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PERSPECTIVE

Measures of Efficacy Using G Proteins as Endpoints: Differential Engagement of G Proteins through Single Receptors

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The concept of agonism has been evaluated extensively for ligands that bind to receptors coupled to G proteins, with the classification of ligands into inverse, partial, or full agonists, or neutral antagonists, most often performed using effectors or effector-driven phenomena as endpoints. Many receptors are coupled not to a single G protein but instead to a variety of G proteins. Because G proteins need not interact equally well with a receptor and because a given receptor might adopt more than one activating conformation, the idea that G proteins can be engaged to different extents through a single receptor depending on the agonist has emerged (Kenakin, 1997). Commonly framed within schemes pertaining to strength of signaling or agonist-directed trafficking of receptor stimulus, the idea of agonist-dependent differences in G protein activation has enormous importance in understanding modes of receptor action and provides the basis for postulating not only quantitative but qualitative differences in cell response as a function of agonist concentration and time.

Inferences of G protein activation based on effectors or subsequent signaling can be difficult. Effectors are almost always subject to regulation by more than one G protein, and measures of second messengers (as well as many effectors) usually require the intact cell. Confounding issues are nonlinearity in signal transmission, cross-regulation of effectors, and receptor desensitization. Consequently, several groups have turned to measurements of G protein activation directly (Gettys et al., 1994; Hartman and Northup, 1996; Cordeaux et al., 2000; Wenzel-Seifert and Seifert, 2000; Akam et al., 2001; Heise et al., 2001; Seifert et al., 2001). The report by Cussac et al. (2002) in this issue of *Molecular Pharmacology* describes the activation of G proteins in Chinese hamster ovary (CHO) cells expressing the VSV (edited) isoform of the human 5-hydroxytryptamine$_{2c}$ (5-HT$_{2c}$) receptor. This group demonstrates through agonist-promoted [^{35}S]GTPγS binding that the 5-HT$_{2c}$ receptor couples to G$_i$ (ostensibly G$_{i3}$ in CHO cells) and G$_q$ (G$_{q/11}$) and that G$_q$ is more readily activated than G$_i$ by all agonists working through the receptor; one agonist activates G$_q$ alone. Thus, for at least some agonists at some concentrations, G$_q$ is activated preferentially through the 5-HT$_{2c}$ receptor.

The use of agonist-promoted binding of [^{35}S]GTPγS in one form or another to evaluate interactions between receptors and G proteins is not new. The assay exploits a property fundamental to transduction; i.e., an activated receptor promotes exchange of GDP for GTP (or related nucleotide, in this instance [^{35}S]GTPγS) on the G protein α subunit. The use of [^{35}S]GTPγS is based on its resistance to hydrolysis, its high affinity (usually) for the Gα subunit, and its relatively high specific activity. Among the earliest but still quite commonly used forms of the assay are those that examine the rate of binding of [^{35}S]GTPγS to membranes without resolution of individual G proteins. Filtration of membranes is used to separate bound from free [^{35}S]GTPγS; hence, the advantage of this kind of assay is speed of processing. One of the disadvantages is that the binding represents an average among different G proteins with no clear picture of binding for any single one. Other disadvantages include often high backgrounds that limit signal-to-noise ratios. Cussac et al. (2002) compare assays involving the membrane alone and subsequently resolved G proteins (see below). Results for the two assays are congruent, due primarily to the adept use of pertussis toxin in the former to resolve (through inhibition) G$_i$ from other G proteins; the latter conceivably include G$_s$, G$_q$, G$_{12}$, and G$_{13}$ (and G$_z$ in some cells). Although PTX-resistant G proteins other than G$_q$ were not evaluated in this study, the VSV form of the 5-HT$_{2c}$ receptor is thought not to couple to G$_{13}$ (Price et al., 2001), and no evidence exists for any coupling of the receptor to G$_s$.

The assay that best permits analysis of differential G protein engagement is, of course, one in which G proteins are resolved. Resolution is achieved using antibodies directed toward specific G protein α subunits after exposure of the membranes to [^{35}S]GTPγS plus agonists and extraction of the membranes with detergent. The specificity of the antibodies is critical, as is

ABBREVIATIONS: CHO, Chinese hamster ovary; 5-HT, 5-hydroxytryptamine; [^{35}S]GTPγS, guanosine 5′-(γ-[^{35}S]thio)-triphosphate.

the ability of the antibodies to immunoprecipitate G protein α subunits under nondenaturing conditions. This form of assay was developed initially simply to determine which G proteins a given receptor might activate without resort to inferences based on second messengers (Barr et al., 1997). More germane to the study by Cussac et al. (2002), however, is the potential of [^{35}S]GTPγS binding when evaluated for G proteins to provide an estimate of efficacy proximal to the receptor.

Assays based on binding of [^{35}S]GTPγS are not without pitfalls, some of which bear directly on measurements of efficacy. The single most important issue to consider is the fact that conditions employed for assay of one G protein often differ from those employed for assay of another. Concentrations of GDP, for example, are commonly varied in the assay of different families of G proteins to enhance signal-to-noise ratios. This is not surprising, because G proteins often have quite different affinities for GDP, such that some G proteins have a propensity to bind GTPγS independently of receptor whereas others do not. Differences in assay conditions can obviously confuse analyses of efficacy. How can one know unequivocally whether conditions used in vitro to analyze coupling in fact place a G protein in a state of sensitivity to activated receptor similar to that existing in the intact cell? Might G_q, for example, be more readily activated than G_i in vitro but not in the intact cell? In this sense, the regulation of second messenger systems in the intact cell—previously noted deficiencies notwithstanding—might provide useful corroboration. Another consideration is sensitivity. In only a few instances has [^{35}S]GTPγS-binding been defined for agonists working through endogenous receptors and G proteins. Generally, receptor or G protein (or both) is overexpressed to achieve measurable binding. Overexpression, however, can have considerable impact on efficacy. Overexpression of a receptor in particular can lead to a situation in which G proteins become limiting ("receptor reserve"), so that partial agonists seem instead to be full agonists. Cussac et al. (2002), employing 5-HT_{2C} receptor overexpression, deal very carefully with this issue, using endogenous G proteins as readouts and varying levels of functional receptor using an alkylating reagent. The data for G_{i3} are particularly good, demonstrating the lack of receptor reserve for this G protein and defining [^{35}S]GTPγS-binding as a function of receptor occupancy; the ranking of agonists according to efficacy was found not to change with receptor expression. The alkylation experiments reveal a substantial receptor reserve for several of the agonists for G_q, however, preventing a detailed an analysis of relative efficacy in this case. The key finding that 5-HT_{2C} receptors are coupled more efficiently to G_q than to G_{i3}, at least under the conditions of the assay, is nevertheless clearly borne out. One other important but often overlooked consideration in the analysis of [^{35}S]GTPγS-binding is the verification for each G protein that binding is linear with respect to time, in that it is the rate of [^{35}S]GTPγS binding, not the final extent, that is the desired parameter.

True agonist-directed trafficking of receptor stimulus, in which agonists variously stabilize different conformations of receptor that are in turn selective for different G proteins (Kenakin, 1997), is most easily invoked when the rank-order of efficacies characterizing the agonists differs from one G protein to another. This is not the case for activation of G_i and G_q through the 5-HT_{2c} receptor, where the ordering of agonists according to efficacy seems to be the same (to the extent analyzed) for both G proteins. The data therefore conform more to differences in degree of G protein activation according to strength of signaling. Differences in degree of coupling to two or more G proteins can be anticipated to typify the situation for a very large number of receptors and is an important concept in its own right—a G protein (G_q) can be activated at a time when another (G_i) is to all intents not, whether by a weak agonist at any concentration or, by extension, a strong agonist at low concentrations, although the receptor couples potentially to both G proteins. Depending on the rise and fall of serotonin concentrations at the receptor physiologically, G_q and G_i might also be activated sequentially, with the temporal relationship of the two events critical to cell function depending on the extent to which they trigger downstream phenomena.

A number of questions can be asked at this point. Why exactly would agonists working through the 5-HT_{2c} receptor activate G_q more easily than G_i? Elements of Gα structure are certainly relevant, however differences in targeting to microdomains and ancillary factors cannot be precluded. Might the converse be true for certain other receptors? Data for the M_1, M_3, and adenosine A_1 receptors indeed suggest that receptors can differ from each other, at least in relative strength of signaling to G_i and G_q (Cordeaux et al., 2000; Akam et al., 2001). Would differences identified here for CHO cells hold true for other cells or if G_{i1} or G_{i2} were examined instead of G_{i3} in these other cells? Differences between $G_{i1/2}$ and G_{i3} have been cited previously, to the extent, in fact, of supporting true agonist-directed targeting of receptor stimulus (Gettys et al., 1994). How are the data affected by differences in relative levels of G proteins, and how might they be extended to receptors that communicate with G_{12} and G_{13}? Finally, how can observations of efficacy established for G proteins best be integrated with concepts of intrinsic efficacy on the one hand and physiological consequences on the other?

References

Akam EC, Challiss RA, and Nahorski SR (2001) $G_{q/11}$ and $G_{i/o}$ activation profiles in CHO cells expressing human muscarinic acetylcholine receptors: dependence on agonist as well as receptor-subtype. *Br J Pharmacol* **132:**950–958.

Barr AJ, Brass LF, and Manning DR (1997) Reconstitution of receptors and GTP-binding regulatory proteins (G proteins) in Sf9 cells. *J Biol Chem* **272:**2223–2229.

Cordeaux Y, Briddon SJ, Megson AE, McDonnell J, Dickenson JM, and Hill SJ (2000) Influence of receptor number on functional responses elicited by agonists acting at the human adenosine A_1 receptor: evidence for signaling pathway-dependent changes in agonist potency and relative intrinsic activity. *Mol Pharmacol* **58:**1075–1084.

Cussac D, Newman-Tancredi A, Duqueyroix D, Pasteau V, Millan MJ (2002) Differential activation of Gq/11 and Gi_3 proteins at 5-hydroxytryptamine$_{2C}$ receptors revealed by antibody capture assays: influence of receptor reserve and relationship to agonist-directed trafficking. *Mol Pharmacol* **62:**578–589.

Gettys TW, Fields TA, and Raymond JR (1994) Selective activation of inhibitory G-protein α-subunits by partial agonists of the human 5-HT_{1A} receptor. *Biochemistry* **33:**4283–4290.

Hartman JL and Northup JK (1996) Functional reconstitution in situ of 5-hydroxytryptamine$_{2c}$ (5HT_{2c}) receptors with α_q and inverse agonism of 5HT_{2c} receptor antagonists. *J Biol Chem* **271:**22591–22597.

Heise CE, Santos WL, Schreihofer AM, Heasely BH, Mukhin YV, MacDonald TL, and Lynch KR (2001) Activity of 2-substituted lysophosphatidic acid (LA) analogs at LPA receptors: discovery of a LPA_1/LPA_3 receptor antagonist. *Mol Pharmacol* **60:**1173–1180.

Kenakin T (1997) *Pharmacologic Analysis of Drug-Receptor Interaction*, 3rd ed. Lippincott-Raven, Philadelphia.

Price RD, Weiner DM, Chang MSS, and Sanders-Bush E (2001) RNA editing of the human serotonin 5-HT_{2C} receptor alters receptor-mediated activation of G_{13} protein. *J Biol Chem* **276:**44663–44668.

Seifert R, Wenzel-Seifert K, Gether U, and Kobilka BK (2001) Functional differences between full and partial agonists: evidence for ligand-specific receptor conformations. *J Pharmacol Exp Ther* **297:**1218–1226.

Wenzel-Seifert K and Seifert R (2000) Molecular analysis of β_2-adrenoceptor coupling to G_s-, G_i- and G_q-proteins. *Mol Pharmacol* **58:**954–966.

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