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Mass-dependent signaling between G protein coupled receptors

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Abstract

The present study provides evidence that G protein coupled receptor (GPCR) signaling pathways participate in an interactive signaling network governed by the principles of mass action. Using an inducible thromboxane A_2 receptor (TPR)/platelet activating factor receptor (PAFR) co-expressing cell model, TPR or PAFR expression was independently up-regulated. Immunostaining and radioligand binding experiments demonstrated that this receptor up-regulation resulted in increased GPCR : G protein mass ratios. This increase in mass ratio impacted both TPR and PAFR ligand affinity. Specifically, up-regulating TPR expression not only decreased TPR ligand affinity, but also decreased the ligand affinity of PAFRs. A similar effect on ligand affinities was observed when PAFRs were up-regulated. In addition, increasing the GPCR : G protein mass ratio for TPRs led to desensitization of the calcium mobilization response to PAFR activation, and increasing PAFR mass desensitized the TPR-mediated calcium response. Finally, it was observed that an increased TPR : G protein mass ratio was associated with a shift in the TPR signaling response, and revealed an additional TPR signaling pathway through G_S. Collectively, these results describe a novel mechanism, i.e., mass-dependent GPCR signaling, by which cells can modulate their GPCR signaling pathways and signaling priorities.

Keywords: G-protein coupled receptors (GPCR); G protein; Signaling; Thromboxane A_2 Receptor (TPR); Platelet activating factor receptor (PAFR); Induction; Adenosine 3′, 5′-cyclic monophosphate (cAMP)

1. Introduction

While substantial research efforts have focused on individual signaling pathways in cells, very little information is currently available concerning the potential interactions between these pathways, or the mechanisms by which these interactions are regulated. Nevertheless, previous studies have demonstrated that cross signaling is a common biological phenomenon in numerous mammalian cell types [1–6]. This process of cross signaling is presumably associated with different molecular mechanisms which can lead to either a down regulation (desensitization) [7–10] or an up regulation (synergism) of the biological response [4,5,11–14]. Historically, the most well characterized mechanism of this signal modulation process is the phenomenon of desensitization. Thus it is well established that the exposure of cells to a single agonist can lead to a reduced responsiveness to that agonist (homologous desensitization) [15–17], and if the strength of the initial stimulus is sufficiently strong, subsequent responsiveness to other agonists may also be reduced (heterologous desensitization) [7,10,17,18]. Such desensitization responses have been linked to multiple mechanisms including receptor phosphorylation, G protein phosphorylation, receptor internalization, among others [19–24]. The occurrence of synergism between the same family of GPCRs or between different families of GPCRs is also well described. For example, several studies [2,13,25] have documented such synergistic interactions between the D1 and D2 dopamine receptors. In addition, Jordan and Devi

Abbreviations: GPCR, G-protein coupled receptors (GPCR); TPR, Thromboxane A_2 Receptor; PAFR, Platelet activating factor receptor; PRP, platelet rich plasma; CHO-TPR/PAFR, TPR/PAFR co-expressing CHO cell line; CHO-TPR^{ind}/$PAFR^{ind}$, Inducible TPR/PAFR co-expressing CHO cell line; CHO-TPR^{ind}, Inducible TPR expressing CHO cell line; cAMP, adenosine 3′, 5′-cyclic monophosphate.

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[26] observed that co-expression and co-activation of the δ and κ opioid receptors led to greater effects on mitogen-activated protein kinase (MAPK) phosphorylation and adenylyl cyclase (AC) inhibition than were produced by the addition of either agonist alone. Other studies have also provided evidence for the existence of cross signaling between different families of GPCRs. Thus, co-stimulation of platelets with the thrombin activating peptide (TRAP, PAR1 agonist) and U46619 (TPR agonist) generated synergistic effects on platelet aggregation [5]. Similarly, Cilluffo et al. [27] observed a synergistic interaction between muscarinic and α2-adrenergic receptors (α2-AR), such that agonist co-activation led to elevated calcium mobilization in epithelial cells. Okajima and Kondo [28] also demonstrated that co-stimulating bradykinin receptors and pertussis toxin (PT) sensitive G protein coupled receptors resulted in synergistic calcium responses.

While receptor dimerization [26,29], oligomerization [30] and co-localization [31] have all been proposed as mechanisms for these cross signaling processes, it appears that other mechanisms can also lead to GPCR signal modulation. In this regard, we previously provided evidence that G proteins can redistribute amongst GPCRs in response to ligand activation (a process we define as ligand-dependent cross signaling). The present work provides evidence that this phenomenon of ligand-dependent cross signaling represents only one manifestation of a general process (mass action) by which cells can organize, integrate and dynamically adjust their GPCR signaling responses. Specifically, we hypothesize that these principles of mass action govern the formation of GPCR-G protein complexes within cells such that changes in either the GPCR : G protein coupling affinities or changes in the GPCR : G protein mass ratios will alter the distribution of functional GPCR : G protein complexes, and hence the cellular signaling profile. While classic receptor theory has historically focused on the mass relationships for individual GPCR-ligand interactions, the ability of GPCRs to function as a signaling network regulated by mass considerations has not been previously documented. In order to investigate this notion, TPR or PAFR expression levels were independently up-regulated using an inducible TPR/PAFR co-expressing CHO cell line. Radioligand binding experiments demonstrated that an increased GPCR : G protein mass ratio was associated with a decrease in the ligand affinity for each receptor. Furthermore, it was found that increasing either the TPR : G protein mass ratio or the PAFR : G protein mass ratio resulted in a decreased ligand affinity and a reduced G_q-mediated calcium response for the other receptor. Finally, an increase in the TPR : G protein mass ratio also resulted in the development of a novel TPR signaling pathway through adenosine 3′, 5′-cyclic monophosphate (cAMP), indicating mass-dependent TPR coupling to G_s. Taken together, the present results provide evidence that GPCR signaling pathways participate in an interactive signaling network, and that the relationship between these signaling pathways is modulated by alterations in the GPCR : G protein mass ratios. Since the magnitude of the mass changes reported in this work are routinely encountered in both cellular development and disease [32–49], we propose that mass-dependent GPCR signaling represents a novel mechanism by which cells can dynamically adjust their signaling profiles and ultimately their phenotype.

2. Material and methods

2.1. Reagents

$G\alpha_{13}$, $G\alpha_q$ and PAFR IgG polyclonal antibody (Ab) were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA); HRP-conjugated goat anti-rabbit IgG (H+L) was from BioRad (Hercules, CA); BCA Protein Assay Kit was from Pierce, Inc. (Rockford, IL). CHO cells were from ATCC. TPR cDNA (generous gift from Dr. C.D. Funk) was cloned from human placenta [50]. PAFR cDNA was generously supplied by Dr. R.D Ye; pcDNA3.1 pIND/Hygro, pcDNA5/TO, pVgRXR, T-REx-CHO cells, LipofectAMINE Plus, G418, zeocin, hygromycin, blastidin and Ponasterone A were purchased from Invitrogen (Carlsbad, CA), ^{3}H-PAF, and ^{3}H-SQ29,548 were from Amersham Pharmacia Biotech (Piscataway, NJ). 3-isobutyl-1-methylxanthine (IBMX) was purchased from Sigma (Saint Louis, MO). The PAF antagonist Web2086 was a generous gift from Boehringer Ingelheim (Ingelheim, Germany). cAMP enzyme immnoassay (EIA) kit was purchased from Biomedical Technologies Inc. (Stoughton, MA).

2.2. Cell culture and transfection

CHO and T-REx-CHO cells were maintained in Ham's F-12 medium containing 10% fetal bovine serum (BSA), 100 units/ml penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml amphotericin B. The cells were transfected with TPR, PAFR cDNAs using LipofectAMINE Plus according to the manufacturer's instructions. Briefly, the cells were grown 60–80% confluent on 6-well plates and the culture medium was replaced with serum-free medium. TPR or PAFR cDNA was mixed with Plus reagent and incubated for 15 min, and then LipofectAMINE reagent was added and incubated for another 15 min. The LipofectAMINE Plus cDNA complexes were added to the cells. After 5 h incubation, one volume of 20% fetal bovine serum medium was added. Forty-eight hrs after transfection, the cells were re-suspended, a series of dilutions was made, and the cells were seeded in 96-well plates with antibiotics for single clone selection. For inducible TPR/PAFR co-expression, the ecdysone-inducible system [51,52] and T-REx inducible systems were used [53]. Specifically, TPR cDNA was subcloned to the pIND vector, and PAFR cDNA was subcloned to the pcDNA5/TO vector. Then, the two vectors