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COMMENTARY

G-protein-coupled receptor dephosphorylation at the cell surface

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Abbreviations: GPCR, G-protein-coupled receptor; GRK, G-protein-coupled receptor kinase; PKA, protein kinase A; PKC, protein kinase C

Agonist-induced desensitization of G-protein-coupled receptors (GPCRs) usually involves phosphorylation of the receptor by G-protein-coupled receptor kinases (GRKs) or second messenger-dependent protein kinases such as PKC and PKA. Phosphorylation by GRKs promotes arrestin binding to the receptor, which not only uncouples receptor and G protein but also targets the receptor to clathrin-coated pits for internalization. The internalized GPCR is either trafficked to lysosomes for degradation or alternatively is dephosphorylated by phosphatase enzymes in an acidic endosomal compartment before being recycled to the membrane for further rounds of agonist stimulation. These GPCR regulatory pathways were identified largely as a result of the seminal studies by the Lefkowitz laboratory in the U.S.A. (reviewed in Lefkowitz, 2004), and have reached the status of dogma in the field of GPCR biology.

However, some new studies, including one in this issue of the *British Journal of Pharmacology* (Iyer *et al*., 2005), suggest that the role of internalization in GPCR dephosphorylation is less clear than previously thought. This work is particularly elegant because the authors have developed antibodies to detect β_2-adrenoceptor phosphorylation by PKA at Ser262 in the receptor's third intracellular loop and by GRKs at Ser355,356 in the COOH-terminus (Tran *et al*., 2004). They show that when internalization of the phosphorylated β_2-adrenoceptor is blocked, either by expression of a dominant-negative mutant form of dynamin or with hypertonic sucrose, the receptor still undergoes dephosphorylation of both PKA and GRK sites. Indeed, the rate of dephosphorylation of the receptor is the same whether or not the receptor is able to internalize. Furthermore, they show that the β_2-adrenoceptor still undergoes dephosphorylation following selective phosphorylation of PKA site Ser262 subsequent to the addition of a very low concentration of isoprenaline (300 pM), which does not induce receptor internalization. These results show conclusively that the β_2-adrenoceptor does not have to internalize in order to become dephosphorylated (Figure 1). Does this apply to other GPCRs? Apparently it does; a recent study (Gardiner *et al*., 2001) shows that the agonist-phosphorylated G_s-coupled D_1 dopamine receptor undergoes the same rate of dephosphorylation whether or not it is able to undergo internalization. Furthermore, a very recent study shows that the agonist-activated G_q-coupled thyrotropin-releasing hormone receptor is rapidly dephosphorylated at the cell membrane in mouse embryo fibroblast cells from arrestin-knockout animals where these receptors are unable to undergo significant internalization (Jones & Hinkle, 2005).

How to reconcile these recent findings with the earlier results (Yu *et al*., 1993; Pippig *et al*., 1995; Krueger *et al*., 1997)? Iyer *et al*. (2005) suggest that the interpretation of earlier studies on the β_2-adrenoceptor is made difficult because the ^{32}P labelling technique reflects a combination of PKA and GRK phosphorylation of the receptor. This may be particularly relevant since the kinetics and agonist concentration dependence of PKA and GRK phosphorylation of the β_2-adrenoceptor are different (Tran *et al*., 2004). However, although most of the data provided by Iyer *et al*. (2005) indicates that the β_2-adrenoceptor undergoes dephosphorylation at the cell surface, they do report some experimental conditions (longer agonist

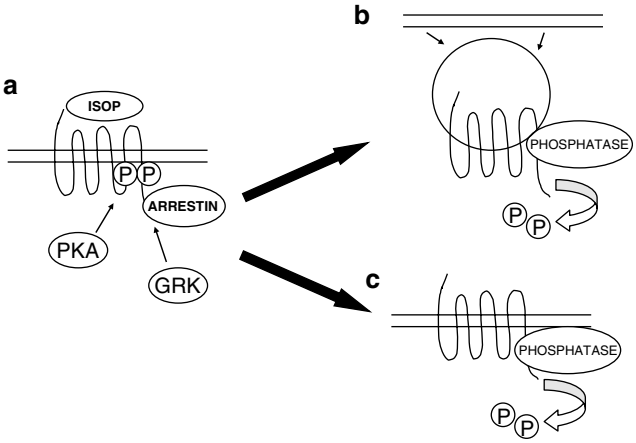

Figure 1 Dephosphorylation of β_2-adrenoceptors. (a) In response to prolonged agonist treatment (isoprenaline; ISOP), the receptor at the cell membrane is phosphorylated by PKA and GRK, and arrestins bind to the receptor. (b) In the classical view, receptor is internalized to endosomes where it undergoes dephosphorylation. The receptor can then be recycled to the membrane in a resensitized form. (c) Recent data indicates that the β_2-adrenoceptor can be effectively dephosphorylated at the cell membrane, without the need for internalization.

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treatment time in the presence of hypertonic sucrose) where dephosphorylation is inhibited. Thus, the experimental conditions used (e.g. agonist concentration, length of agonist treatment, presence of antagonist in the wash buffer, use of ^{32}P labelling *versus* phosphosite-specifc antibodies) may explain some of the differences. In all likelihood, the β_2-adrenoceptor is able to undergo dephosphorylation both at the cell membrane and intracellularly in endosomes.

Important questions remain. For example, the work of Iyer *et al*. (2005) does not explain why inhibitors of internalization can block resensitization, as shown in the earlier studies (Yu *et al*., 1993; Pippig *et al*., 1995). Furthermore, do GPCRs that undergo dephosphorylation at the cell surface resensitize immediately, and does this provide for more rapid resensitization following agonist-induced desensitization than for an internalized receptor? Is the role of GPCR internalization more concerned with the activation of alternative signalling pathways, or with downregulation, than with dephosphorylation? Finally, what determines the activity and localization of phosphatase enzymes at the plasma membrane? One possibility here may be the family of A-kinase-anchoring proteins, which associate with at least some GPCRs and which, by acting as a scaffold protein, can localize both kinase and phosphatase enzymes to the vicinity of the receptor in the cell membrane (Fraser *et al*., 2000; Lin *et al*., 2000).

In summary, Iyer *et al*. (2005) show that the β_2-adrenoceptor can undergo dephosphorylation without having to undergo internalization. The functional consequences of this interesting finding for GPCR signalling remain to be explored in detail.

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