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COMMENTARY

The ups and downs of Gs- to Gi-protein switching

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Abbreviations: PKA, protein kinase A; RGS, regulator of G-protein signalling; MAP kinase, mitogen-activated protein kinase

The β_2-adrenoceptor has proved to be an excellent model system for unravelling the details of receptor coupling to Gs-proteins, activation of adenylyl cyclase and intracellular signalling via the second messenger cyclic AMP (Lefkowitz *et al*., 2002). However, more recently studies of the effect of β_2-adrenoceptor stimulation on other signalling pathways has led to considerable debate concerning the ability of this receptor to utilise alternative G-proteins to mediate its agonist effects. It has been known for some time that G-protein-coupled receptors can be promiscuous in their association with particular heterotrimeric G-proteins, particularly at high levels of receptor expression (Wenzel-Seifert & Seifert, 2000). Evidence has been presented that signalling from the β_2-adrenoceptor to the mitogen-activated protein kinase (MAP kinase) pathway may involve a switch in receptor coupling from Gs- to Gi-proteins (Daaka *et al*., 1997). This has been proposed to be because of a feedback phosphorylation by protein kinase A (PKA) of the β_2-adrenoceptor itself following stimulation of the Gs-protein pathway by β_2-agonists (Daaka *et al*., 1997). Thus, following stimulation of adenylyl cyclase via Gsα, cyclic AMP is produced and leads to activation of PKA. Phosphorylation of PKA consensus sites within the third intracellular loop and C-terminal tail of the β_2-adrenoceptor then causes an attenuation of receptor – Gs coupling and facilitates coupling of the receptor to Gi-proteins. This leads in turn to activation of the MAP kinase pathway. Pertussis toxin, which ADP-ribosylates the Giα subunit and prevents receptor – Giα coupling, has been shown to inhibit β_2-adrenoceptor-mediated MAP kinase activation in these cells (Daaka *et al*., 1997).

However, others have shown that signalling to the MAP kinase cascade from the β_2-adrenoceptor does not involve pertussis toxin-sensitive Gi-proteins and can be explained by Gs/PKA activation of the small G-protein Rap1 and the serine – threonine kinase B-Raf (Schmitt & Stork, 2000; Friedman *et al*., 2002). Many of these conflicting sets of data have been obtained in the HEK 293 cells, and it may be that differences in the role of Gs/Gi switching is a consequence of variability among different 'HEK 293' cell lines (Lefkowitz *et al*., 2002). What remains unclear are the reasons for these differences and the molecular mechanisms underlying them. However, it is likely to involve heterogeneity in the amount of particular G-proteins or their ancillary signalling proteins (e.g. levels of Gi-specific regulator of G-protein signalling proteins; Friedman *et al*., 2002) present in the cells. Once unravelled, however, this may provide important insights into β_2-adrenoceptor signalling in health and disease.

Evidence for coupling of β_2-adrenoceptors to Gi-proteins is not, however, confined to transformed cell lines and has been observed in cardiac myocytes (Xiao *et al*., 1999). Dual coupling of β_2-adrenoceptors (but not β_1-adrenoceptors) to both Gs- and Gi-proteins has also been demonstrated in human atrial membrane preparations (Kilts *et al*., 2000). In this issue of the *British Journal of Pharmacology*, Hasseldine *et al*. (2003) show that isoprenaline can elicit both positive and negative inotropic effects via β_2-adrenoceptor stimulation in left atria isolated from transgenic mice (TG4) overexpressing the β_2-adrenoceptor. The negative inotropic effects of the β_2-adrenoceptor stimulation appear to be mediated via Gi-proteins since they can be prevented by pertussis toxin treatment. However, perhaps the most interesting aspect of this work concerns the effect of pretreatment with isoprenaline or the PKA activator 8-bromo-cAMP. These interventions inhibited the positive inotropic effects of β_2-adrenoceptor stimulation, but were without effect on the Gi-mediated negative inotropic responses. The authors suggest that PKA-mediated phosphorylation of the β_2-adrenoceptor is responsible for the desensitisation of the positive inotropic effects seen and thus is consistent with a switch from Gs- to Gi-coupling. However, it remains to be established whether the Gi-mediated negative inotropic responses are indeed all because of an interaction with a phosphorylated receptor, or whether at the high receptor expression level used here, the unmodified β_2-adrenoceptor can also couple directly to Gi-proteins. If this latter hypothesis is true, however, the data obtained by Hasseldine *et al*. (2003) effectively mean that PKA-mediated phosphorylation of the β_2-adrenoceptor reduces the receptor – Gs interactions but not the receptor – Gi – protein interaction.

An interesting feature of some of the earlier studies with the TG4 mice (Bond *et al*., 1995) was the presence of constitutive β_2-adrenoceptor (Gs-mediated) activity, leading to an elevated force of contraction in the absence of agonist stimulation (compared to litter mates lacking the transgene). These original studies in this model system allowed the demonstration of inverse agonist responses (i.e., decreases from basal) with the β_2-adrenoceptor antagonist ICI 118,551. These inverse agonist responses were antagonised by alprenolol, and the extent of constitutive activity was reduced by depletion

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of the β_2-adrenoceptor population with pindobind (Bond *et al*., 1995). The presence of constitutive activity was therefore a consequence of β_2-adrenoceptor overexpression and agonist-independent coupling of receptors to Gs-proteins. However, it now appears from the study of Hasseldine *et al.* (2003) that the phenotype of the TG4 mice has changed so that constitutive receptor activity is no longer apparent and Gi-mediated negative inotropic actions are now present. Thus, in a similar fashion to the work described above for HEK 293 cells and MAP kinase signalling, subtle changes may have occurred in these mice that have affected the final signalling readouts. This could be explained by a change in the stoichiometry between receptor, Gs- and Gi-proteins particularly as physiological changes have already been reported. For example, an increase in cardiac expression of Gαi2-proteins has been observed with age, which results in enhanced coupling of this Gi-protein to the β_2-adrenoceptor (Kilts *et al*., 2002).

Positive (Gs-mediated) and negative (Gi-mediated) effects of β_2-adrenoceptor stimulation on contraction rate in cardiac myocytes have recently been reported in β_1-adrenoceptor knockout mice (Xiang *et al*., 2002). These data are entirely consistent with the results of Hasseldine *et al.* (2003). However, the study of Xiang *et al*. (2002) has also shown that β_2-adrenoceptors are localised within caveolae in the membrane of cardiac myocytes. These small 'flask-like' invaginations in the plasma membrane (that can be depleted by cholesterol-lowering agents such as β-cyclodextrin or filipin) are enriched in a number of signalling proteins, including Gi-proteins (Xiang *et al*., 2002). Thus, changes in the coupling of β_2-adrenoceptors to different G-proteins may depend not only on the relative expression of different components of the receptor – G-protein signalling complex, but also on their location within microdomains of the plasma membrane. The potential involvement of microdomains, and therefore all the other signalling molecules therein, may further add to the heterogeneity of responses, and thus the differences reported in Gs- to Gi- switching between cell lines and within tissues.

The study of Hasseldine *et al*. (2003) and others raises a number of important questions regarding the impact of the receptor – G-protein stoichiometry on signalling from the β_2-adrenoceptor to Gs- and Gi-proteins. However, it remains to be established in cardiac myocytes whether phosphorylation of the β_2-adrenoceptor by PKA is an essential prerequisite for Gi coupling. If not, then the data suggest that the nonphosphorylated form of the β_2-adrenoceptor can, under certain conditions, couple directly to Gi-proteins, independently of any subsequent actions of PKA. It will be interesting to follow how this debate progresses.

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