

# When a G protein-coupled receptor does not couple to a G protein

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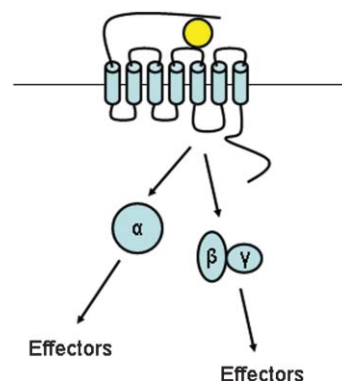
Classically, G protein-coupled receptors (GPCRs) relay signals by directly activating heterotrimeric guanine nucleotide-binding proteins (G proteins). Increasing evidence indicates that GPCRs may also signal through G protein-independent pathways. JAK/STATs, Src-family tyrosine kinases, GRKs/ $\beta$ -arrestins, and PDZ domain-containing proteins have been suggested to directly relay signals from GPCRs independent of G proteins. In addition, our laboratory recently reported that the  $\beta_2$  adrenergic receptor ( $\beta_2$ AR) could switch from G protein-coupled to G protein-independent ERK (extracellular signal-regulated kinase) activation in an agonist dosage-dependent manner. This finding provides a novel mechanism for G protein-independent GPCR signaling. This review focuses on recent progress in understanding the mechanisms by which G protein-independent GPCR signaling occurs.

## Introduction

G protein-coupled receptors (GPCRs), also called seven-transmembrane receptors, constitute the largest membrane receptor gene family of the human genome. These receptors are activated by various ligands, including light, odor, chemokines, hormones, growth factors and neurotransmitters. GPCRs play important roles in regulating many vital physiological functions of eukaryotic cells<sup>1</sup> and thus are major targets for therapeutic drugs.<sup>2</sup>

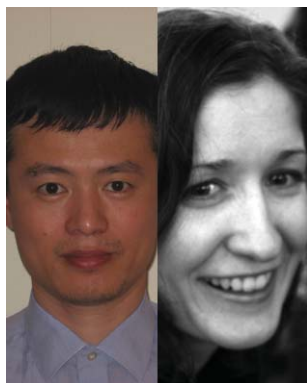
The classical textbook view purports that GPCR signaling is mediated solely by heterotrimeric G proteins (composed of  $\alpha$ ,  $\beta$  and  $\gamma$  subunits) and their downstream effectors<sup>3</sup> (Fig. 1). Heterotrimeric G proteins can be grouped into four families:  $G_s$ ,  $G_i$ ,  $G_q$  and  $G_{12}$  based on sequence homology and functional similarities of their  $\alpha$  subunits<sup>4</sup>. Once GPCRs are occupied by their ligands, they become activated and bind directly to heterotrimeric G proteins, leading to the nucleotide exchange of GDP for GTP bound to the  $G\alpha$  subunit and the

dissociation of the  $G\alpha$  subunit from the  $G\beta\gamma$  complex. Subsequently,  $G\alpha$  and  $G\beta\gamma$  subunits can relay signals to a wide variety of downstream effectors, such as adenylyl cyclases, phosphodiesterases, phospholipases, ion channels,



**Fig. 1** The classical textbook view of GPCR signaling. A ligand activates a GPCR which relays the signal by activating heterotrimeric G proteins. The activated G proteins then dissociate into their  $\alpha$  and  $\beta\gamma$  subunits to act on their respective effectors.

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protein tyrosine kinases and guanine nucleotide exchange factors (Fig. 1).

As early as the mid-1990s, evidence from experiments performed with Dictyostelium raised suspicions that GPCRs cheated on their G protein “spouses” by coupling directly to other effectors (reviewed in reference 5). A rapidly growing body of evidence supports this notion that GPCRs can indeed transduce environmental signals in the absence of heterotrimeric G proteins.<sup>5–7</sup> In this review, we focus on the recent progress in understanding the mechanisms through which GPCRs transduce signals that bypass heterotrimeric G-proteins.

## GPCRs transduce signals in the absence of heterotrimeric G proteins

In addition to heterotrimeric G proteins, more and more biochemical and cellular studies indicate that GPCRs may relay signals independent of heterotrimeric G protein activation.<sup>5–7</sup> For example, G protein-independent pathways are involved in the activation of ERK (extracellular signal-regulated kinase)<sup>8</sup> and in the promotion of Na<sup>+</sup>–H<sup>+</sup> exchange<sup>9</sup> by the  $\beta_2$  adrenergic receptor ( $\beta_2$ AR). Muscarinic receptors activate an inward Na<sup>+</sup> current in mouse pancreatic  $\beta$ -cells in the presence of PTX or guanosine-5'-O-(3-thiotriphosphate) (GTP- $\gamma$ -S) or guanosine-5'-O-(2-thiotriphosphate) (GTP- $\beta$ -S).<sup>10</sup> An angiotensin II type 1A receptor mutant (AT1a-i2m; Asp125, Arg126, Tyr 127 and Met134 in the second intracellular loop mutated to Gly125, Gly126, Ala127 and Ala134, respectively), which cannot couple to G proteins, still activates the Src/Ras/ERK pathway in CHO cells,<sup>11</sup> and causes hypertrophy and bradycardia when cardiac-specifically over-expressed in transgenic mice.<sup>12</sup> A chimeric neurokinin 1 receptor- $\beta$ -arrestin 1 fusion protein activates ERK in the absence of G protein-coupling.<sup>13</sup> Also, long-term activation of ERK by the parathyroid hormone (PTH) receptor is not mediated by G proteins.<sup>14</sup> Mossy fiber stimulation evokes an excitatory postsynaptic response mediated by activation of type 1 metabotropic glutamate receptors which is independent of G proteins but requires Src tyrosine kinase.<sup>7,15</sup> Activation of type 1 metabotropic glutamate receptors in the same cell was also reported to inhibit the slow afterhyperpolarization current in a G protein-dependent manner. Thus it appears that G protein-dependent and G protein-independent signaling through the same type of receptor are not exclusive of each other.

Numerous examples of GPCRs signaling independent of G proteins have been reported. However, the mechanisms of G protein-independent GPCR signaling are still largely unknown. Other than heterotrimeric G proteins, GPCRs also interact with a wide variety of intracellular proteins, such as  $\beta$ -arrestins, tyrosine kinases (e.g. Src and JAK), and PDZ-domain containing proteins (e.g. NHERF, PSD-95, MUPP1 and Grb2). Thus, these GPCR binding molecules might potentially mediate G protein-independent GPCR signaling.

## JAK/STAT

The Janus protein kinase/signal transducers and activators of transcription (JAK/STAT) pathway is one of the major

mechanisms by which cytokine receptors regulate intracellular signaling. The association of ligands with cytokine receptors leads to receptor dimerization, and Janus protein kinase (JAK) activation *via* tyrosine phosphorylation. Subsequently, cytokine receptors are phosphorylated by JAKs and recruit STATs (signal transducers and activators of transcription) to form a receptor/STAT complex. Upon phosphorylation by JAKs, STATs dissociate from the receptor, forming homo- or heterodimers which translocate to the nucleus.

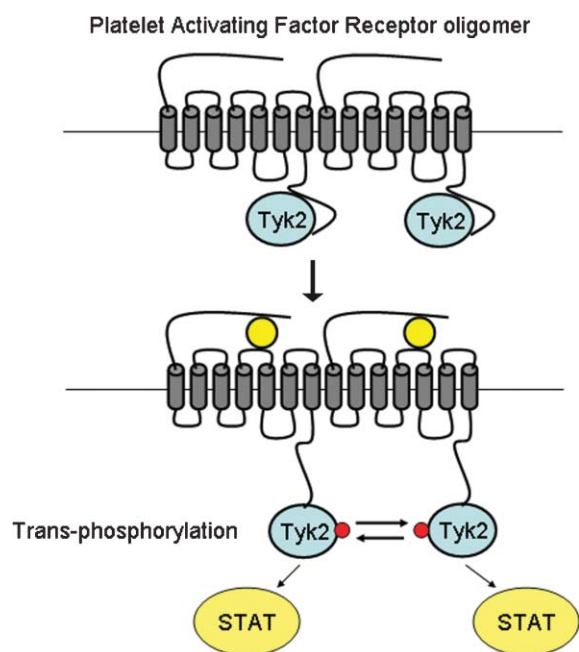
Some reports have indicated that GPCRs can also activate JAK/STAT signaling. Activation of the AT1R,<sup>16</sup> the platelet-activating factor receptor<sup>17</sup> and chemokine receptors CCR2,<sup>18</sup> CCR5<sup>19</sup> and CXCR4,<sup>20</sup> leads to tyrosine phosphorylation and activation of the JAK/STAT pathway. It was first reported that the AT1R binds to JAKs (JAK2 and Tyk2) directly stimulating their activation,<sup>16</sup> suggesting the existence of a G protein-independent pathway. However, more recent evidence indicates that Ca<sup>2+</sup>, PLC $\delta$  and Pyk2, are required for JAK2 activation by Ang II, which implies that JAK2 activation by Ang II may still require some form of G protein-dependent signaling.<sup>21</sup> Further biochemical investigation is needed to determine whether JAK2 activation by Ang II is truly G protein-independent. Direct evidence could be obtained by examining whether the mutant AT1Rs, AT1a-i2m<sup>11</sup> and DRY/AAY,<sup>22</sup> could directly bind to and activate JAK2.

Interestingly, platelet-activating factor (PAF) can activate Tyk2 through a platelet-activating factor receptor (PAFR) mutant that cannot couple to any G proteins.<sup>17</sup> Tyk2 also co-immunoprecipitates with the mutant PAF receptors even in the absence of PAF. Although it is not clear whether the association of Tyk2 with PAFR has any implication for kinase activation, the current hypothesis (Fig. 2) is that the trans-phosphorylation of Tyk2 in the oligomeric PAFR complex could be induced by PAFR conformational changes after ligand binding since the PAF receptor can form oligomers in the absence of ligand.<sup>23</sup>

These reports suggest that GPCRs can signal through JAK/STATs. In the case of the PAFR and Tyk2, this signaling appears to be G protein-independent. Further studies are needed to determine if this is also the case for other members of the JAK family and other GPCRs.

## Tyrosine kinase Src

Src is a non-receptor tyrosine kinase involved in regulating many physiological responses, including cell proliferation, survival, differentiation, adhesion, migration and cell cycle control.<sup>24,25</sup> Src family tyrosine kinases have been shown to be activated by GPCRs.<sup>26,27</sup> Src is involved in the activation of ERK downstream of the  $G\alpha_s$ -coupled  $\beta_2$ AR. Depending on cell type and even clonal variants of the same cell type, the molecular mechanisms by which Src participates in ERK activation by  $\beta_2$ AR seem to be different.<sup>28</sup> For example, in HEK293 cells, the binding of  $\beta$ -arrestin to the activated  $\beta_2$ AR recruits Src, leading to receptor desensitization/internalization, which initiates a second wave of signaling including the ERK MAPK pathway.<sup>29</sup> The  $\beta_2$ AR may also switch its G-protein-coupling from  $G\alpha_s$  to  $G\alpha_i$  in a PKA-dependent manner. The activation of Src by  $G\beta\gamma$  released from  $G_i$  leads to ERK



**Fig. 2** Model of PAFR activation of the JAK/STAT pathway in a G protein-independent manner. PAFRs are known to oligomerize. Upon ligand binding, the PAFR oligomer undergoes a conformational change that allows Tyk2 bound to individual PAFRs in the oligomer to undergo transphosphorylation. Phosphorylated Tyk2 can then activate STATs.

activation.<sup>30</sup> However, some other studies indicate that ERK activation by  $\beta_2$ ARs is mediated by  $G\alpha_s$ , PKA, Rap1, and B-Raf, in a manner that is Src-dependent and PTX (pertussis toxin)-insensitive.<sup>31–33</sup> In other cell types such as CHO cells, PC12 cells, and NIH3T3 cells, the activation of ERK by  $G_s$ -coupled receptors also appears to involve  $G_s$ , PKA, and Src proteins.<sup>34</sup>

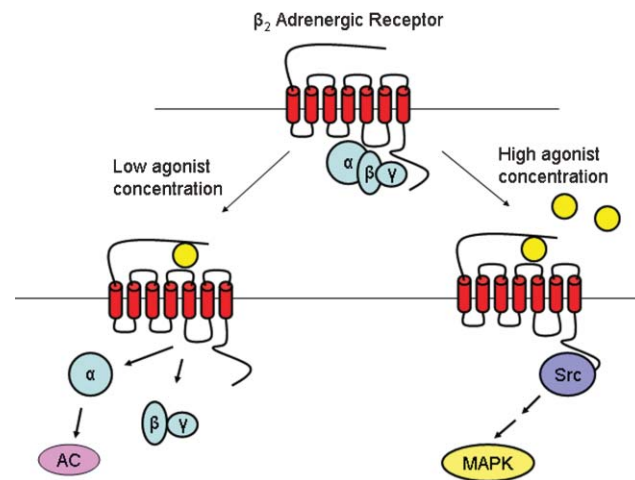
In MEF (mouse embryonic fibroblast) cells<sup>8</sup> or COS-7 cells,<sup>35</sup> the dose-response curve of ERK activation by the  $\beta_2$ AR agonist is biphasic. To study whether  $G\alpha_s$  and Src were involved in ERK activation by  $\beta_2$ ARs in MEF cells, we explored ERK activation by  $\beta_2$ AR in  $G\alpha_s$ - and Src-family tyrosine kinase knockout cells.<sup>8</sup> In  $G\alpha_s$ -knockout cells,  $\beta_2$ AR fails to increase intracellular cAMP.<sup>8,36</sup> MEF cells from Src, Yes and Fyn (SYF cells) triple knockout mice are devoid of any known Src-family tyrosine kinase activity.<sup>37</sup> Both  $G\alpha_s$ -knockout and SYF cells express a similar level of  $\beta_2$ AR as wild type MEF cells.<sup>8</sup> In  $G\alpha_s$ -knockout cells, low concentrations of  $\beta_2$ AR agonist (<100 nM) fail to activate ERK while high concentrations of agonist (>100 nM) can still activate ERK,<sup>8</sup> indicating that  $G\alpha_s$  is required for the first phase of the biphasic dose-response curve of ERK activation by  $\beta_2$ ARs. In SYF cells, low concentrations of  $\beta_2$ AR agonist (<100 nM) activate ERK while high concentrations of agonist (>100 nM) fail to further increase the ERK activity. Re-expression of c-Src in SYF cells restores the biphasic dose-response curve of ERK activation,<sup>8</sup> suggesting that Src is essential for the second phase of the response.

Neither PTX nor expression of RGS4 [a GTPase-activating protein (GAP) for  $G\alpha_q$  and  $G\alpha_i$  proteins] and the RGS-like

domain of p115 Rho-GEF (a GAP for  $G\alpha_{12}$  and  $G\alpha_{13}$  proteins) in  $G\alpha_s$ -knockout cells could abolish ERK activation by a high concentration (10  $\mu$ M) of agonist.<sup>8</sup> The dose-response curves of ERK activation by  $\beta_2$ ARs in  $\beta$ -arrestin 2-knockout cells and  $\beta$ -arrestin 1/ $\beta$ -arrestin 2-knockout cells are similar to that of wild type MEF cells.<sup>8</sup> Thus, we concluded that the second phase of the dose-response curve of ERK activation by  $\beta_2$ AR is G protein- and  $\beta$ -arrestin-independent but Src-dependent.

There are several different mechanisms through which  $\beta_2$ ARs activate Src, including by PKA phosphorylation,<sup>38</sup> by direct  $G\alpha_s$  interaction,<sup>39</sup> and by  $\beta$ -arrestin recruitment.<sup>29</sup> Considering the fact that Src can be activated by  $\beta_2$ AR in  $G\alpha_s$ -knockout and  $\beta$ -arrestin 1/ $\beta$ -arrestin 2-knockout cells,<sup>8</sup> we hypothesized that  $\beta_2$ ARs activate Src through direct interaction since a previous report showed that Src could be co-immunoprecipitated with  $\beta_2$ ARs in cells with or without agonist stimulation.<sup>40</sup> Also the  $\beta_3$ AR has been reported to co-immunoprecipitate with Src in an agonist-dependent and PTX-sensitive manner.<sup>41</sup> Using purified proteins, including GST-tagged  $\beta_2$ AR C-terminus, c-Src and  $\beta_2$ AR, we established that  $\beta_2$ ARs directly bind to and activate Src *in vitro*.<sup>8</sup> Furthermore, in  $\beta_1$ AR/ $\beta_2$ AR -knockout cells, a  $\beta_2$ AR mutant defective in Src binding is associated with an absence of the second phase of the biphasic dose-response curve of ERK activation by  $\beta_2$ ARs.<sup>8</sup> Thus, in the case of ERK activation by  $\beta_2$ ARs, the receptor signals are transduced through two mechanisms: one that is  $G\alpha_s$ -coupled and one that is G protein-independent but Src-mediated with agonist dosage acting as the switch (Fig. 3).

Recently, another study found that Src-family kinase member, Fyn, interacts directly with the C-terminal region of the 5-HT<sub>6</sub> receptor (5-HT<sub>6</sub>R).<sup>42</sup> Activation of the 5-HT<sub>6</sub> receptor led to Fyn-dependent activation of ERK1/2. 5-HT<sub>6</sub>R expressing CHO cells showed a partial decrease in activation of ERK1/2 when treated with a PKA inhibitor. However, a larger decrease in ERK activation in response to 5-HT treatment was



**Fig. 3** Model of  $\beta_2$ AR signaling at low and high agonist concentrations. At low agonist concentrations, the  $\beta_2$ AR signals through G proteins. At high concentrations of agonist, a switch occurs whereby  $\beta_2$ ARs directly couple to Src. Src activation, in turn, leads to activation of the ERK/MAPK pathway.

seen when the SH3 domain of Fyn (which would interfere with the receptor-Fyn binding) was overexpressed in these cells.<sup>42</sup> These results suggest that the 5-HT<sub>6</sub>R-induced ERK activation proceeds mainly *via* a Fyn-dependent pathway and also partially through a PKA-dependent pathway. It remains to be investigated whether the Fyn-mediated ERK activation pathway was a G protein dependent process or not.

These studies indicate that GPCRs can signal directly through Src in a G protein- and  $\beta$ -arrestin-independent manner. Further study is needed to determine if GPCRs can signal through other Src-family members in the same manner. The novel finding that agonist dosage acts as a switch between G protein-dependent and -independent signaling by a GPCR provides a mechanism to explain how a GPCR could signal through alternative pathways in response to the same signal, in the same cells under different physiological conditions.

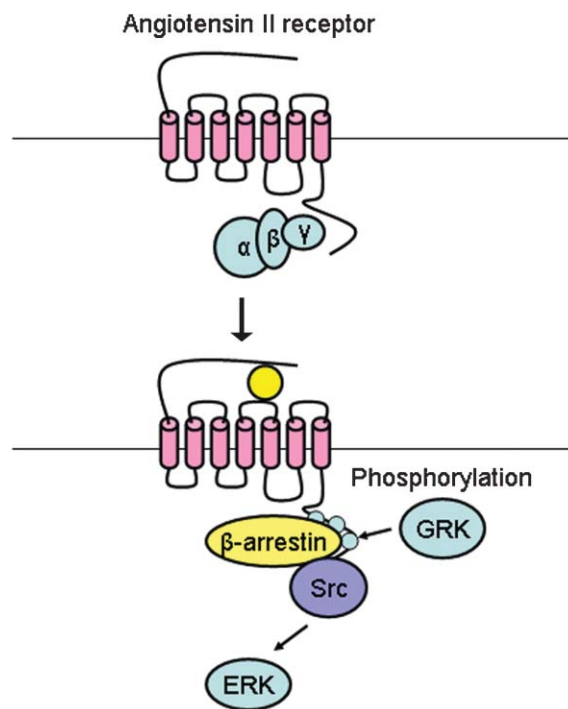
### GRKs/ $\beta$ -arrestins

$\beta$ -arrestins are scaffold proteins that play important roles in GPCR desensitization, internalization and signal transduction.<sup>43,44</sup> The binding of  $\beta$ -arrestins to GPCRs requires the phosphorylation of activated GPCRs by G protein-coupled receptor kinases (GRKs).<sup>45</sup> There are seven GRKs in the GRK family, which is divided into three subfamilies: GRKs 1 and 7 (almost exclusive to retinal rod and cone cells); the pleckstrin homology (PH) domain-containing GRKs 2 and 3 whose membrane recruitment depends on interaction with the G $\beta\gamma$  subunits of heterotrimeric G proteins and phosphatidylinositol 4,5-bisphosphate; and GRKs 4, 5 and 6, which are constitutively associated with membranes.<sup>46</sup> It is possible that activated GPCRs could be directly phosphorylated by the GRKs that are constitutively located on the membrane and that the binding of  $\beta$ -arrestins to the phosphorylated GPCRs is thus independent of G proteins. Upon association with GPCRs,  $\beta$ -arrestins recruit signaling molecules involved in receptor translocation and signal transduction, such as AP2 (adaptor protein complex 2) and clathrin (for receptor endocytosis),<sup>47,48</sup> Src (for receptor endocytosis and ERK activation),<sup>29</sup> Hck (for endocytosis of granules),<sup>49</sup> and c-Raf-1 (for ERK activation).<sup>50</sup> Recruitment of these signaling molecules may also initiate G protein-independent GPCR signaling.

One of the well-studied  $\beta$ -arrestin-dependent GPCR signaling pathways is the activation of the ERK1/2 MAP (mitogen-activated protein) kinase cascade. Data suggest that  $\beta$ -arrestin-dependent ERK activation by some GPCRs could be G protein-independent. Angiotensin II (Ang II) activates ERK 1/2 and tyrosine kinase, Src, through Angiotensin II type 1 receptors (AT1Rs). Surprisingly, Ang II can also activate ERK1/2 and Src through an AT1R mutant (AT1a-i2m), which lacks heterotrimeric G protein coupling.<sup>11</sup> C-terminal truncation of AT1R fails to activate Src, suggesting that the C-terminus of the AT1R is important for Ang II-induced Src activation.<sup>11</sup> Challenging the wild type AT1R with an Ang II analogue ([sarcosine1, Ile4, Ile8]Ang II) or another AT1R mutant (DRY/AAY) with Ang II does not result in activation of heterotrimeric G proteins but does recruit  $\beta$ -arrestin 2 and does activate ERK1/2. The activation of ERK1/2 by Ang II analogue ([sarcosine1, Ile4, Ile8] Ang II) or the AT1R mutant

is blocked by  $\beta$ -arrestin-2 depletion.<sup>22</sup> These studies indeed support a model (Fig. 4) whereby the AT1R receptor can signal independently of G proteins to activate ERK. Furthermore, a recent report shows that a chimeric GPCR, a neurokinin 1 receptor- $\beta$ -arrestin 1 fusion protein, constitutively activates ERK1/2 although the receptor is G protein-uncoupled and resides almost entirely in an intracellular endosomal compartment.<sup>13</sup>

A characteristic property for  $\beta$ -arrestin-dependent ERK activation is a more prolonged duration than that of G protein-mediated ERK activation. Using  $\beta$  arrestin RNA interference and a mutant AT1R (DRY/AAY), Ahn *et al.* showed that G protein-dependent ERK activation is rapid and transient while  $\beta$  arrestin-dependent ERK activation is slower and more sustained.<sup>51</sup> Similar results have also been shown for ERK activation by the parathyroid hormone (PTH) receptor and the  $\beta_2$ AR.<sup>14,52</sup> PTH activates ERK1/2 through the type 1 PTH/PTH-related peptide receptor.<sup>14</sup> The time course of ERK1/2 activation is biphasic with an early peak and a later sustained ERK1/2 activation. Inhibition of PKA or PKC reduces the early phase of PTH-stimulated ERK1/2 activity while the down-regulation of  $\beta$ -arrestins blocks the later sustained ERK1/2 activation. Interestingly, PTH analogues, [Trp1]PTHrp-(1-36) and [D-Trp12, Tyr34]PTH-(97-34), can selectively activate G $\beta$ /PKA-mediated or G protein-independent/ $\beta$ -arrestin-dependent ERK activation.<sup>14</sup> The kinetics of the ERK activation by the  $\beta_2$ AR in HEK293 cells also shows a rapid, transient and G protein-mediated ERK1/2 activation and a slower in onset but more prolonged  $\beta$ -arrestin-dependent ERK1/2 activation.<sup>52</sup>



**Fig. 4** Model of AT1R signaling through GRKs/ $\beta$ -arrestins to activate the ERK/MAPK pathway independently of G proteins. GRKs phosphorylate the C-terminal tail of the activated receptor which leads to  $\beta$ -arrestin binding which, in turn, activates Src leading to activation of the ERK/MAPK pathway.

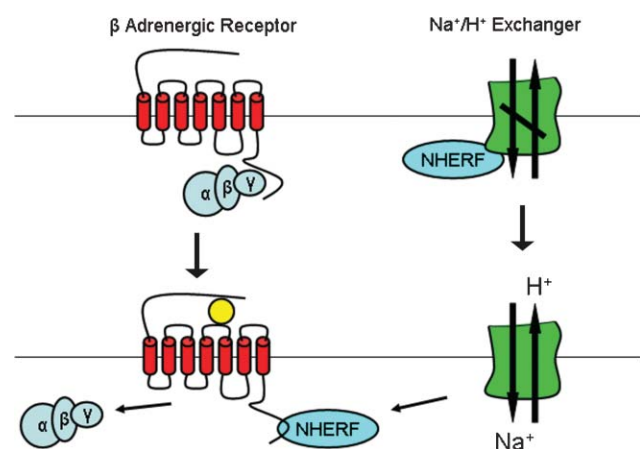
These data indicate that both G protein-dependent and G protein-independent  $\beta$ -arrestin-dependent ERK/MAPK activation occur through GPCRs. Furthermore, the difference in kinetics of ERK activation suggests that these two ERK activation pathways may serve different physiological functions.

### PDZ domain-containing proteins

PDZ domains primarily bind proteins containing a C-terminal S/TXV(L/I) motif, which is found in some GPCRs. GPCRs might use the interaction with PDZ domain-containing proteins to relay intracellular signals that bypass the requirements of heterotrimeric G proteins. One example is the interaction of the  $\beta_2$ AR with the  $\text{Na}^+/\text{H}^+$  exchanger regulatory factor (NHERF).<sup>53</sup> It is known that the activation of  $\text{G}_s$ -coupled receptors can increase intracellular cAMP and activate protein kinase A (PKA), leading to the association of NHERF with the renal  $\text{Na}^+/\text{H}^+$  exchanger, thus inhibiting ionic exchange.<sup>54</sup> Paradoxically, agonist activation of the  $\beta_2$ AR increases, rather than decreases,  $\text{Na}^+/\text{H}^+$  exchange. On the other hand, forskolin (an activator of adenylyl cyclase) inhibits the transporter. NHERF contains a PDZ domain that interacts with the C-terminus of the  $\beta_2$ AR. This interaction can be abolished by a single Ala substitution for Leu at the C-terminus of the  $\beta_2$ AR. Activation of this mutant receptor increases intracellular cAMP and inhibits  $\text{Na}^+/\text{H}^+$  exchange.<sup>9</sup> These results were interpreted to indicate that the activation of  $\text{Na}^+/\text{H}^+$  exchange by the  $\beta_2$ AR is not mediated by G proteins but rather is the result of the activated  $\beta_2$ AR competing with the  $\text{Na}^+/\text{H}^+$  exchanger for NHERF binding, thus leading to the increase of  $\text{Na}^+/\text{H}^+$  exchange (Fig. 5). In addition, the NHERF proteins play an important role in the regulation of  $\beta_2$ AR trafficking.

### Conclusions

The classical heterotrimeric G protein-coupling and second-messenger-generating mechanisms are not sufficient to



**Fig. 5** Model of  $\beta_2$ AR directly signaling through PDZ domain-containing protein, NHERF, in a G protein-independent manner. NHERF binds to the  $\text{Na}^+/\text{H}^+$  exchanger inhibiting its exchange activity. When the  $\beta_2$ AR becomes activated, it competes with the  $\text{Na}^+/\text{H}^+$  exchanger for NHERF binding thus activating  $\text{Na}^+/\text{H}^+$  exchange.

interpret all the biological responses regulated by GPCRs. Increasing evidence indicates that GPCR signal transduction is not always mediated by heterotrimeric G proteins. However, the mechanisms and physiologic functions of G protein-independent GPCR signaling still need further investigation.

GPCRs are one of the most important targets for novel therapeutic drugs, including agonists and antagonists.<sup>2</sup> However, GPCR signaling is a complex signal transduction network and the final physiological outcome depends on the receptor expression, effector-coupling specificity and the cellular context. Thus, ligands with pathway-selective activities could potentially represent a new and extremely useful generation of drugs. It is possible to develop new drugs distinct from conventional agonists or antagonists, which can preferentially activate/inhibit G protein-coupled/-independent GPCR signaling.

These pathway-specific ligands could not only be therapeutically useful but would also be useful tools for furthering our understanding of GPCR signaling. The GPCR mutant receptors deficient in G protein-coupling described in this review have proven useful for studying G protein-independent GPCR signaling. We look forward to the development of similar mutants of other GPCRs which could provide insights into G protein-dependent and -independent GPCR signaling in a range of physiological processes.

The work on the AT1R and the  $\beta_2$ AR described herein suggest that there are two distinct active conformations of GPCRs—one that is compatible with G protein-coupling and one that is not compatible with G protein-coupling but still signals in a G protein-independent manner. The current lack of any structure of the active conformation(s) of a GPCR leaves a gap in our knowledge. Such structures would have important implications for drug design and provide insight into how GPCRs signal both through G proteins and independently of them.

We speculate that some drugs currently on the market may elicit their therapeutic effect only *via* a G protein-dependent pathway or only *via* a G protein-independent pathway and that side effects could be reduced through modification of drugs such that they only activated or inactivated the pathway concerned. Also it would be interesting to examine dosage of currently marketed drugs to investigate whether both G protein-dependent and -independent pathways are activated/inhibited at the therapeutically effective dose.

The implications of the results of the studies discussed in this review are profound, and, although there is still much to be learned about G protein-independent GPCR signaling, we suggest that it is time to reconsider the textbook paradigm of GPCR signaling.

### List of abbreviations

GPCR	G protein-coupled receptor
G protein	guanine nucleotide-binding protein
JAK	Janus protein tyrosine kinase
STAT	signal transducers and activators of transcription
GRK	G protein-coupled receptor kinase
$\beta_2$ AR	$\beta_2$ adrenergic receptor

ERK	extracellular signal-regulated kinase
PAF	platelet-activating factor
MAPK	mitogen-activated protein kinase
PTX	pertussis toxin
NHERF	Na <sup>+</sup> -H <sup>+</sup> exchanger regulatory factor

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