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Dissociation between intracellular calcium mobilization and insulin secretion in isolated rat islets of Langerhans

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The neuropeptide bombesin provoked a dose-dependent stimulation of $^{45}Ca^{2+}$ efflux from pre-loaded islets of Langerhans. This response occurred rapidly, was not sustained and did not depend on the presence of extracellular calcium, suggesting that it resulted from the mobilization of intracellular calcium stores. Under conditions when large increases in $^{45}Ca^{2+}$ efflux were observed, bombesin completely failed to stimulate the rate of insulin secretion. Similar results were also obtained with the muscarinic cholinergic agonist, carbachol. The data suggest that the release of calcium from intracellular pools is not sufficient to induce an increase in insulin secretion in normal islet cells.

Insulin secretion; $^{45}Ca^{2+}$ efflux; Ca^{2+} mobilization; Bombesin; (Islets of Langerhans)

1. INTRODUCTION

Stimulation of insulin secretion from the pancreatic B-cell is a calcium-dependent process [1,2] which can be directly elicited by a rise in the cytosolic free calcium concentration, over the range 0.1–10 μM [3,4]. Studies with pharmacological calcium channel agonists and antagonists have revealed that the B-cell is equipped with voltage-sensitive calcium channels which facilitate the entry of extracellular calcium when the cell membrane is depolarised [5,7]. This mechanism therefore ensures that elevated rates of insulin secretion are sustained during prolonged periods of stimulation.

Recently, it has also been demonstrated that islet cells contain labile pools of intracellular calcium which can be mobilized by inositol (1,4,5)-trisphosphate (IP_3) [8,9]. Since both nutrient and some hormonal stimuli can provoke IP_3 formation in islets [10,11] the idea has emerged that mobilization of intracellular calcium may be an important early component in the initiation of an insulin secretory response [1,2]. However, recent evidence suggests that, like insulin secretion, the generation of IP_3 can also be a calcium-mediated process in islet cells [12]. Thus, the ability of glucose to induce IP_3 formation has been attributed, in part, to an increased rate of calcium influx [13]. This in turn, suggests that, in glucose-stimulated islets, intracellular calcium release induced by IP_3 is unlikely to be a primary response; which casts doubt on its significance for the stimulation of insulin secretion.

In the present study we have directly assessed the potential role of intracellular calcium mobilization in the initiation of insulin secretion. We have used the neuropeptide bombesin which activates inositol lipid hydrolysis and IP_3 formation in a variety of cells [14,15] and has been reported to exert direct effects on insulin secreting cells in culture [16]. The results demonstrate that bombesin elicits significant calcium release from isolated rat islets of Langerhans but that this response is not accompanied by any increase in the rate of insulin secretion. The data suggest that the mobilization of intracellular pools of calcium is not, itself, sufficient to stimulate insulin secretion in normal islet cells.

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2. EXPERIMENTAL

Islets of Langerhans were freshly isolated from male Wistar rats by collagenase digestion [17] for each experiment. The isolation medium was a bicarbonate-buffered saline solution, pH 7.4 [18], containing 4 mM glucose and 2 mM $CaCl_2$. For islet incubations this medium was supplemented with 1 mg/ml bovine serum albumin (fraction V; Wilfred Smith).

$^{45}Ca^{2+}$ efflux experiments were performed as described in [19]. In brief, groups of 100 islets were pre-loaded with $^{45}Ca^{2+}$ for 30 min before washing and transfer to perifusion chambers. They were perifused at a flow rate of 1 ml/min for a stabilization period of 30 min before the start of the experiment, and successive 1 min fractions were then collected to determine the extent of $^{45}Ca^{2+}$ efflux.

The rate of insulin secretion was monitored using both islet perifusion and static incubation conditions. For the latter experiments, groups of 3 isolated islets were incubated for 60 min at 37°C in 500 μl of incubation medium containing test reagents. After this time samples of the medium were removed for measurement of their insulin content by radioimmunoassay.

3. RESULTS AND DISCUSSION

Bombesin activates inositol lipid hydrolysis and formation of the calcium mobilizing second messenger IP_3 in a variety of cells [14,15]. Consistent with the operation of this system in islets, bombesin induced a dose-dependent stimulation of $^{45}Ca^{2+}$ efflux from pre-loaded islets (fig.1). This effect occurred rapidly, being observed within 1 min of stimulation, and was transient, with the rate of $^{45}Ca^{2+}$ efflux returning to basal levels within 10 min. $^{45}Ca^{2+}$ efflux was measurably enhanced by 1 nM bombesin and was maximally stimulated by 100 nM (fig.1). Bombesin also markedly stimulated $^{45}Ca^{2+}$ efflux when islets were perifused with medium containing no added calcium, demonstrating that the response was not caused by exchange or displacement of intracellular calcium by influent calcium, but that it reflected true intracellular calcium mobilization (fig.2).

In agreement with previous data [20–22] exposure of islets to 1 mM carbachol also induced a large efflux of $^{45}Ca^{2+}$ from islets perifused in the absence of calcium (fig.2). These results probably reflect the mobilization of calcium from an endoplasmic reticulum pool in the islet cells [23] mediated by an agonist induced rise in IP_3 [11,24,25].

If intracellular calcium mobilization plays an important role in the initiation of insulin secretion

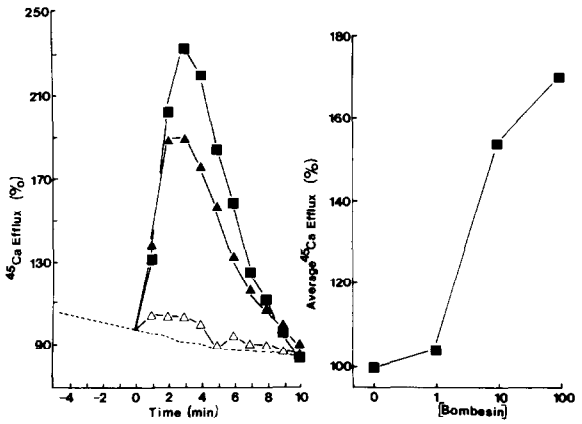

Fig.1. Effect of increasing concentrations of bombesin on $^{45}Ca^{2+}$ efflux from pre-loaded islets of Langerhans. Isolated islets of Langerhans were pre-loaded with $^{45}Ca^{2+}$ and perifused in the presence of 4 mM glucose and 2 mM $CaCl_2$ for 30 min prior to the start of the experiment. (Left panel) Bombesin (Δ, 1 nM; ▲, 10 nM; ■, 100 nM) was introduced ($t=0$) and the rate of $^{45}Ca^{2+}$ efflux measured at 1 min intervals. The control rate is shown by the dotted line. Data are presented as % of the rate averaged over the 5 min preceding addition of bombesin. Results are from a representative experiment. (Right panel) The data were integrated to show the average rate of $^{45}Ca^{2+}$ release over the 10 min following bombesin addition and plotted relative to the control rate (100%).

in islet cells, then it would be expected on the basis of the $^{45}Ca^{2+}$ efflux profiles, that bombesin and carbachol would each have elicited at least a transient increase in insulin secretion; perhaps equivalent to the first phase of the response to glucose. However, at concentrations which caused significant calcium mobilization, neither agent induced any increase in insulin secretion from islets incubated under static conditions (table 1). Furthermore, in perifusion experiments, no significant change in insulin secretion rate could be detected during the time period that $^{45}Ca^{2+}$ efflux was maximally stimulated by bombesin (fig.3). Similar results were also obtained with carbachol (not shown; [25]). This lack of a secretory response was not due to any general deficiency in islet responsiveness since under both static incubation and perifusion conditions, addition of 20 mM glucose elicited a marked increase in insulin secretion (table 1 and fig.3).

One possible explanation for the present results is that the bombesin-sensitive calcium pool that we have monitored is not located in the insulin secreting B-cells, but is confined to another cell type within the islets. We cannot completely ex-

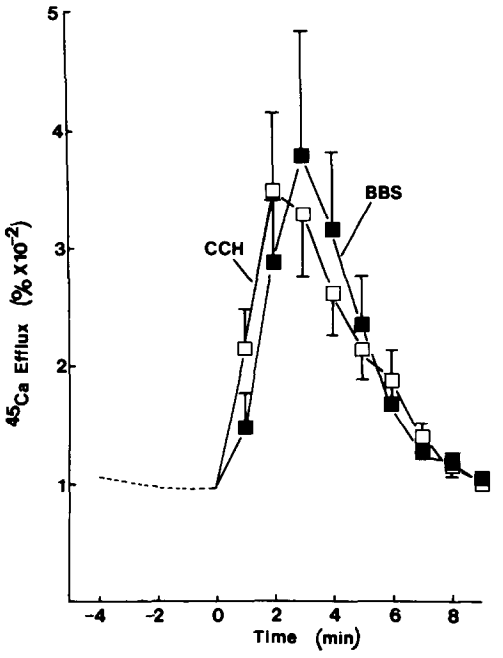

Fig.2. Effect of removal of extracellular calcium on $^{45}Ca^{2+}$ efflux in response to bombesin. Islets were pre-loaded with $^{45}Ca^{2+}$ and perifused in medium containing 4 mM glucose but no added calcium. After 30 min ($t = 0$) either 100 nM bombesin (□) or 1 mM carbachol (■) was introduced and $^{45}Ca^{2+}$ efflux was determined at 1 min intervals. Data are mean values ± SE from 3 perifusions in each case.

clude this possibility although it seems unlikely since B cells comprise approx. 70% of the cell population in rat islets [26], and similar results were also obtained after addition of the muscarinic agonist carbachol, which is in accord with other data [27,28]. Furthermore, bombesin has been shown to interact directly with insulin secreting cells in culture [16,29]. However, in those cells (HIT-T15) bombesin was reported to induce up to 25-fold increases in insulin secretion [16] which is at variance with our results using isolated rat islets. The reasons for this discrepancy are unclear, but it may imply that the intracellular events which control insulin secretion are altered in the clonal cells compared to normal islet B-cells.

The present results should not be taken as evidence against a direct role for calcium in mediating increases in insulin secretion, since this has been well established [1-3,30]. They do, however, suggest that the release of intracellular stored calcium is not, itself, sufficient to induce an increase in insulin secretion rate. We have not

Table 1

Effect of bombesin and carbachol on insulin secretion from islets incubated under static conditions

Incubation conditions			Insulin secretion (ng/islet per h)
[Glucose] (mM)	[Bombesin] (nM)	[Carbachol] (mM)	
4	-	-	0.95 ± 0.09
4	1	-	1.10 ± 0.09[a]
4	10	-	0.86 ± 0.06[a]
4	100	-	0.79 ± 0.09[a]
4	-	1	1.07 ± 0.13[a]
20	-	-	3.88 ± 0.42[b]

[a] Not significantly different from 4 mM glucose alone
[b] $p < 0.001$ relative to 4 mM glucose alone

Islets were incubated in groups of 3 in media containing 2 mM $CaCl_2$ and the test agents shown, for 60 min at 37°C. Samples of the medium were removed and their insulin content measured. Results represent mean data ± SE from 6-12 observations

measured directly cytosolic free calcium concentrations in this study, but it does not seem likely that calcium ions would be released from intracellular sites and then extruded from the cell without also passing through the cytosol. It must be assumed therefore that, following addition of carbachol or bombesin, the resultant mobilization of calcium produced an increase in cytosolic free calcium concentration that was either too small or of insufficient duration to lead to activation of the secretory mechanism.

The present data have important implications for our understanding of the functional significance of the inositol lipid signalling system in islets of Langerhans. It has been established that islet cells contain a phospholipase C which hydrolyses phosphatidylinositol 4,5-bisphosphate (PIP_2) to yield I(1,4,5)P_3 [31], and that this enzyme can be activated by both nutrients and neurotransmitters [10,11]. In the latter case, the response is not dependent on calcium entry and can occur under low calcium conditions [25,32]. However, as we now demonstrate, the resultant calcium release does not lead to increased insulin secretion. Furthermore, recent evidence suggests that much of the increase in IP_3 that occurs in nutrient-stimulated islets, may result from a secondary activation of phospholipase C by influent calcium [12,13]. Thus, in this situation, IP_3 formation is mediated by a prior rise in cytosolic calcium. This

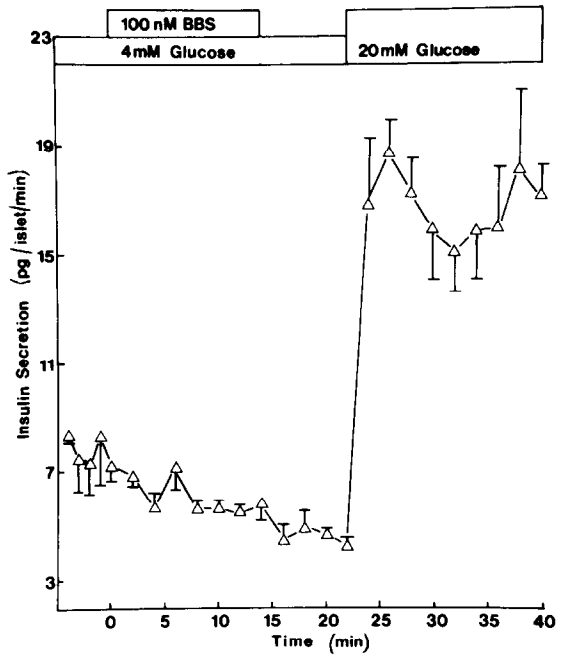

Fig.3. Effect of bombesin on insulin secretion from perifused islets. Isolated islets were perifused with medium containing 4 mM glucose and 2 mM $CaCl_2$ for 30 min. Bombesin was then added and perifusion continued for another 15 min. The bombesin was removed and, 5 min later, 20 mM glucose introduced. Insulin secretion was determined by radioimmunoassay of samples of the perifusion medium. Data represent mean values ± SE from 4 perifusions.

does not accord with a primary role for IP_3-induced calcium release in the initial events that follow glucose stimulation. Indeed, on this basis, intracellular stores of calcium would only be released following prior membrane depolarisation and gating of voltage-dependent calcium channels.

These considerations call into question the perceived role of PIP_2 hydrolysis and IP_3 generation in islets, and suggest that intracellular calcium mobilization does not directly initiate insulin secretion in normal islet B-cells.

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