Regulation of G protein subunit composition in cardiomyocytes: pharmacological implications

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It is now well established that any given ligand for a G protein-coupled receptor (GPCR) stabilizes a unique ligand-specific receptor conformation with specific signaling capabilities (Kobilka and Deupi 2007; Kenakin and Christopoulos 2012; Venkatakrishnan et al. 2013). Evidence for the existence of ligand-specific receptor conformations has been obtained in functional experiments with GPCR-transfected mammalian cells (Galandrin et al. 2008) and insect cell membranes expressing mammalian GPCRs and G proteins (Wenzel-Seifert and Seifert 2000), fluorescence spectroscopy studies with purified receptors (Swaminath et al. 2005), and structural studies using crystallographic and nuclear magnetic resonance approaches (Warne et al. 2011; Nygaard et al. 2013). Ligand-specific GPCR activation results in ligand-specific activation of downstream coupling partners and is also referred to as biased signaling (Rajagopal et al. 2010). Among the proteins to which GPCRs couple are not only the name-conferring heterotrimeric G proteins but also the arrestins; these are originally assumed to be exclusively involved in receptor desensitization (Rajagopal et al. 2010). Ligand-specific receptor activation holds great therapeutic potential in terms of specifically activating certain cell functions while reducing unwanted side effects (Rajagopal et al. 2010; Kenakin and Christopoulos 2012). A major problem is that it is not easy to examine this concept in native cells with endogenously expressed GPCRs. One approach to address this important question is to examine the effects of multiple ligands at a given GPCR on multiple cellular parameters including second messenger generation and cell functions. In the next step, one can correlate potencies and efficacies of ligands for the various parameters in webs of potencies and efficacies. Deviations

from symmetric patterns in these webs are indicative for ligand-specific receptor conformations (Reher et al. 2012a). These types of studies are just beginning to emerge but so far support the concept that ligand-specific receptor conformations also exist in native systems (Reher et al. 2012a, b).

The β_1 -adrenergic receptor (β_1 AR) and β_2 -adrenergic receptor (β_2 AR) are well-studied paradigms for ligand-specific receptor conformations. For example, in a seminal study, Michel Bouvier's group showed that the pharmacological profile of the β_1 AR with regard to cAMP accumulation is quite different from the profile with respect to the activation of extracellular signal-regulated kinase (ERK) pathways (Galandrin et al. 2008). Similarly, the pharmacological profile of the recombinant β_2 AR with respect to coupling to G_i and G_s proteins in insect cell membranes is different (Wenzel-Seifert and Seifert 2000), and certain stereoisomers of β_2 AR ligands differ in their capacity to direct β_2 AR signaling towards G_i versus G_s proteins (Woo et al. 2009; Seifert and Dove 2009). Coupling of a given GPCR to a specific G protein is not only influenced by the ligand bound to the receptor but also by the expression level of the receptor, the concentration of the G protein in the vicinity of the receptor, and the particular cell type examined (Kenakin 2007, 2009; Schneider and Seifert 2010). One approach to address this problem is the GPCR–G protein α -subunit fusion protein approach, ensuring a close proximity of the coupling partners and a 1:1 stoichiometry (Milligan et al. 2007; Schneider and Seifert 2010). However, evidently, this system is not physiological. Because of this critique, recent research efforts in our group regarding the concept of ligand-specific receptor conformations have shifted away from fusion proteins towards native human systems, specifically peripheral blood cells (Reher et al. 2012a, b), accepting the fact that such systems offer much less opportunity for genetic manipulation than recombinant systems and possess higher intrinsic variability.

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Cardiomyocytes express both the β_1 AR and the β_2 AR (Brodde and Michel 1999). The expression level of G proteins changes in heart disease. Most prominently, expression of $G_{i\alpha 2}$ is increased in heart failure (El-Armouche et al. 2003). It is controversially discussed whether $G_{i\alpha 2}$ signaling is beneficial or detrimental in heart failure (Kaur et al. 2012). Accordingly, the role of $G_{i\alpha 2}$ must be studied in greater detail. Moreover, ligand-specific β_2 AR coupling to G_i and G_s proteins has been observed in cardiomyocytes (Woo et al. 2009). Furthermore, several therapeutically relevant β AR antagonists clinically used for the treatment of cardiovascular diseases including chronic heart failure, hypertension, and coronary heart disease constitute, in fact, not simple antagonists but rather ligands exhibiting biased signaling (Galandrin et al. 2008; Rajagopal et al. 2010). Long-term activation of the β_1 AR exhibits detrimental effects on heart function (Engelhardt et al. 1999), while activation of the β_2 AR may exert beneficial effects, at least in some systems (Patterson et al. 2004; Chakir et al. 2011). Hence, it is conceivable that the altered expression of G_i proteins in heart failure is not only an adaptive change to the disease but also offers a therapeutic opportunity for pharmacological intervention. The clinically most relevant approach would be to examine heart tissue from patients, but evidently, the amount of available material does not allow for comprehensive pharmacological studies.

On this complex conceptual and pathophysiological background, the groups of Thomas Wieland (University of Heidelberg, Germany), Bernd Nürnberg (University of Tübingen), and Susanne Lutz (University of Göttingen, Germany) present a very interesting model system in which G protein expression pattern is manipulated in neonatal rat cardiomyocytes (Hippe et al. 2013). As starting point, they observed that in heart tissue (constituting a mixture of various cell types including cardiomyocytes, fibroblasts, smooth muscle cells, and endothelial cells), the expression of $G_{i\alpha 3}$ is moderately increased in $G_{i\alpha 2}$ -deficient mice, whereas the expression of $G_{i\alpha 2}$ is moderately enhanced in $G_{i\alpha 3}$ -deficient mice. While these differences are probably too small to conduct studies aimed at the dissection of pharmacological differences between β AR ligands in terms of biased signaling, nonetheless, the data provided the first hints for reciprocal regulation of G protein α -subunits at the organ level.

In the next step, Hippe and collaborators established an elegant expression system in which neonatal rat cardiomyocytes were infected with adenoviruses encoding for various G protein subunits. Most strikingly, with the adenovirus encoding $G_{i\alpha 2}$, this G protein α -subunit can be expressed at up to sixfold higher levels in cardiomyocytes. This is a much larger overexpression of $G_{i\alpha 2}$ than in heart failure, amounting just up to twofold. One can argue that the adenovirus system is artificial, but for pharmacological studies, it may be very useful because it can magnify effects below the analytical detection level in a heart failure model. In the

spectrum of systems ranging from “native” to “recombinant,” the system developed by Hippe et al. (2013) is somewhere located in the middle. In parallel with increased $G_{i\alpha 2}$ expression, the expression of $G_{i\alpha 3}$ and of $G_{s\alpha}$ decreases, and $G_{\beta\gamma}$ complexes are not significantly changed. In addition, overexpression of $G_{s\alpha}$ diminishes the expression of $G_{i\alpha 2}$ and $G_{i\alpha 3}$ but not of $G_{\beta\gamma}$. The lack of effect of G_{α} overexpression on $G_{\beta\gamma}$ level argues against nonspecific effects of the adenovirus infection. This notion is further supported by the finding that enhanced expression of $G_{i\alpha 3}$ does not change the expression of $G_{i\alpha 2}$, $G_{s\alpha}$, and $G_{\beta\gamma}$. Moreover, adenoviral expression of either $G_{i\alpha 2}$ or $G_{s\alpha}$ had no effect on expression of $G_{o\alpha}$ or $G_{q\alpha}$. Furthermore, enhanced adenoviral expression of $G_{\beta\gamma}$ prevented the loss of endogenous $G_{i\alpha}$ expression. Lastly, the authors show that overexpression of $G_{i\alpha 2}$ reduces cAMP accumulation induced by isoproterenol, a nonselective full β_1 AR/ β_2 AR agonist.

The focus of the paper by Hippe et al. (2013) is the establishment of a “semi-native/semi-recombinant” system, manipulation of specific G protein levels, and analysis of compensatory changes in endogenous G protein isoforms, while the functional consequences are just beginning to be explored. It is already clear from the few functional data presented in the paper that the system developed by the group of Thomas Wieland is a nice model system to reflect pathophysiological changes in G protein expression observed in heart failure and is well suited for future pharmacological studies. It will be very important to generate complete concentration/response curves for a large set of β AR ligands comprising classic “antagonists” such as propranolol and partial agonists such as dichloroisoproterenol, ephedrine, and salbutamol. It will also be important to dissect the contributions of the β_1 AR and β_2 AR to cAMP responses by use of β_1 AR- and β_2 AR-selective antagonists. As a complementary strategy, cardiomyocytes from mice deficient in either β_1 AR or β_2 AR could be used as model systems (Zhou et al. 2000).

When designing pharmacological studies in this field, an additional level of complexity needs to be taken into consideration. Many β AR ligands have one or two chirality centers, but routinely, racemic mixtures are used in most studies (Seifert and Dove 2009), availability to researchers being the major reason. However, considering that fact that defined stereoisomers of β AR ligands stabilize unique receptor conformations and exhibit distinct signaling capacities (Seifert et al. 2001; Woo et al. 2009), it is highly important to examine pairs of chemically pure stereoisomers. Unfortunately, this is not trivial since for most important β AR ligands, only the potent and supposedly “active” enantiomer is commercially available, whereas the less potent and supposedly “inactive” diastomer is not. Some diastomers may be obtained from academic medicinal chemistry laboratories or pharmaceutical companies, but overall, acquisition of stereoisomer pairs for many ligands is difficult or impossible. Also, preparation of highly pure stereoisomers from racemic mixtures is only a

task that can be accomplished by specialized medicinal chemistry laboratories (Beigi et al. 2006). We can only hope that increased awareness of the importance of pharmacological studies with defined stereoisomer pairs will improve the availability of such compounds by standard reagent suppliers.

In this context, it will also be crucial to examine other signaling pathways than the adenylyl cyclase/cAMP pathway. Most importantly, analysis of the ERK pathway will be most informative. Last but not least, spatiotemporal analysis of signal transduction should be performed. Wieland and co-workers analyzed the concentrations of G protein subunits in crude cell membranes (Hippe et al. 2013). It is well known, and the authors are fully aware of this fact that GPCRs, G proteins, and effector proteins are compartmentalized (Ostrom et al. 2012). The differential localization of signaling proteins can have a major impact on second messenger generation. With fluorescent cAMP sensors, spatiotemporal distribution of cell signaling can be assessed (Nikolaev et al. 2006). In conclusion, Hippe and co-workers have established a very important and useful “semi-native” or “semi-recombinant” model system for studying β AR-mediated signal transduction in a pathophysiologically relevant setting. I look forward to seeing the complex matrix of pharmacological parameters that can now be obtained with this system.

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