ELSEVIER

European Journal of Pharmacology 389 (2000) 125–130

ejp

www.elsevier.nl/locate/ejphar

Activation of multiple mitogen-activated protein kinases by recombinant calcitonin gene-related peptide receptor

Narayanan Parameswaran [a], Jyoti Disa [b], William S. Spielman [a], David P. Brooks [b], Ponnal Nambi [b], Nambi Aiyar [b,*]

[a] *Department of Physiology, Michigan State University, East Lansing, MI 48824, USA*

[b] *Department of CV Pharmacology, SmithKline Beecham Pharmaceuticals, UW2510, 709 Swedeland Road, Box 1539, King of Prussia, Philadelphia, PA 19406-0939, USA*

Received 2 November 1999; accepted 3 December 1999

Abstract

Calcitonin gene-related peptide is a 37-amino-acid neuropeptide and a potent vasodilator. Although calcitonin gene-related peptide has been shown to have a number of effects in a variety of systems, the mechanisms of action and the intracellular signaling pathways, especially the regulation of mitogen-activated protien kinase (MAPK) pathway, is not known. In the present study we investigated the role of calcitonin gene-related peptide in the regulation of MAPKs in human embryonic kidney (HEK) 293 cells stably transfected with a recombinant porcine calcitonin gene-related peptide-1 receptor. Calcitonin gene-related peptide caused a significant dose-dependent increase in cAMP response and the effect was inhibited by calcitonin gene-related peptide(8–37), the calcitonin gene-related peptide-receptor antagonist. Calcitonin gene-related peptide also caused a time- and concentration-dependent increase in extracellular signal-regulated kinase (ERK) and P38 mitogen-activated protein kinase (P38 MAPK) activities, with apparently no significant change in cjun-N-terminal kinase (JNK) activity. Forskolin, a direct activator of adenylyl cyclase also stimulated ERK and P38 activities in these cells suggesting the invovement of cAMP in this process. Calcitonin gene-related peptide-stimulated ERK and P38 MAPK activities were inhibited significantly by calcitonin gene-related peptide receptor antagonist, calcitonin gene-related peptide-(8–37) suggesting the involvement of calcitonin gene-related peptide-1 receptor. Preincubation of the cells with the cAMP-dependent protein kinase inhibitor, H89 [{*N*-[2-((*p*-bromocinnamyl)amino)ethyl]-5-isoquinolinesulfonamide, hydrochloride}] inhibited calcitonin gene-related peptide-mediated activation of ERK and p38 kinases. On the other hand, preincubation of the cells with wortmannin {[1*S*-(1α,6bα,9aβ,11α, 11bβ)]-11-(acetyloxy)-1,6b,7,8,9a,10,11,11b-octahydro-1-(methoxymethyl)-9a,11b-dimethyl-3*H*-furo[4,3,2-*de*]indeno[4,5-*h*]-2-benzopyran-3,6,9-trione}, a PI3-kinase inhibitor, attenuated only calcitonin gene-related peptide-induced ERK and not P38 MAPK activation. Thus, these data suggest that activation of ERK by calcitonin gene-related peptide involves a H89-sensitive protein kinase A and a wortmannin-sensitive PI3-kinase while activation of p38 MAPK by calcitonin gene-related peptide involves only the H89 sensitive pathway and is independent of PI3 kinase. This also suggests that although both ERK and P38 can be activated by protein kinase A, the distal signaling components to protein kinase A in the activation of these two kinases (ERK and P38) are different.

Keywords: CGRP (calcitonin gene-related peptide); ERK (extracellular signal-regulated kinase); P38; Forskolin; HEK 293 cell

1. Introduction

Mitogen-activated protein kinases (MAPK's) are proline directed serine/threonine kinases that are activated in response to a variety of extracellular stimuli. There are five distinct groups of MAPK's in mammalian cells. At present, the best characterized are extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and P38 mitogen-activated protein kinase (P38 MAPK) (Denhardt, 1996). Activation of growth factor receptors, G-protein-coupled receptors, inflammatory cytokine receptors and induction of stress by ultraviolet light or high osmolarity have been shown to activate one or more of these MAPK pathways resulting in varied biological responses including cell proliferation, cell death, matrix production, secretion etc. Each of these pathways prototypically con-

* Corresponding author. Tel.: +1-610-270-5004; fax: +1-610-270-5080.

E-mail address: nambi_aiyar-1@sbphrd.com (N. Aiyar).

0014-2999/00/$ - see front matter
PII: S0014-2999(99)00874-2

sists of a small G-protein (e.g., ras) activating a mitogen-activated protein kinase kinase kinase (MAPKKK) or MEKK (e.g., raf). Activated MEKK phosphorylates a mitogen-activated protein kinase kinase (MAPKK or MEK) (e.g., MEK1/2) and activates it. MEK is a dual function kinase that can phosphorylate threonine and tyrosine residues. When activated, MEK phosphorylates and activates a MAPK (e.g., ERK, JNK, P38 MAPK) (Denhardt, 1996; Neary, 1997; Robinson and Cobb, 1997). Activation of MAPK plays important roles in integrating the effects of extracellular signals on multiple cellular functions, including differentiation, proliferation and transformation.

Calcitonin gene-related peptide is a neuropeptide with diverse biological effects including potent vasodilator activity (DeFeudis, 1992; Poyner, 1995; Brain and Cambridge, 1996). The receptor for calcitonin gene-related peptide has been cloned from various species including human, rat and pig (Wimalawansa, 1996). The receptor displays 7 transmembrane domains and shows significant homology with a subfamily of G-protein-coupled receptors that includes calcitonin, vasoactive intestinal peptide, secretin, glucagon and corticotropin releasing factor. Messenger RNA encoding the calcitonin gene-related peptide receptor is expressed in relatively high levels in human heart and lung (Wimalawansa, 1996). Expression of the recombinant calcitonin gene-related peptide receptor in stably transfected human embryonic kidney (HEK) 293 cells has enabled study of their ligand-stimulated signal transduction. Like other receptors of this family, the recombinant calcitonin gene-related peptide receptor was shown to be capable of activating adenylyl cyclase (Aiyar et al., 1996), as well as rapid increase of intracellular Ca^{2+} through the activation of phospholipase C (Aiyar et al., 1999). The purpose of this study was to delineate the signaling events downstream of cAMP in 293 cells by calcitonin gene-related peptide. Here we show that the recombinant calcitonin gene-related peptide receptor expressed in HEK-293 cells respond to calcitonin gene-related peptide stimulation with increased ERK and P38 activity and that this effect is mediated by the calcitonin gene-related peptide type 1 receptor in a cAMP-dependent manner.

2. Materials and methods

2.1. Materials

Human calcitonin gene-related peptide α (hα-calcitonin gene-related peptide) and human α-calcitonin gene-related peptide-(8–37) (hα-calcitonin gene-related peptide-(8–37)) were purchased from Bachem Biochemicals (King of Prussia, PA). Myelin basic protein, from Sigma (St. Louis). Polyclonal anti-ERK2, anti-P38 MAPK and anti-JNK1 antibodies were purchased from Santa Cruz laboratories (Santa Cruz, CA). GST-cJUN was purchased from Alexis Biochemicals (San Diego, CA). DMEM, fetal bovine serum, penicillin and streptomycin were from Gibco (Grand Island, NY). All other reagents were of high quality available.

2.2. Cell culture

HEK-293 cells, stably transfected with the porcine calcitonin gene-related peptide receptor cDNA (HEK-293-PR), were maintained in minimum essential medium (MEM) supplemented with 10% fetal calf serum in Falcon T-150 flasks at 37°C in 95% air–5% CO2 (Aiyar et al., 1996).

2.3. cAMP assays

HEK 293 cells stably expressing porcine calcitonin gene-related peptide receptor-1 were plated at 2.5×10^3 cells/well in six-well plates. On day 4, the medium was aspirated and the cells were washed with 1 ml of Dulbecco's phosphate-buffered saline containing 0.5 mM isobutylmethylxanthine for 10 min at room temperature. The cells were treated with various concentrations of calcitonin gene-related peptide or the antagonist at 37°C for 10 min. The reaction was stopped by addition of 100 μl of 100% ice-cold trichloroacetic acid to each well, and cAMP in each well was measured following the radioimmunoassay protocol as described (Advanced Magnetics). Each experiment was performed in triplicate and repeated two to three times with different passages of cells.

2.4. Kinase assays

Cells were plated in p100 plates and were serum starved overnight on reaching confluency. The agonist solutions were prepared in the growth media without serum. Cells were treated with the agonists for the indicated time points. The cell lysates were prepared as described (Bogoyevitch et al., 1995; Li et al., 1995). In the meantime specific antibodies (10 μg/reaction) were incubated with protein A agarose (Gibco) for 30 min at room temperature. After normalizing for protein concentration, the cell lysates were incubated with the specific antibody agarose conjugate for 2 h at 4°C with constant shaking. The kinase assays were done after washing the immunoprecipitate three times with HNTG (20 mM HEPES pH 7.5, 150 mM NaCl, 0.1% Triton X-100, 10% glycerol) buffer and two times with kinase buffer (50 mM Tris–HCl, 100 mM NaCl, 10 mM $MnCl_2$ and 0.1 mM sodium ortho vanadate). The functional assay was done in the presence of 50 μM ATP, 5 μCi 32P-ATP, 10 μg of specific substrate (myelin basic protein for ERK and P38 MAPK, and glutathione *S*-transferase-c-jun for JNK), and the immunoprecipitate. The reactions were performed at 30°C for 15 min and then

stopped with sodium dodecyl sulphate buffer. The samples were electrophoresed in 12% polyacrylamide gel with proper molecular weight standards. The gels were dried and subjected to phosphoimager plates. The bands were quantitated using imagequant program. Results are expressed as percent change from the basal of the relative densitometric units or phosphoimager units. The specificity of the antibodies and the immunoprecipitation protocols were tested for their respective kinases by Western blot analysis in our preliminary experiments.

3. Results

3.1. Effect of calcitonin gene-related peptide on cAMP levels in HEK 293 cells stably transfected with recombinant porcine calcitonin gene-related peptide-1 receptor

The threshold, half-maximal, and maximal concentrations of calcitonin gene-related peptide required to stimulate cAMP accumulation in these cells were 0.1, 0.9, and 10 nM, respectively. calcitonin gene-related peptide-(8–37) at 300 nM effectively shifted the calcitonin gene-related peptide dose–response curve to the right in a parallel manner indicating competitive inhibition (Fig. 1).

3.2. Effect of calcitonin gene-related peptide on ERK and P38 MAPK

Calcitonin gene-related peptide at 100 nM caused a time-dependent increase in ERK2 and P38 MAPK activities without any change in JNK1 activity in HEK 293 cells stably transfected with recombinant porcine calcitonin gene-related peptide-1 receptor. ERK2 and P38 activities remained elevated at 15 min after calcitonin gene-related peptide treatment, and came back to basal levels by 60 min of treatment (Fig. 2). Because calcitonin gene-related peptide did not affect JNK1 activity within the time frame tested even with 100 nM calcitonin gene-related peptide, further studies were done only for calcitonin gene-related peptide-activated ERK2 and P38 MAPK. Calcitonin gene-related peptide also caused a concentration-dependent increase in ERK2 and P38 MAPK activities (Fig. 3). The maximum stimulation of ERK2 activity was 176.4 ± 59.8% over basal, which was obtained with 10 nM calcitonin gene-related peptide, whereas the maximum stimulation of P38 was about 97.3 ± 27% over basal. To determine whether calcitonin gene-related peptide-stimulated ERK2 and p38 activities in HEK-293 cells is mediated via calcitonin gene-related peptide receptor, the effect of calcitonin gene-related peptide receptor antagonist was tested. Pretreatment of the cells with calcitonin gene-related peptide-

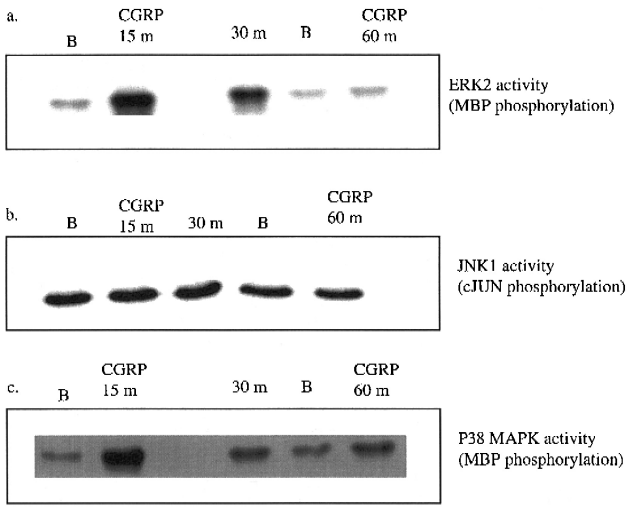

Fig. 2. Representative autoradiograms showing the effect of calcitonin gene-related peptide (CGRP) on (a) ERK2, (b) JNK1, and (c) P38 MAPK activity at 15, 30 and 60 min of treatment in HEK 293 cells expressing CGRP-1 receptor. The kinase activities were measured with the specific kinases, which were immunoprecipitated using specific antibodies, in the presence of myelin basic protein (MBP) for ERK and P38, and cJun for JNK as substrates. The degree of phosphorylation of the substrate by the immunoprecipitated kinase indicates the degree of activity of the respective kinases. The experiment was repeated at least two more times.

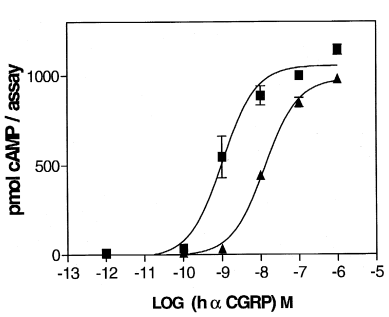

Fig. 1. Concentration-dependent effect of calcitonin gene-related peptide (CGRP) (dark squares) on cAMP response in HEK 293 cells stably expressing porcine calcitonin gene-related peptide-1 receptor (CGRP-1 receptor). CGRP-(8–37) (dark triangles) (at 300 nM) significantly shifted the curve to the right. Experiments were done in triplicates. A representative experiment is shown and each point represents the mean ± S.E. of the triplicate from one experiment. Experiments were repeated at least three times.

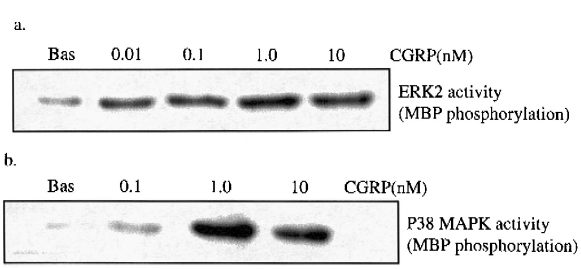

Fig. 3. Representative autoradiograms showing the effect of different concentrations of calcitonin gene-related peptide (CGRP) on (a) ERK2 and (b) P38 MAPK activity in HEK 293 cells expressing CGRP-1 receptor. Kinase activities were done as in Materials and Methods. The experiment was repeated at least three times.

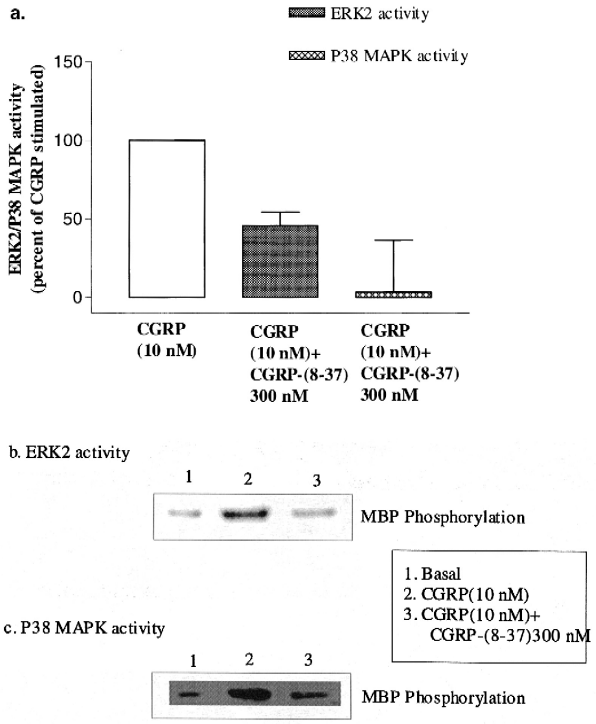

Fig. 4. (a) Effect of calcitonin gene-related peptide-(8–37) (CGRP-(8–37), the CGRP1 receptor antagonist on CGRP-stimulated ERK and P38 activities in HEK 293 cells expressing CGRP-1 receptor (results are mean ± S.E from three independent experiments). A representative autoradiogram is shown in (b) for ERK activity and in (c) for P38 activity. By itself the antagonist did not have any effect on the basal kinase activities.

(8–37) (300 nM) significantly blocked calcitonin gene-related peptide-mediated ERK2 and p38 activation (Fig. 4) suggesting that calcitonin gene-related peptide activation of ERK2 and p38 are mediated via calcitonin gene-related peptide type1 receptor. To further elucidate the mechanisms involved in calcitonin gene-related peptide-mediated ERK2 and p38 activation, cells were treated with different pharmacological inhibitors. As observed for endogenously expressed calcitonin gene-related peptide receptors, recombinant calcitonin gene-related peptide receptor in 293 HEK cells is also coupled to the activation of adenylyl cyclase through G_s protein (Aiyar et al., 1996). In order to identify the involvement of cAMP in calcitonin gene-related peptide-mediated activation of ERK2 and p38 MAPK, we employed forskolin which is capable of increasing cAMP levels in these cells. Forskolin is a direct activator of adenylate cyclase and increases cAMP to very high levels in 293 HEK cells. Forskolin increased ERK2 (7.2 ± 2.9-fold over basal) and P38 MAPK (2.1 ± 0.4-fold over basal) activities significantly above basal levels (Fig. 5), suggesting that cAMP-mediated pathway can lead to the activation of ERK2 and P38 MAPK (Fig. 5) in HEK 293 cells stably transfected with a recombinant calcitonin gene-related peptide receptor.

3.3. Effect of H89 and wortmannin on calcitonin gene-related peptide-stimulated ERK and P38 MAPK activities

In order to identify some of the proximal signaling molecules of calcitonin gene-related peptide-mediated ERK2 and P38 MAPK activation and also to study the mechanism of activation of these two kinases, we have used some selective tools such as a protein kinase-A inhibitor H89 [{*N*-[2-((*p*-bromocinnamyl)amino)ethyl]-5-isoquinolinesulfonamide, hydrochloride}] (Daaka et al., 1997). cAMP-dependent protein kinase is activated by cAMP via a G_s-coupled calcitonin gene-related peptide 1 receptor. The use of this inhibitor was essentially to understand the role, if any, of cAMP-dependent protein kinase molecules in calcitonin gene-related peptide-stimulated ERK and P38. H89 at 20 μM inhibited both calcitonin gene-related peptide-stimulated ERK2 and P38 MAPK activity, at least partially (Fig 6). Furthermore, we also tested for the presence of a wortmannin {[1*S*-(1α,6bα, aβ,11α,11bβ)]-11-(acetyloxy)-1,6b,7,8,9a,10,11,11b-octahydro-1-(methoxymethyl)-9a,11b-dimethyl-3*H*-furo[4,3,2-*de*]indeno[4,5-*h*]-2-benzopyran-3,6,9-trione}-sensitive kinase (possibly PI3 kinase) in the signaling cascade stimulated by calcitonin gene-related peptide, since PI3 kinase activation has been shown to be involved in some of the G-protein-coupled receptor-mediated signaling pathways and biological responses (Rane et al., 1997; Yamauchi et al., 1997; Gutkind, 1998; Lopez-Ilasaca et al., 1998).

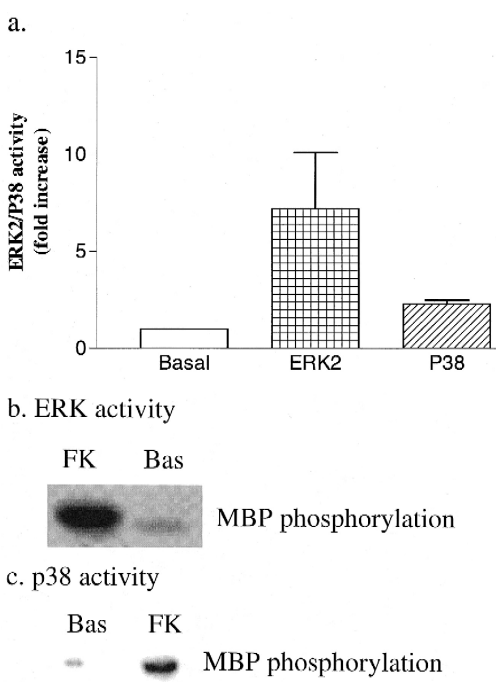

Fig. 5. (a) Effect of forskolin (10 μM) on ERK2 and p38 MAPK activities in HEK 293 cells (Results are mean ± S.E from 3 independent experiments). A representative autoradiogram is shown in (b) for ERK activity and (c) for p38 MAPK activity, indicated by the degree of phosphorylation of myelin basic protein (MBP) by the immunoprecipitated kinases.

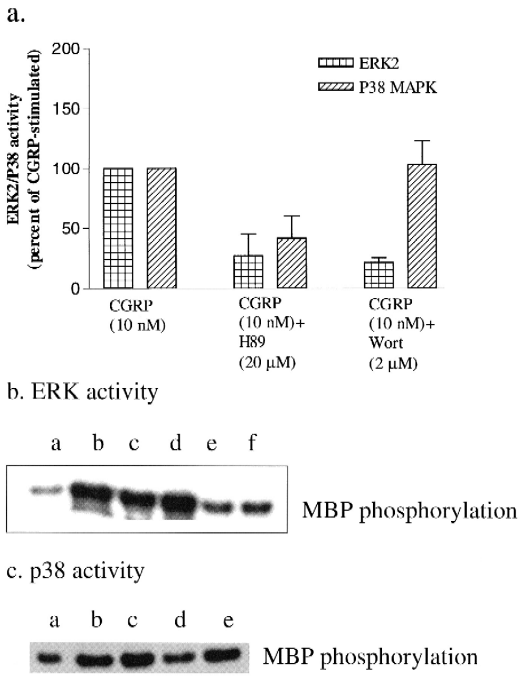

Fig. 6. (a) Effect of H89 (protein kinase A inhibitor) (results are mean ± S.E from three independent experiments), and wortmannin (phosphatidyl inositol 3-kinase inhibitor) (results are mean ± S.E from four independent experiments) on CGRP-stimulated ERK and p38 MAPK activities in HEK293 cells stably expressing CGRP-1 receptor. The inhibitors by themselves did not affect the basal activities at the concentration used. A representative autoradiogram is shown in (b) indicating the ERK activity, and (c) indicating p38 MAPK activity, shown by the degree of phosphorylation of MBP by the immunoprecipitated ERK and p38 respectively.(b) a, Basal; b and c, CGRP (10 nM); d, CGRP + H89 (2 μM); e, CGRP + H89 (20 μM); f, CGRP + wortmannin (2 μM).(c) a, Basal; b, CGRP (10 nM); c, CGRP + H89 (2 μM); d, CGRP + H89 (20 μM); e, CGRP + wortmannin (2 μM).

Wortmannin inhibited only the ERK activity while it did not affect P38 activity stimulated by calcitonin gene-related peptide (Fig. 6). The results with the above inhibitors indicate that ERK2 and P38 have different proximal signaling components, probably distal to cAMP-dependent protein kinase activation.

4. Discussion

Although the second messenger system of calcitonin gene-related peptide receptor stimulation has been characterized quite well, the distal signaling components beyond the second messenger generation is not currently known. Understanding the different signaling components that regulate different biological responses in cells will help us target signaling pathways for drug development. Thus, examining the signaling systems stimulated by calcitonin gene-related peptide will give us better understanding of the complexity of actions calcitonin gene-related peptide. Moreover by examining the different signaling pathways and delineating them for example, one can selectively target one biological response of calcitonin gene-related peptide and not the other.

Calcitonin gene-related peptide acts through a G_s-coupled receptor in HEK 293 cells transfected stably with a recombinant porcine calcitonin gene-related peptide1 receptor resulting in the activation of adenylyl cyclase (Aiyar et al., 1996). Here we show for the first time that, calcitonin gene-related peptide causes a time- and concentration-dependent increase in ERK2 and P38 MAPK activities in HEK 293 cells. The fact that calcitonin gene-related peptide-(8–37) inhibited both these activities indicates that these responses are mediated through the recombinant calcitonin gene-related peptide-1 receptor. Surprisingly both these kinase activities were also elevated by forskolin, a direct activator of adenylate cyclase. These results indicate that elevation of cAMP (by forskolin) can increase ERK2 and P38 MAPK activities individually. Although H89, a potent protein kinase-A inhibitor inhibited calcitonin gene-related peptide-stimulated ERK and P38 activities in these cells, it does not preclude the involvement of protein kinase-A-independent signaling components, such as Ca^{2+} and G-βγ subunits.

Activation of MAPK pathways by G-protein coupled receptor is complicated because of the diversity in proximal signaling components involved. In HEK 293 cells, activation of β-adrenoceptor was shown to result in protein kinase-A-mediated phosphorylation of G_s-coupled β-adrenoceptor resulting in decreased receptor-coupling efficiency to G_s, and increased coupling efficiency to Gi. The βγ subunit of the Gi was then shown to actually be involved in the phosphorylation of MAPK through a series of signaling molecules. Activation of MAPK phosphorylation by β-adrenoceptor was also shown to be pertussis-toxin sensitive further supporting the notion that Gi was involved (Daaka et al., 1997, Zou et al., 1999). In the present study, calcitonin gene-related peptide-stimulated increase in ERK or P38 is not mediated through a switching mechanism to Gi because pertussis toxin did not affect calcitonin gene-related peptide-stimulated ERK or P38 MAPK activity (data not shown). In HEK 293 cells, activation of P38 MAPK by m2 muscarinic receptor was also found to be mediated via G-βγ subunits (Yamauchi et al., 1997). In that study transfection with αs did not affect P38 MAPK activity while transfection with αq and βγ subunits resulted in the activation of P38 MAPK. In our preparations, forskolin a direct activator of adenylate cyclase by itself was able to stimulate P38 MAPK activity. Recently in SK-N-MC cells (human neuroblastoma), dopamine was shown to elevate JNK and P38 MAPK through a cAMP-dependent pathway (Zhen et al., 1998). Our present results using H89, a potent protein kinase-A inhibitor indicates that calcitonin gene-related peptide-stimulated ERK and P38 MAPK activities are at least partly dependent on the activation of cAMP-dependent protein kinase. Although there is not enough evidence in the literature on the mechanism of protein kinase-A-depen-

dent activation of P38 MAPK, protein kinase-A-mediated activation of P38 MAPK via phosphorylation of glial maturation factor has been reported (Lim and Zaheer, 1996).

To identify the proximal signaling components of ERK and P38 activation by calcitonin gene-related peptide, we have used wortmannin, selective inhibitor that inhibits PI3 kinase specifically. Wortmannin is a fungal metabolite and a potent inhibitor of PI3 kinase (Ferby et al., 1994). The results of our experiments using this inhibitor on calcitonin gene-related peptide-stimulated ERK and P38 activities indicates that the two pathways are differentially regulated. That is, ERK activation is dependent on a wortmannin-sensitive kinase whereas P38 activation is not dependent on wortmannin, suggesting different pathways for ERK and P38 activation, possibly downstream of protein kinase A.

The differential regulation of MAPK pathways by calcitonin gene-related peptide that we see in our study leads to a number of questions. Calcitonin gene-related peptide has been shown to have a variety of biological effects (Wimalawansa, 1996). Whether these different effects are mediated via these specific signaling pathways would be the focus of future studies. Delineating these pathways in terms of the biological response will help us selectively target these pathways for therapy.

Durham and Russo (1998) recently showed that the activation of the enhancer for calcitonin gene-related peptide is under the control of mitogen-activated protein kinase pathway. If calcitonin gene-related peptide is able to activate this pathway in cell types where calcitonin gene-related peptide gene transcription is important, it could result in a positive feed back role by calcitonin gene-related itself. Furthermore, it would also be interesting to see if calcitonin gene-related peptide-induced apoptosis in cardiomyocytes or thymocytes involves P38 MAPK activation (Sakuta et al., 1996).

Thus, we show for the first time that calcitonin gene-related peptide causes a time- and concentration-dependent increase in ERK and P38 activities through the recombinant porcine calcitonin gene-related peptide-1 receptor in HEK 293 cells. Our results also indicate that while calcitonin gene-related peptide-stimulated ERK and P38 activities are at least partly through activation of protein kinase-A, only calcitonin gene-related peptide-stimulated ERK activity is sensitive to wortmannin. P38 MAPK activated by calcitonin gene-related peptide in 293 cells is not sensitive to wortmannin. Future studies will focus on the implications of calcitonin gene-related peptide-mediated activation of ERK and P38 and further define the regulatory components that mediate their activation and the biological responses involved.

References

Aiyar, N., Disa, J., Stadel, J.M., Lysko, P.G., 1999. Calcitonin gene-related peptide receptor independently stimulates 3′,5′-cyclic adenosine monophosphate and Ca^{2+} signaling pathways. Mol. Cell. Biochem. 197, 179–185.

Aiyar, N., Rand, K., Elshourbagy, N.A., Zeng, Z., Adamou, J.E., Bergsma, D.J., Li, Y., 1996. A cDNA encoding the calcitonin gene-related peptide type 1 receptor. J. Biol. Chem. 271, 11325–11329.

Bogoyevitch, M.A., Marshall, C.J., Sugden, P.H., 1995. Hypertrophic agonists stimulate the activities of the protein kinases c-raf and A-raf in cultured ventricular myocytes. J. Biol. Chem. 270, 26303–26310.

Brain, S.D., Cambridge, H., 1996. Calcitonin gene-related peptide: vasoactive effects and potential therapeutic role. Gen. Pharmacol. 27, 607–611.

Daaka, Y., Luttrell, L.M., Lefkowitz, R.J., 1997. Switching of the coupling of the β2-adrenergic receptor to different G proteins by protein kinase A. Nature 390, 88–91.

DeFeudis, F.V., 1992. Calcitonin gene-related peptide and cardiovascular function: an overview. Drugs Today 28, 163–173.

Denhardt, D.T., 1996. Signal transduction and protein phosphorylation cascades mediated by ras/rho proteins in the mammalian cells: the potential for multiplex signalling. Biochem. J. 318, 729–747.

Durham, P.L., Russo, A.F., 1998. Serotonergic repression of mitogen-activated protein kinase control of the calcitonin gene-related peptide enhancer. Mol. Endocrinol. 12, 1002–1009.

Ferby, I.M., Waga, I., Sakanaka, C., Kume, K., Shimizu, T., 1994. Wortmannin inhibits mitogen-activated protein kinase activation induced by platelet-activating factor in guinea pig neutrophils. J. Biol. Chem. 269, 30485–30488.

Gutkind, J.S., 1998. The pathways connecting G protein-coupled receptors to the nucleus through divergent mitogen-activated protein kinase cascades. J. Biol. Chem. 273, 1839–1842.

Li, X., Zarinetchi, F., Schrier, R.W., Nemenoff, R.A., 1995. Inhibition of MAP kinase by PGE2 and forskolin in rat renal mesengial cells. Am. J. Physiol. 269, C986–C991.

Lim, R., Zaheer, A., 1996. In vitro enhancement of p38 mitogen-activated protein kinase activity by phosphorylated glia maturation factor. J. Biol. Chem. 271, 22953–22956.

Lopez-Ilasaca, M., Gutkind, J.S., Wetzker, R., 1998. Phosphoinositide 3-kinase γ is a mediator of Gβγ-dependent jun kinase activation. J. Biol. Chem. 273, 2505–2508.

Neary, J.T., 1997. MAPK cascades in cell growth and death. News Physiol. Sci. 12, 286–293.

Poyner, D., 1995. Pharmacology of receptors for calcitonin gene-related peptide and amylin. Trends Pharmacol. Sci. 16, 424–428.

Rane, M.J., Carrithers, S.L., Arthur, J.M., Klein, J.B., McLeish, K.R., 1997. Formyl peptide receptors are coupled to multiple mitogen-activated protein kinase cascades by distinct signal transduction pathways. J. Immunol. 159, 5070–5078.

Robinson, M.J., Cobb, M.H., 1997. Mitogen-activated protein kinase pathways. Curr. Opin. Cell Biol. 9, 180–186.

Sakuta, H., Inaba, K., Muramatsu, S., 1996. Calcitonin gene-related peptide enhances apoptosis of thymocytes. J. Neuroimmunol. 67, 103–109.

Wimalawansa, S.J., 1996. Calcitonin gene-related peptide and its receptors: molecular genetics, physiology, pathophysiology and therapeutic potentials. Endocr. Rev. 17, 533–585.

Yamauchi, J., Nagao, M., Kaziro, Y., Itoh, H., 1997. Activation of P38 mitogen-activated protein kinase by signaling through G protein-coupled receptors — involvement of $G_{\beta\gamma}$ and $G\alpha_{q/11}$ subunits. J. Biol. Chem. 272, 27771–27777.

Zhen, X., Uryu, K., Wang, H.-Y., Friedman, E., 1998. D1 dopamine receptor agonists mediate activation of p38 mitogen-activated protein kinase and c-Jun amino-terminal kinase by a protein kinase A-dependent mechanism in SK-N-MC human neuroblastoma cells. Mol. Pharmacol. 54, 453–458.

Zou, Y., Komuro, I., Yamazaki, T., Kudoh, S., Uozumi, H., Kadowaki, T., Yazaki, Y., 1999. Both G_s and Gi proteins are critically involved in isoproterenol-induced cardiomyocyte hypertrophy. J. Biol. Chem. 274, 9760–9770.