Glucagon receptor causes glucagon-dependent activation of Erk1/2 in H22 stable cell lines

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Introduction

Glucagon plays an important role in the regulation of glucose homeostasis by stimulation of glucose output in response to low blood glucose levels and by the potentiation of glucose-induced insulin secretion. These effects are mediated by the glucagon receptor, a member of the Class II group of receptors within a superfamily of seven transmembrane-spanning receptors that couple to heterotrimeric G proteins (GPCRs). The Class II receptors share few common features with other GPCRs, but nevertheless couple to the same set of G proteins which activate multiple signaling pathways.

Recent studies have demonstrated that subunits of heterotrimeric G proteins modulate mitogen-activated protein kinases (MAP kinases), upon ligand activation of G protein-coupled receptors. The MAP kinases, which include Erk1/2 (p44/p42 MAPK), integrate information from several signaling pathways to initiate cellular differentiation, transformation, and proliferation. Erk1/2 are known to be activated by tyrosine kinase receptors (TRK) which possess intrinsic tyrosine kinase activity, via pathways involving a series of protein-protein interactions and phosphorylation by other kinases. However, the mechanism by which ligand interaction with its G protein-coupled receptor at the cell surface results in the potentiation of MAP kinase activity is still undefined.

Glucagon has been reported to affect MAP kinases leading to a decrease in activity in different cell types [1,2]. In this report, we show that glucagon increased MAP kinase activity and induced a dose-dependent phosphorylation of Erk1/2 in HEK 293 (H22) cells stably expressing the rat glucagon receptor. To sort out the components of the signaling pathway leading from initial glucagon recognition and binding to its receptor at the cell surface, to the phosphorylation of Erk1/2 in H22 cells, we examined the functional coupling of the glucagon receptor and endogenous GPCRs with G proteins in H22 cells, using assays for hormone-dependent production of cAMP, inositol phosphates, and calcium flux. MAP kinase activation was assessed by immunoblot analysis of H22 cell lysates with anti-phospho-p44/42 MAP kinase polyclonal antibody.

Results and Discussion

Stable expression of the glucagon receptor in H22 cells was demonstrated by the ability to generate hormone-dependent increases in cAMP and intracellular calcium, with potencies similar to those of native receptor. These effects are known to be mediated by coupling primarily to G_s [3]. In H22 cells, increase in inositol phosphates induced by glucagon was negligible compared to the response generated by G_q-mediated carbachol stimulation. Treatment of H22 cells with glucagon increased phosphorylation of Erk1/2 in a dose dependent manner (Fig. 1A) with an EC_{50} of 160±10 pM (Fig. 1B). Carbachol also increased Erk1/2 phosphorylation, but somatostatin had no effect, which indicated that G_q-coupled muscarinic receptors activated MAP kinase but G_i-coupled somatostatin receptors did not. These observations showed that while carbachol utilizes G_q for both MAPK

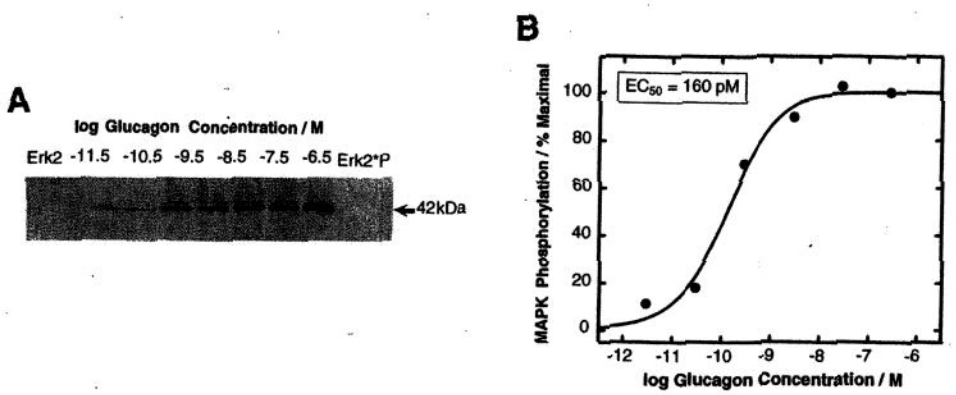

Fig. 1. Glucagon-induced phosphorylation of Erk1/2 in H22 cells. A) Immunoblot with anti-phospho-p44/42MAPK. B). Plot of band intensities vs. log glucagon concentration.

stimulation and IP_3 production, the glucagon receptor does not activate G_q to increase Erk1/2 phosphorylation in these cells. H22 cells were incubated with pertussis toxin (PTX) to test whether G_i and G_o were involved in glucagon-dependent MAPK activation. Erk1/2 phosphorylation was not attenuated by treatment with pertussis toxin when cells were stimulated with glucagon, carbachol, EGF, or isoproterenol. But PTX treatment eliminated G_i-coupled somatostatin receptor-mediated calcium increase in H22 cells assayed in parallel, which indicated that in H22 cells, glucagon activation of MAPK was not mediated by a PTX-sensitive G protein such as G_i or G_o. In addition, the calcium chelator BAPTA did not reduce glucagon activation of Erk1/2, indicating that the glucagon-stimulated increase in $[Ca^{+2}]_i$ could not account for its effects on MAP kinase. The cAMP-dependent protein kinase (PKA)-responsive MEK activator B-Raf is known to be present in these cells. Treatment of these cells with the MEK inhibitor PD 98059 blocked glucagon activation of Erk1/2.

We have shown that glucagon induced a dose-dependent phosphorylation of the MAP kinase isoforms Erk1/2 in H22 cells. The activity did not involve phosphoinositide turnover, and was not inhibited by pretreatment with either pertussis toxin or a calcium chelator. This led to the conclusion that glucagon-dependent activation of MAP kinases in H22 cells is mediated predominantly by G_s, and likely proceeds via a cAMP-dependent pathway involving PKA, B-Raf, and MEK.

Acknowledgments

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