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Biased G protein-coupled receptor agonism mediates Neu1 sialidase and matrix metalloproteinase-9 crosstalk to induce transactivation of insulin receptor signaling

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ABSTRACT

G protein-coupled receptors (GPCR) can participate in a number of signaling pathways, and this property led to the concept of biased GPCR agonism. Agonists, antagonists and allosteric modulators can bind to GPCRs in different ways, creating unique conformations that differentially modulate signaling through one or more G proteins. A unique neuromedin B (NMBR) GPCR-signaling platform controlling mammalian neuraminidase-1 (Neu1) and matrix metalloproteinase-9 (MMP9) crosstalk has been reported in the activation of the insulin receptor (IR) through the modification of the IR glycosylation. Here, we propose that there exists a biased GPCR agonism as small diffusible molecules in the activation of Neu1-mediated insulin receptor signaling. GPCR agonists bombesin, bradykinin, angiotensin I and angiotensin II significantly and dose-dependently induce Neu1 sialidase activity and IR activation in human IR-expressing rat hepatoma cell lines (HTC-IR), in the absence of insulin. Furthermore, the GPCR agonist-induced Neu1 sialidase activity could be specifically blocked by the NMBR inhibitor, BIM-23127. Protein expression analyses showed that these GPCR agonists significantly induced phosphorylation of IRβ and insulin receptor substrate-1 (IRS1). Among these, angiotensin II was the most potent GPCR agonist capable of promoting IRβ phosphorylation in HTC-IR cells. Interestingly, treatment with BIM-23127 and Neu1 inhibitor oseltamivir phosphate were able to block GPCR agonist-induced IR activation in HTC cells *in vitro*. Additionally, we found that angiotensin II receptor (type I) exists in a multimeric receptor complex with Neu1, IRβ and NMBR in naïve (unstimulated) and stimulated HTC-IR cells with insulin, bradykinin, angiotensin I and angiotensin II. This complex suggests a molecular link regulating the interaction and signaling mechanism between these molecules on the cell surface. These findings uncover a biased GPCR agonist-induced IR transactivation signaling axis, mediated by Neu1 sialidase and the modification of insulin receptor glycosylation.

1. Introduction

The insulin receptor (IR) is a transmembrane receptor tyrosine kinase (RTK) that is activated by insulin and insulin growth factors-I and -II. Metabolically, insulin-induced IR tyrosine kinase activation is essential for the initiation of downstream insulin signaling and glucose homeostasis. A deregulated process of insulin-induced IR activation may manifest in a range of clinical disease including insulin resistance [1], type 2 diabetes mellitus, obesity, hypertension and cardiovascular disorders, and cancer [2–4]. Specifically, insulin resistance occurs when insulin-sensitive tissues, including skeletal muscle, adipose tissue, and liver, cannot respond to insulin and elicit insulin receptor signaling pathways [5,6] and consequently, may develop several of these metabolic diseases [7–9].

The precise mechanism(s) involved in insulin resistance is unknown. Accumulating evidence, however, demonstrates a link between insulin resistance and aberrant cell membrane glycosylation [10–13]. Notably, the sialylation state of glycans modulated by sialyltransferases

Abbreviations: GPCR, G-protein coupled receptor; 4MUNANA or 4-MU, 2′-(4-methylumbelliferyl)-α-D-*N*-acetylneuraminic acid; OP, oseltamivir phosphate; MMP, matrix metalloproteinase; NMBR, neuromedin B receptor; BR_2, bradykinin receptor; AT2R or AngIIR1, angiotensin II receptor type I; Neu1, neuraminidase-1; IRβ, insulin receptor β subunit; IRS1, insulin receptor substrate-1; HTC-IR, human IR-expressing rat hepatoma cell line; PDGFβR, platelet-derived growth factor β receptor

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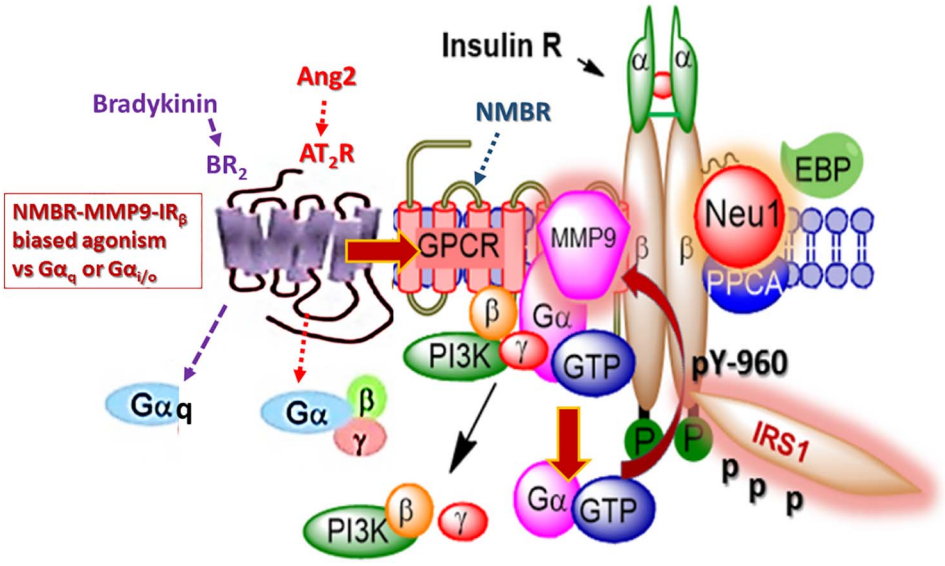

Fig. 1. Bradykinin (BR_2) and Angiotensin II receptor type I (AT_2R) exist in a multimeric receptor complex with NMBR, IRβ, and Neu1 in naïve (unstimulated) and stimulated HTC-IR cells. Here, a molecular link regulating the interaction and signaling mechanism(s) between these molecules on the cell surface uncover a biased GPCR agonist-induced IRβ trans-activation signaling axis, mediated by Neu1 sialidase and the modification of insulin receptor glycosylation. The biased G-protein-coupled receptor (GPCR)-signaling platform potentiates neuraminidase-1 (Neu1) and matrix metalloproteinase-9 (MMP-9) crosstalk on the cell surface that is essential for the activation of the insulin receptor β subunit (IRβ) tyrosine kinases.
Notes: Insulin-binding receptor α subunits (IRα) as well GPCR agonists potentiate a biased NMBR-IRβ signaling and MMP-9 activation to induce Neu1 sialidase. Activated MMP-9 is proposed to remove the elastin-binding protein (EBP) as part of the molecular multi-enzymatic complex that contains β-galactosidase/Neu1 and protective protein cathepsin A (PPCA). Activated Neu1 hydrolyzes α-2,3 sialyl residues of IRβ at the ectodomain to remove steric hindrance to facilitate IRβ subunits association and tyrosine kinase activation. Activated phospho-IRβ subunits phosphorylate insulin receptor substrate-1 (pIRS1), which initiates intracellular insulin signaling *via* the Ras-MAPK and the PI3K-Akt pathway, among others.
Abbreviations: PI3K: phosphatidylinositol 3-kinase; GTP: guanine triphosphate; IRS1: insulin receptor substrate-1; p: phosphorylation.

and sialidases (neuraminidases) has been recognized as a critical factor modulating molecular recognition, adhesion, interaction and signal transduction inside the cell, among cells, the cells and extracellular matrix, and the cells and exogenous pathogens. Four mammalian neuraminidases (Neu1–4) have been described, each varying in cellular localization and affinity for specific glycosidic linkages [14]. Of the four, Neu1 is the only sialidase in which a genetic deficiency and functional mutation manifest clinically in humans. The higher expression levels of Neu1, both intracellular and on the plasma membrane, has been suggested to allow Neu1 to compensate for any deficiencies in Neu2–4 sialidase activities. Although Neu1 has a higher specificity for α2,3-linked sialic acid-galactose (Neu5Ac-Gal) residues, it can also hydrolyze α2,6-linkages primarily cleaved by the other neuraminidases [14,15]. Indeed, Neu1-mediated desialylation process has been identified as a critical mechanism regulating the conformational changes that precede receptor activation and signaling (Fig. 1). These receptors include the nerve growth factor (NGF) TrkA [16–18], IR [19,20], insulin growth factor receptor-1 (IGF-R1) [21], various TOLL-like receptors (TLRs) [22–28], epidermal growth factor receptor (EGFR) [29], and other receptors [14,15]. An extensive review by Pshezhetsky and colleagues summarizes the key pathways in which desialylation of cell surface receptors provides a new dimension for cellular signaling and molecular targeting [15].

Several other signaling molecules have been identified as critical players in the regulation of insulin-induced IR activation. Among these, G protein-coupled receptors (GPCR) have recently been implicated in intracellular crosstalk pathways with IR [30–33]. The integration of GPCR and receptor tyrosine kinase (RTK) signaling including IR upon ligand stimulation is eloquently reviewed by Pyne and colleagues [34–37], Abdulkhalek et al. [38] and Haxho et al. [39]. Other reports have also shown that IR can interact with $G\alpha_i$ subunits upon receptor activation [40–42]. Rozengurt et al. have shown that an upregulation of IR expression and its activation is significantly associated with higher levels of downstream G protein signaling cascades [32]. To explain these latter observations, Onfroy et al. have proposed that G protein stoichiometry dictates biased agonism through distinct receptor-G protein partitioning [43]. Here, they explained that the expression levels of Gα subunits influence the biased profiling of β-agonists as well as antagonists, in that they determine both their activity and efficacy by affecting different membrane distribution of receptor-G protein populations. In the naïve state, the level of Gα expression influences the partitioning of not only Gα but also the co-expressed receptor in different membrane domains [43]. This intriguing concept could explain the results of Rozengurt et al. where GPCR activation mutations correlated with increased downstream IR signaling, in the complete absence of insulin [31]. Collectively, these findings uncover a unique mode of control for IR activation and present an innovative approach to targeting insulin signaling *via* GPCR complexes.

The fact that GPCRs can adopt more than one active state is a phenomenon called 'biased agonism,' 'functional selectivity' or 'ligand-directed signaling' [44–46]. Similarly, there is an increasing array of allosteric ligands with different degrees of modulation, called 'biased modulation' that can vary dramatically in a probe- and pathway-specific manner which cannot be due to simple differences in orthosteric ligand efficacy or stimulus-response coupling [44,47,48]. To that end, Alderton et al. demonstrated that the platelet-derived growth factor β receptor (PDGFβR) forms a complex with a GPCR called Myc-tagged endothelial differentiation gene-1 in cells co-transfected with these receptors [49]. PDGF stimulated tyrosine phosphorylation of the inhibitory $G\alpha_i$ subunit to increase p42/p44 mitogen-activated protein kinase (MAPK) activation and consequently, induction of cell proliferation. Notably, GPCR kinase 2 and β-arrestin-1 are each able to form a complex with PDGFβR and regulate its endocytosis, a requirement for the activation of p42/p44 MAPK. The unresolved issues involving 'biased modulation' are that the release of S1P induced by PDGF can regulate and activate S1P1 receptors to stimulate migration, while S1P2 can inhibit PDGF-induced cell migration in the same cell type [50]. Waters et al. have provided evidence for the basal constitutive activity of the S1P1 receptor, which enhances PDGF-induced migration, while S1P2 activation inhibits migration [50]. This inverse agonism of the S1P1 receptor using SB649146, a novel inverse agonist of the S1P1 receptor, blocks the PDGF-induced endocytosis of the PDGFβ-S1P1 receptor complex in promoting p42/p44 MAPK activation and cell migration. These observations suggest an essential role of the constitutively active S1P1 receptor in regulating G_i-dependent endocytosis of the PDGFβR-S1P1 receptor complex in response to PDGF. The constitutive activity of the PDGFβR-S1P1 receptor complex is not dependent on the PDGFβR growth factor. The novelty of this signaling platform is that G-protein subunits are pre-associated with the complex of growth factor receptor (RTK) and GPCR, and are activated by the