. At least 4 experiments were performed under each experimental setting

amide, 10 nmol/l CCK-8S, and 50 μmol/l MOG was dependent, to a large extent, on the ambient glucose level. For example, at 2.75 mmol/l glucose peak first phase insulin release to this agonist combination averaged 111 ± 8 pg·islet⁻¹·min⁻¹ while release 30 min after the onset of stimulation was 74 ± 3 pg·islet⁻¹·min⁻¹ ($n=3$). In contrast, at 5.5 mmol/l glucose these responses to the same combination now averaged 1825 ± 191 pg·islet⁻¹·min⁻¹ and 2306 ± 98 pg·islet⁻¹·min⁻¹ ($n=4$), respectively. Insulin release from the B cell provoked by the combination of forskolin, tolbutamide and CCK-8S was also entirely reversible. Removal of these agonists resulted in basal insulin secretory rates within 20 min (results not shown).

Finally, it should be emphasised that the effects of MOG are both reversible and dependent on calcium influx (Fig. 3). The presence of the calcium channel antagonist nitrendipine (0.5 μmol/l) virtually obliterated secretion in response to 20 mmol/l glucose plus MOG. Despite being exposed to MOG, these islets were able, however, to respond to a subsequent exposure to 20 mmol/l glucose.

Discussion

When the glucose concentration is 5.5 mmol/l, addition of 10 nmol/l CCK-8S or a low dose of tolbutamide alone does not have an appreciable effect on insulin secretion (Fig. 1). However, the combination of the two agents induces a biphasic insulin secretory response (Fig. 1, right). These data argue that CCK-8S and tolbutamide interact in a synergistic fashion. The simplest ex-

planation, based on the available evidence, is that the major effect of CCK-8S is on phosphatidylinositol 4,5 bisphosphate (PIP₂) hydrolysis with the production of inositol 1,4,5-trisphosphate (Ins 1,4,5P₃) and diacylglycerol. The major effect of tolbutamide is to stimulate Ca²⁺ influx, a process which may lead to a further activation of PIP₂ hydrolysis [17]. The CCK-8S-induced rise in Ins 1,4,5P₃ leads to the release of sequestered intracellular [Ca²⁺]. When this change in intracellular [Ca²⁺] is coupled to the tolbutamide-induced increase in Ca²⁺ influx, the resulting change in Ca²⁺ metabolism is sufficient to induce the initial phase of insulin secretion. Activation of adenylate cyclase by forskolin (Fig. 2, center) causes a considerable enhancement of the insulin secretory response to the combination of CCK-8S plus tolbutamide even though an increase in intracellular [cAMP] causes a partial inhibition of CCK-8S-induced PI hydrolysis [18].

If the glucose concentration is low (2.75 mmol/l), then CCK-8S alone or tolbutamide alone do not induce a significant increase in insulin secretion [11], but if combined with forskolin, they will induce a first phase of secretion but no second phase [11]. Hence, glucose appears to have an independent effect on the metabolism of diacylglycerol. We previously postulated that at a very low glucose concentration (2.75 mmol/l), CCK-8S induces an increase in DAG production, but not in DAG accumulation [11]. Hence, there is no activation of protein kinase C and thus no second phase of insulin secretion. In islets incubated in 5.5 mmol/l glucose, addition of 10 nmol/l CCK-8S alone also does not induce any second phase of insulin secretion, but when combined with tolbutamide, it does so. A simple interpretation of this interaction is based on observations in other systems in which it has been shown that both an increase in Ca²⁺ influx and an increase in DAG production are necessary to produce a sustained activation of protein kinase C [8]. In the present circumstance (5.5 mmol/l glucose), 10 nmol/l CCK-8S is postulated to cause a small increase in the DAG content of the tissue, but this alone is not sufficient to evoke a significant second phase of insulin secretion. However, when combined with an agent (tolbutamide) that stimulates Ca²⁺ influx, it does bring about a sufficient activation of C-kinase to induce a second phase of insulin secretion. If under these circumstances MOG, an inhibitor of DAG kinase [15], is added (Fig. 2, left), then an increased accumulation of DAG and a further activation of protein kinase C occur leading to an enhanced secretory response. If the same manipulations are performed on islets incubated in the presence of 1 μ mol/l forskolin, then a massive MOG-dependent second phase of insulin secretion is seen (Fig. 2, center). All four signals (MOG, CCK-8S, forskolin, and tolbutamide) are necessary for this amplified response: CCK-8S induces an increase in both Ins 1,4,5P₃ and DAG production, MOG inhibits the further metabolism of DAG, tolbutamide increases Ca²⁺ influx, and forskolin

raises the cAMP concentration in islets. A remarkable fact is that the only natural secretagogue which appears to produce all four of these biochemical changes is high glucose.

It has to be emphasised that the simultaneous presence of moderate glucose level (5.5 mmol/l) controls in an absolute sense the secretory response of the islets to this combination of agonists. If the glucose level is maintained at 2.75 mmol/l, the combination of CCK-8S, forskolin, tolbutamide and MOG induces a weak insulin secretory response. However, at 5.5 mmol/l glucose, a level of glucose which by itself has no significant stimulatory effect on insulin secretion, a dramatically amplified, biphasic insulin secretory response occurs in response to MOG, CCK-8S and tolbutamide. An obvious question to be addressed is the nature of the metabolic change induced by glucose which permits such a dramatic difference in responsiveness. In other words, what effector system changes in response to such a small alteration in the ambient glucose level from 2.75 to 5.5 mmol/l, concentrations which by themselves have no significant insulin secretory effect. It is perhaps more than coincidental that almost complete closure of the ATP-sensitive potassium channel occurs between 2.75–6 mmol/l glucose [19]. It is tempting to speculate that an increase in ATP levels [20], brought about by a small increase in glucose usage rates over this concentration range [21], promotes closure of these channels and poises the system to respond to a secretory signal. This change, however, is insufficient in and of itself to induce insulin secretion. In other words, the permissive effect of moderate glucose on this agonist combination resides in the ability of glucose, mediated by the generation of ATP, to close ATP-regulated potassium channels.

Previous work by Dunlop and Larkins [22] had shown that in neonatal rat islets an increase in glucose concentration leads to the *de novo* synthesis of diacylglycerol. More recently, Peter-Reisch et al. [14] have shown that glucose causes a prompt and sustained increase in the tissue content of DAG by a mechanism other than that occurring via PI hydrolysis. Hence, a second important effect of glucose is that of increasing the size of the DAG pool in islets. The fact that MOG causes a further enhancement of glucose-dependent insulin secretion suggests that even at high glucose and hence presumably high rates of dephosphorylation of PA to DAG [23], there are also high rates of DAG kinase activity. Addition of MOG under this circumstance would lead to higher levels of DAG and higher rates of protein kinase C activity.

From a practical point of view, the present results along with our previous study of CCK-8S-glucose interactions provide evidence that it is possible to regulate, by pharmacological means, the separate phases of insulin secretion. In clinical situations characterised by altered temporal patterns of insulin secretion, it might be possible to manipulate B-cell function to normalise in-

sulin secretory patterns. Finally, it must be noted that the foregoing discussion is based on the concept that MOG acts specifically to inhibit the enzyme DAG kinase and, thereby, cause an increase in the DAG content of B cells. As a result of this change in DAG content, a further activation of protein kinase C is thought to occur. These assumptions remain to be validated in this tissue.

Acknowledgements. This work was supported by grant AM19813 from the National Institutes of Health. The expert secretarial assistance of Ms. N. Canetti is gratefully acknowledged.

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Received: 2 November 1988
and in revised form: 23 March 1989

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