A muscarinic view on Gq

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Quantification of agonist action can be challenging for the experimental pharmacologist, as the intensity of response to an agonist may vary depending on the effectiveness of signal transduction within the biological system. Signal amplification is addressed by terms such as “receptor reserve” and “spare receptors”. Signal amplification can be quantified by operational models of agonism (Black and Leff 1983; Rajagopal et al. 2011; Kenakin et al. 2012; Kenakin and Christopoulos 2013). As a consequence of signal amplification, a partial agonist may—under experimental conditions of a receptor reserve—achieve the same maximum effect as the physiological full agonist. Furthermore, if the endogenous agonist already induces maximum activation of the system, super-agonism of an artificial compound, i.e., more-than-physiological receptor activation, is prone of being overlooked (Schrage et al. 2013). Therefore, with respect to preclinical pharmacology, primary and native cells as well as conventional isolated tissue preparations have high value. Worth mentioning, it was the isolated beating guinea pig atrium as a muscarinic M₂ receptor model and alkane-bis-ammonio compounds such as W84 which first disclosed allosteric interactions at G protein-coupled receptors (Lüllmann et al. 1969). In addition, using the M₂ receptor a physiological function of the allosteric vestibule has recently been proposed (Bock et al. 2012).

Odagaki et al. (this issue of the journal) employed the “Antibody-capture [³⁵S]GTPγS scintillation proximity assay” (Porter et al. 2002; for review, see DeLapp 2004) to measure modulation of muscarinic M₁ receptor signaling by a variety of orthosteric, allosteric, and bitopic

orthosteric/allosteric (“dualsteric”) compounds in membranes from rat cerebral cortex and hippocampus. There are five subtypes of muscarinic receptors. The odd-numbered receptors M₁, M₃, and M₅ preferentially couple to the Gq-pathway, the even-numbered receptors M₂ and M₄ favor Gi (Wess et al. 2007). Levey et al. (1991) found that M₁, M₂, and M₄ “accounted for the vast majority ... of muscarinic sites in rat brain”. In the applied assay (Fig. 1), in order to focus on activation of the M₁ subtype, selectivity for the Gq-signaling pathway is provided by an antibody which binds the Gαq subunit of the Gq protein. Therefore, [³⁵S]GTPγS bound to M₂ and M₄, which both prefer signaling into the Gi-route, is not recognized by the assay. If M₁ signaled into the Gi- (Offermanns et al. 1994) or the Gs-pathway (Burford and Nahorski 1996), the assay would not respond either.

The muscarinic acetylcholine receptor is archetypal for the allosteric modulation of G protein-coupled receptors (Lüllmann et al. 1969; Clark and Mitchelson 1976; Stockton et al. 1983; Jepsen et al. 1988; Tuček et al. 1990) and crystal structures of the inactive M₂ and M₃ receptor reveal a rather voluminous allosteric vestibule in the entrance of the ligand binding pocket (Haga et al. 2012; Kruse et al. 2012). Consequently, Odagaki et al. characterize the receptor interaction of various agonistic and inverse agonistic allosteric ligands as well as of McN-A 343 which is a bitopic allosteric/orthosteric agonist at the M₂ subtype (Valant et al. 2008). Noteworthy, the intrinsic efficacy of some compounds was rather low compared with findings reported for cell lines, suggesting that physiological cells operate with comparably low signal amplification.

Taken together, Odagaki et al. provide almost encyclopedic information on M₁ receptor-mediated Gq-signaling of dozens of muscarinic receptor ligands. Next, it would be interesting to learn more on how the ligands compare with regard to promiscuous and biased signaling.

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Fig. 1 Gαq-activation triggered by the M₁ muscarinic acetylcholine receptor as measured with the “Antibody-capture [³⁵S]GTPγS scintillation proximity assay” (for review, see DeLapp 2004) in the study of Odagaki et al. (this issue of the journal). Test compounds target either the orthosteric acetylcholine binding site located in the depth of the ligand binding pocket, or the allosteric site in the entrance of the binding pocket, or—in the case of dualsteric ligands—both sites simultaneously. Employed drugs include agonists (green) and inverse agonists (gray). Upon activation, G proteins recruit and irreversibly bind [³⁵S]GTPγS. The G protein of interest, i.e., Gq, is “fished” with a selective antibody (rabbit anti-Gαq). The latter antibody then mediates linkage with antibody-coated scintillation beads, thus allowing transformation of Gαq-bound [³⁵S]GTPγS radiation into light flashes

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