Bombesin and platelet-derived growth factor stimulate phosphatidylcholine breakdown by a common mechanism

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The two Swiss 3T3 cell mitogens, bombesin and platelet-derived growth factor (PDGF) both stimulate the phosphoinositide C-catalysed hydrolysis of phosphatidylinositol 4,5-bisphosphate (Ptdlns(4,5)P2). Bombesin stimulates rapid generation of inositol 1,4,5-trisphosphate (Ins[1,4,5]P3) in these cells [1], whereas there is a distinct lag before PDGF-stimulated InsP3 generation is observed [2, 3]. Bombesin is believed to stimulate phosphoinositidase C via an interaction with a receptor–G-protein complex [4]. However, recent evidence suggests that PDGF-stimulated Ptdlns(4,5)P2 hydrolysis occurs via tyrosine phosphorylation of PLC-γ with no G-protein involvement [5]. We have recently demonstrated that bombesin stimulates phosphatidylycholine (PtdCho) hydrolysis in Swiss 3T3 cells [6] which may be important in generating a sustained increase in diacylglycerol (DAG) levels and thus protein kinase C activity. PDGF-stimulated PtdCho hydrolysis has been demonstrated in 3T3-L1 cells [7]. Since the two mitogens regulate Ptdlns(4,5)P2 hydrolysis by different mechanisms, we investigated the interrelationship between Ptdlns(4,5)P2 and PtdCho breakdown in response to each agonist.

For assay of PtdCho hydrolysis, Swiss 3T3 cells were labelled with 2 µCi of [methyl-3H]choline chloride in Dulbecco’s modified Eagle’s medium (DMEM) containing 2% (v/v) calf serum for 48 h. After treatment with the agonists, 3H-choline metabolites were separated on Dowex-50W×8 cation-exchange columns according to Cook & Wakelam [6]. Assay of agonist-stimulated increases in Ins(1,4,5)P3 mass was achieved using the competitive binding assay of Palmer et al. [8]. Both PDGF and c-sis were used and gave similar results.

Bombesin (617 nM) stimulated the rapid elevation of Ins(1,4,5)P3, which was maximal at 5 s and rapidly returned to unstimulated levels by 30 s (typical experiment: basal, 3.15 ± 0.31 pmol; stimulated, 33.13 ± 2.65 pmol at 5 s). This response occurred with a concentration required to give 50% of maximal response (EC50) of 5.88 ± 3.66 nm, which is similar to that for bombesin-stimulated DAG production [9] and DNA synthesis [1]. Maximum concentrations of PDGF (30 ng/ml) also stimulated the formation of Ins(1,4,5)P3 in Swiss 3T3 fibroblasts. As expected, the onset of the response was slower than that obtained for bombesin, with a lag time of 10 s before any increase in mass Ins(1,4,5)P3 was observed. This lag period is, however, considerably less than previously reported in studies using [3H]inositol-labelled cells [2, 3]. Furthermore, although the magnitude of the response was smaller than that for bombesin (bombesin, 10–12-fold, n = 8; PDGF, 2.5–3.5-fold at 20 s, n = 3), the increased levels were maintained for up to 5 min.

We have previously shown that bombesin stimulates PtdCho hydrolysis subsequent to Ins(1,4,5)P3 production. Since PDGF stimulates Ins(1,4,5)P3 production by a mechanism fundamentally different to bombesin, the effects of PDGF upon PtdCho metabolism were investigated. PDGF-stimulated increases in choline were apparent between 10 and 20 s and peaked at 30 s (basal, 249 ± 38 d.p.m.; 30 s, 461 ± 100; data from a single typical experiment) after which basal and stimulated values paralleled each other for the remainder of the time course. The generation of choline preceded any minor changes in phosphocholine suggesting that PDGF, like bombesin, stimulates the hydrolysis of PtdCho by a phospholipase D mechanism. Increases in choline generation did not precede increases in Ins(1,4,5)P3.

PDGF-stimulated production of total [3H]inositol phosphates (IP) and choline exhibited similar EC50 values ([3H]IP response, 3–10 ng/ml; [3H]choline, 1–4 ng/ml; n = 3 and 4, respectively) suggesting interaction with a single receptor mediated both responses.

It has previously been shown that 12-O-tetradecanoylphorbol 13-acetate (TPA) stimulates an increase in choline production [10] suggesting a role for protein kinase C in PtdCho hydrolysis. To test this we examined bombesin- and PDGF-stimulated choline generation in cells which had been pretreated with 400 nm TPA for 48 h in order to down-regulate protein kinase C activity [11]. The ability of both bombesin and PDGF to stimulate [3H]choline generation was completely abolished in these down-regulated cells (Fig. 1). These results are consistent with the hypothesis that both bombesin and PDGF stimulate PtdCho hydrolysis through the prior activation of protein kinase C, and, while the mechanism for this differs for the two agonists, the effect upon PtdCho metabolism is similar. Therefore, PtdCho hydrolysis may be a consequence of both forms of mitogenic stimulation and may contribute to the generation of potent messenger molecules such as phosphatidic acid and DAG.

Abbreviations used: PDGF, platelet-derived growth factor; Ptdlns(4,5)P2, phosphatidylinositol 4,5-bisphosphate; Ins(1,4,5)P3, inositol 1,4,5-trisphosphate; PtdCho, phosphatidylcholine; DAG, diacylglycerol; IP, inositol phosphate; TPA, 12-O-tetradecanoylphorbol 13-acetate; DMEM, Dulbecco’s modified Eagle’s medium.
Evidence for the presence of low molecular mass GTP-binding proteins in rat islets of Langerhans

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Guanine nucleotide-binding (G) proteins have been implicated in regulation of the secretory activity of endocrine cells, including the pancreatic β-cell [1]. These proteins have not been well-characterized in the cells of isolated islets of Langerhans, but toxin-mediated [32P]labelling experiments have revealed the presence of both Gα [2] and a member of the G family [3, 4]. Immunocytochemical analysis has also indicated the presence of Gαi in islet cells [5]. These proteins are all of similar molecular size and evidence suggests that they are involved in the coupling of cell surface receptors to intracellular effectors.

Recently, it has emerged that cells also contain another class of G-proteins which are of lower molecular mass than those involved in receptor-signalling and which may play important roles in control of intracellular processing events associated with secretion [6, 7]. In the present study we have used [α-32P]GTP binding to investigate whether such proteins are present in the cells of isolated islets of Langerhans.

Islets were isolated from Wistar rats by collagenase digestion. Groups of 400-600 islets were ultrasonically disrupted in 50 mM-Tris buffer, pH 7.4, before dilution with buffer containing SDS and incubation for 5 min at 100°C. The proteins were then separated by electrophoresis on SDS/polyacrylamide mini-gels (12% w/v) and transferred to nitrocellulose. Protein transfer was confirmed by staining with Ponceau Red.

To label G-proteins, the nitrocellulose strips were incubated at 15-20°C for 30 min in buffer containing 50 mM-Tris, pH 7.4, 0.1% (v/v) Tween-20, and [α-32P]GTP (0.6 nm). The strips were washed three times in the same buffer without GTP, and air-dried. Radiolabelled proteins were identified by autoradiography and their molecular masses estimated by reference to marker proteins. Examination of the autoradiographs obtained in GTP labelling experiments revealed the presence of several radiolabelled proteins in the molecular mass range 14-28 kDa (Fig. 1). Comparison of autoradiograms from several preparations suggested that at least six separate G-proteins were present having molecular masses of 14, 18, 19, 23, 26 and 28 kDa. Consistent with data from other cell types [6], no radiolabelled bands were found in the region of the gel expected to contain Gαs, Gαi and Gαq, suggesting that these proteins do not retain their GTP-binding capacity following SDS treatment. However, a further 32P-labelled band corresponding to a protein with a molecular mass of 49 kDa was consistently observed in the islet preparations (Fig. 1).

Labelling of the lower molecular mass G-proteins was reduced by addition of the GTP analogue guanosine 5′-[γ-thio]triphosphate (GTP[S]) to the incubation buffer. This effect was dose dependent and labelling was completely inhibited in the presence of 10 μM-GTP[S]. ATP and CTP were much less effective at displacing [α-32P]GTP suggesting that the labelled proteins bind GTP with higher affinity than other nucleotides.

These results demonstrate the presence of several novel G-proteins in rat islets, but further studies are required to elucidate the subcellular distribution and the function of these molecules.

Abbreviation used: GTP[S], guanosine 5′-[γ-thio]triphosphate.

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Fig. 1. Autoradiographic visualization of 32P-labelled G-proteins in rat islets of Langerhans

Proteins from rat islets (approximately 15 μg lane) were separated by SDS/polyacrylamide-gel electrophoresis and transferred to nitrocellulose. The resultant blots were incubated with [α-32P]GTP to label the G-proteins, then dried and exposed to X-ray film for 18-24 h at −20°C. The molecular masses of labelled proteins were determined by comparison with marker proteins run in parallel with the islet preparations. (The 14 kDa protein is not visible in this example.)

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