

Chapter 2

Functional Selectivity: Theoretical Considerations and Future Directions

Terry Kenakin

Abstract The selective activation of receptors by some agonists to emphasize some but not all aspects of the receptor signaling capability was proposed on theoretical grounds in 1995 because of data showing reversal of relative orders of potency for different stimulus pathways linked to a single receptor. These data precluded the notion that all agonists produce a single receptor active state. Since that time, a number of different lines of evidence indicate that ligands can bias receptor toward different pathways in cells. Conformational selection within the ensemble of conformation receptors formed during normal function theoretically is capable of producing functional selectivity; this chapter discusses the thermodynamic nature of this effect. Finally, although functional selectivity is a well-documented pharmacological phenomenon duplicated in many laboratories, it is still unclear whether it can be harnessed to produce therapeutically unique effect; it is hoped that studies in translational medicine with functionally selective ligands will furnish the link to therapy.

Keywords Receptor theory, Functional selectivity, Receptor signaling

2.1 Introduction: Linear View of Efficacy

The concept of agonist efficacy was required when it was observed that agonist occupancy curves and functional response curves did not coincide with respect to location along the concentration axis (functional curves are shifted to the left of occupancy curves). To accommodate this, it is necessary to invoke some property of the agonist operative in the production of tissue response, specifically a property of the agonist variously referred to as “intrinsic activity” (1), efficacy (2) and intrinsic efficacy (3). These were not terms rooted in physiology but rather were mathematical

T. Kenakin

Department of Biological Reagents and Assay Development

GlaxoSmithKline Research and Development Research Triangle Park, NC 27709

e-mail: Terry.P.Kenakin@gsk.com

terms inserted to make experimental results coincide with theory. A more physiological approach yielded the standard that largely has replaced these early theories, namely Operational Theory where efficacy and tissue sensitivity is quantified by a term τ (4). Although the settings for these efficacies have changed over the years, the basic assumption driving all of them has not, namely that efficacy stems from a single activated receptor state. This assumption has furnished the basis for receptor and drug classification such as agonist potency ratios. The necessity for invoking functional selectivity as described in this volume stems from violations of these predictions of classical receptor theory.

When considering equiactive concentrations of agonist under null conditions, it is assumed that the ability of each agonist to produce response is subject to identical cellular constraints leaving the difference in potency to be solely due to molecular features controlling ligand affinity and efficacy. If this premise is violated, then the resulting potency ratio loses this immutable property of being dependent on chemical features of the agonists and takes on tissue-specific characteristics. This can be illustrated by analyzing potency ratios for agonists at different stages of stimulus–response coupling. Figure 2.1 shows the effects of β -adrenoceptor agonists on two cardiac functions in rat atria that are both mediated by elevation of cytosolic cyclic AMP through the same receptor activation process (5). At some point in the cell, the two processes utilize the cyclic AMP to yield positive inotropy (increased strength of contraction) and the other to elicit lusitropy (increased rate of relaxation). It also is observed that the lusitropic response is more sensitive to agonist stimulation probably because of a more efficient coupling of the relaxation mechanism to elevated cyclic AMP. It can be seen in Fig. 2.1 that relative potency ratios of two β -adrenoceptor agonists, isoproterenol and pirbuterol, yield the same relative potency when each is compared within the same stimulus–response pathway. This is consistent with equal ratios of τ since this term reflects the efficacy of the agonists (a constant molecular term), the receptor density (constant for both agonists since testing is done in the same tissue), and K_E (reflecting the efficiency of coupling of receptors to the response cascade, which is common for the two agonists when the same pathway is compared).

When relative potencies are compared across two different stimulus–response coupling cascades, then different K_E values for the response are operative. It can be seen that when this prerequisite is violated (compare relative potency for inotropy vs. lusitropy), the potency ratio is much different and not reflective of only agonist efficacy. However, within a given pathway (inotropy or lusitropy), potency ratios agree, a finding consistent with each agonist producing a single receptor active state. In general, such potency ratios have been very consistent for receptor and agonist classification over the years, since the definition of efficacy by Stephenson (2,) up to approximately 10 years ago. What has changed since that time is that the number of vantage points to view receptor activation has greatly increased with improving technology. Now there are many methods available to measure agonist interaction with the receptor and the resulting change in receptor behavior beyond simple organ response (i.e., guinea pig ileal contraction as was available to Stephenson), and these increased vantage points have shown an astounding

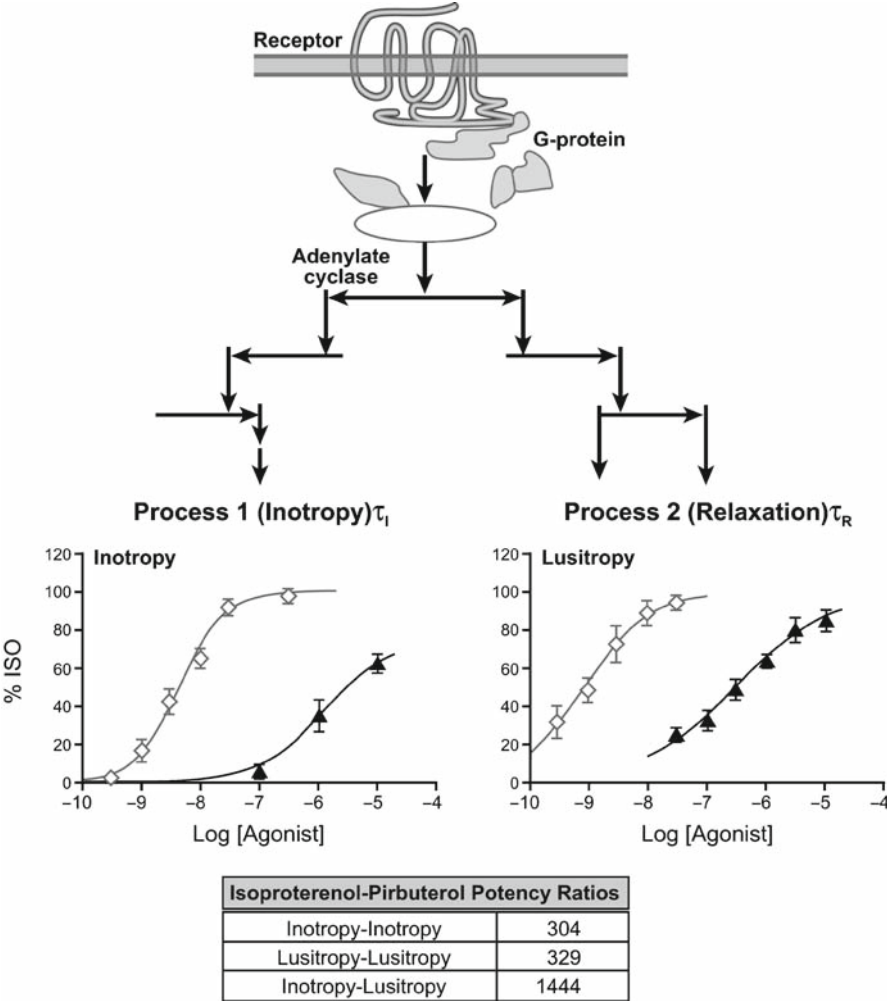


Fig. 2.1 The coupling of cardiac β -adrenoceptors to adenylate cyclase which, in turn, activates stimulus–response systems to increase cardiac inotropy and myocardial relaxation rate. The coupling is more efficient for relaxation than it is for inotropy, therefore agonists such as isoproterenol (*diamonds*) and pirbuterol (*triangles*) are more potent for lusitropy (relaxation). Within a given stimulus–response cascade, potency ratios are preserved in agreement with receptor theory predictions (tissue effects cancel). However, if this is violated and potencies are compared across stimulus–response coupling cascades the potency ratio is different

heterogeneity in receptor behavior with activation by different ligands. The other important observation is the fact that various receptors have been shown to be pleiotropic with respect to the number of G-proteins with which they interact (particularly with Family B (secretin) receptors). This phenomenon allowed some of the first

early opportunities to quantitatively measure more than one consequence of receptor activation by an agonist, namely the effect on separate G-protein pathways.

2.2 Multiple G-Proteins and Functional Selectivity

Although the most simple model dictates that a receptor couples monotonically to a single signaling pathway, there is no a priori reason for this to be the case for all receptors. Moreover, the demonstration that receptors pleiotropically couple to multiple G-proteins suggests that multiple coupling with differential signaling is possible. An early formal model depicting such multi receptor coupling behavior is based on two G-proteins interacting with one receptor (6) – see linkage model schematic Fig. 2.2. Intrinsic to such models is the fact that receptor species bound to ligand and/or G-proteins are energetically different than those that are not. Therefore, given two G-proteins, G_1 and G_2 , the energy required to form the two ternary species ARG_1 and ARG_2 will fundamentally be different. This furnishes thermodynamic reasons for a given ligand to not be equally adept at producing two such ternary species. The same argument applies to different spontaneous receptor/G-protein complexes, an idea supported by the fact that receptors have different intrinsic affinities for different G-proteins biochemically. The model also fits with the notion that proteins adopt a variety of conformations in accordance to variations in thermal energy (7–12). For example, mutation data, such as that reported for the α_2 -adrenoceptor, indicate that multiple receptor conformations are able to activate G-proteins, i.e., there can be multiple receptor active conformations. The model shown in Fig. 2.2 accommodates

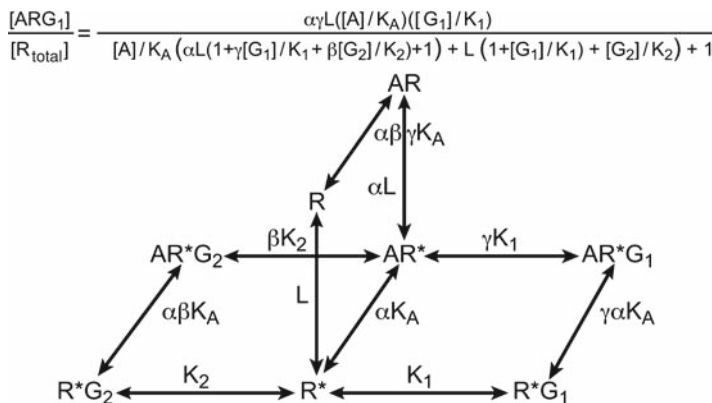


Fig. 2.2 Model of one receptor interacting with two G-proteins (G_1 and G_2). The affinity of the receptor differs for each G-protein (K_1 and K_2) as does that of the ligand-bound receptor (γK_1 βK_2). The receptor forms the active state of the receptor through selective affinity (α). The equation for production of one of the ternary complex species can be used to demonstrate how different agonists can traffic stimulus to different G-proteins and thus how reversals in potency ratios for agonists can occur through selective values of β and γ . This model was presented on theoretical grounds six years before the first experimental evidence to show such behavior was described in the literature (6)

this by the presence of specific parameters γ and β , which denote the possibility of differing affinities of the ligand-bound receptor for each G-protein. Interestingly, the model also predicts that different ligands have the capability of actually reversing their relative potency for different G-proteins (6).

The link between differential G-protein coupling and receptor conformation comes from mutation study data indicating that different regions of the receptor interact with different G-proteins. Under these circumstances, it would be highly unlikely that different receptor conformations would expose different regions of the receptor protein in identical ways. The corollary to this idea then is that different receptor conformations in systems that couple to multiple G-proteins would lead to differential activation of the G-protein pathways. The only theoretical piece missing to link these ideas to ligand functional selectivity is the ability of ligands to stabilize different receptor conformations.

A model of efficacy proposed by Burgen (13), namely conformational selection, is useful in imagining the interaction of a ligand with multiple receptor conformations. Burgen's view is that ligands stabilize various conformations by having selectively higher affinities for them (these will preferentially be stabilized). In turn, the preferential stabilization of some conformations in a system of freely interchangeable conformations necessitates that favored conformations will be formed at the expense of other conformations (Le Chatelier's principle, '*... If a dynamic equilibrium is disturbed by changing the conditions, the position of the equilibrium moves to counteract the change...*'). Therefore, when a ligand enters a collection of conformations (to be referred to as an ensemble), it could, by selective micro-affinities, create a new preferred ensemble (Fig. 2.3). Interestingly, since

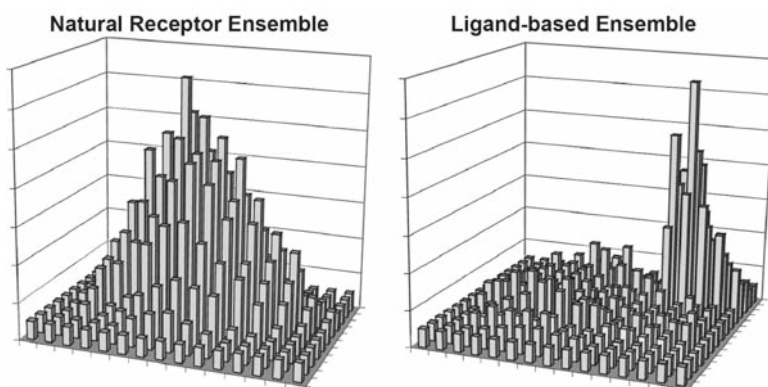


Fig. 2.3 Histograms depicting the relative abundance of receptor conformations for a receptor at rest (*left panel*) and in the presence of a ligand that has different affinities for the various states (*right panel*). In the latter case, the conformations for which the ligand has high affinity are stabilized and therefore enriched at the expense of other conformations. The composition of the new collection of conformations depends upon the molecular structure of the agonist; therefore, there is no a priori reason to suppose that the same ligand-bias will be formed by every agonist

multiple conformations are involved, it need not be that each type of ligand would stabilize an identical ensemble of conformations. In fact, since affinity is specific to chemical structure, it might be postulated that different ligands would *not* form identical ensembles, i.e., that ligands would produce different bias in the receptor conformation that subsequently interacts with the cell (14).

It can be shown that a ligand with varying affinities for a range of receptor conformations necessarily will change the composition of the conformational collection through binding. Assume an ensemble of receptor conformations R (denoted as the root “inactive” state) to R_i . It can be shown that the fraction of receptors not in the R state in the absence of a ligand is given by (15):

$$\rho_{\text{nonR}} = \frac{\sum_{i=1}^n L_i}{\left(1 + \sum_{i=1}^n L_i\right)} \quad (2.1)$$

Where L_1 to L_i are the allosteric constants for the various states ($L_i = [R_i]/[R]$). In the presence of a ligand A having an affinity of K for R and $\psi_1 K_1$ to $\psi_i K_i$ for each of the other states, this expression changes to:

$$\rho_{\text{nonR}} = \frac{\sum_{i=1}^n L_i + [A]/K \sum_{i=1}^n \psi_i L_i}{[A]/K \left(1 + \sum_{i=1}^n \psi_i L_i\right) + \left(1 + \sum_{i=1}^n L_i\right)} \quad (2.2)$$

It can be seen that (2.2) reduces to (2.1) (i.e., there will be no change in the make-up of the conformational ensemble) *only* if ψ_1 to $\psi_i = 1$, i.e., only if the affinity of the ligand for every single conformation is *identical*. If this is not the case, then the fraction of conformations different from R in the absence and presence of a ligand will change. By definition, this means that the binding of the ligand will change the nature of the ensemble of the receptor conformations present.

Thermodynamic and theoretical predictions indicate that ligands have the ability to stabilize different receptor conformations, and that these conformations interact with multiple components in the cell membrane (16). In addition, the expectation would be that if these components interact with different regions of the receptor protein, then heterogenous interaction with differing conformations would result. This puts all of the theoretical pieces in place to describe ligand-specific functional selectivity. At this point in time, it remained for an experimental system to combine these various elements to demonstrate this effect. Early data to suggest this came from studies on the PACAP receptor, and this led to the first formal mechanistic model of ligand-specific functional selectivity (17); this model is shown in Fig. 2.4a. The model, formally identical to the one shown in Fig. 2.2, was invoked to describe a particularly striking experimental phenomenon seen in the literature with PACAP receptors, a pleiotropic receptor that activates pathways to elevate cyclic AMP and IP_3 . Specifically, it was seen that two PACAP peptide fragments

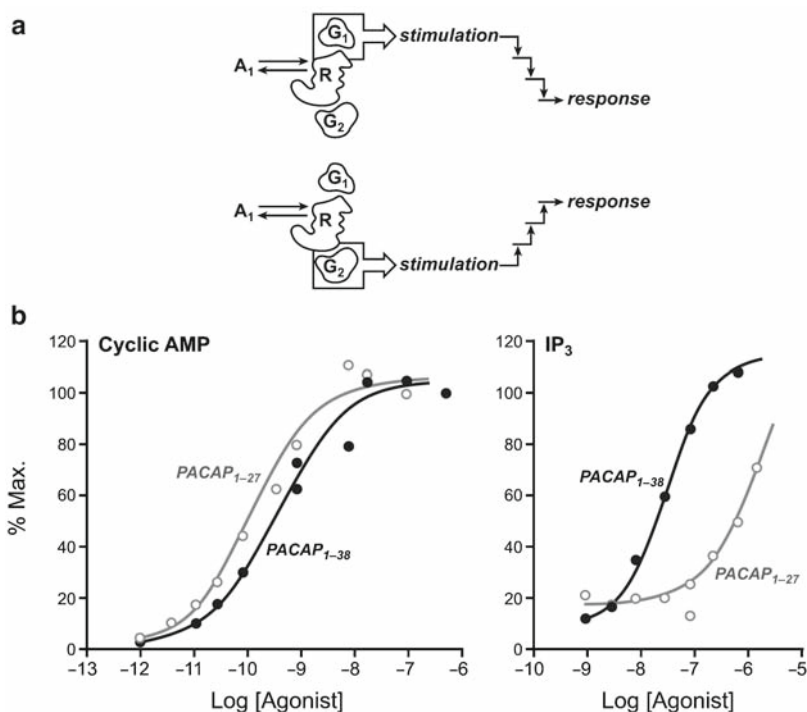


Fig. 2.4 Theoretical model of biased agonism (17) based on a one-receptor/two G-protein model. The first data to support this model were reported for PACAP receptors where reversed relative potencies of PACAP₁₋₂₇ and PACAP₁₋₃₈ are clearly inconsistent with a single receptor state produced by these two agonists (model from (17); data from (18))

(PACAP₁₋₂₇ and PACAP₁₋₃₈) produced elevated cyclic AMP and IP₃ in cells but the relative potency of these two agonists for these pathways was *reversed* (18). Thus, the relative efficacy of PACAP₁₋₂₇ for cyclic AMP elevation is higher than that for PACAP₁₋₃₈ but lower for elevation of IP₃. This phenomenon is not compatible with these agonists producing a single active state of the receptor that goes on to activate these two pathways. In contrast, it suggests that PACAP₁₋₂₇ produces an active state with higher efficacy for cyclic AMP stimulus components (relative to PACAP₁₋₃₈) and that PACAP₁₋₃₈ produces an active state with higher efficacy for IP₃ stimulus components.

The model depicted in Fig. 2.4 is sufficient to describe the differential signaling properties of PACAP₁₋₂₇ and PACAP₁₋₃₈ but no doubt other models are capable of doing this. The more important outcome of the analysis of the PACAP data is the demonstration of total inconsistency of such behavior with a single receptor active state model of agonist function. These data provided a serious question to the assumption that agonists form only one receptor active state to induce response but it should be noted that the conceptual thread described here is not the only one questioning the linear concept of agonist efficacy (*vide infra*).

This phenomenon originally was labeled as “stimulus trafficking” when first described (17) but subsequently has been referred to in the literature by a number of labels including “biased agonism,” “collateral efficacy,” “receptor-based functional selectivity,” “conformation-based functional selectivity,” and simply “functional selectivity.” In subsequent years, versions of this phenomenon, namely differential signaling by different agonists acting on the same receptor, also have been described in a variety of settings beyond multiple G-protein activation including desensitization, phosphorylation, receptor internalization, and, recently and notably in β -arrestin/receptor interactions (19–25). Also over the past decade, advances in technology have led to independent data to support the notion that different ligands stabilize different conformations of the same receptor (26–30).

2.3 Links to Established Allosteric Mechanisms

The previous discussion is concerned with ligands that bind in special ways to the receptor to produce an active effect. However, there is no mechanistic difference between this effect and long established models describing allosteric effects of molecules, i.e., standard allosteric molecules such as muscarinic modulators that bind to receptors to stabilize certain conformations that have special properties with respect to their interaction with natural ligands. The operational differences between these established mechanisms and functionally active ligands may involve the geography of binding (i.e., functional antagonists may or may not bind to the natural orthosteric endogenous agonist binding site), and the fact that the allosteric effect is expressed through an active receptor property (functional agonism) as opposed to modification of the effects of other ligands (allosteric modulation). However, the lines become blurred in these distinctions with allosteric agonists such as alcuronium where effects on endogenous agonists are mixed with a direct agonism by the allosteric ligand (31).

Functional selectivity is remarkably similar to classical allosteric modulation. Thus the binding of an allosteric modulator can impose functional selectivity on endogenous agonism through bias of the conformations possible with the binding of the endogenous agonist. For example, in systems containing CRTH2 receptors, the allosteric modulator $N\alpha$ -tosyltryptophan causes the natural agonist prostaglandin D2 to change from G_i and β -arrestin activation to solely G_i -activation (with no concomitant β -arrestin interaction; (32)). Similarly, the allosteric modulator AMD3100 blocks natural agonist (SDF-1 α)-mediated chemotaxis via CXCR4 receptor but not the effects of peptide fragments RSVM and ASLW (33). Also, the natural agonist neurokinin A acts via NK2 receptors to activate G_s and G_q , while the allosteric modulator LP1805 changes this pattern to one of enhanced G_q activation and blockade of G_s activation (34). The key to these effects is the fact that an allosteric mechanism allows the receptor to be permissive and edit the effects of other ligands by cobinding with them. The relationship between functional agonism and classical allosteric mechanisms is illustrated schematically in Fig. 2.5.

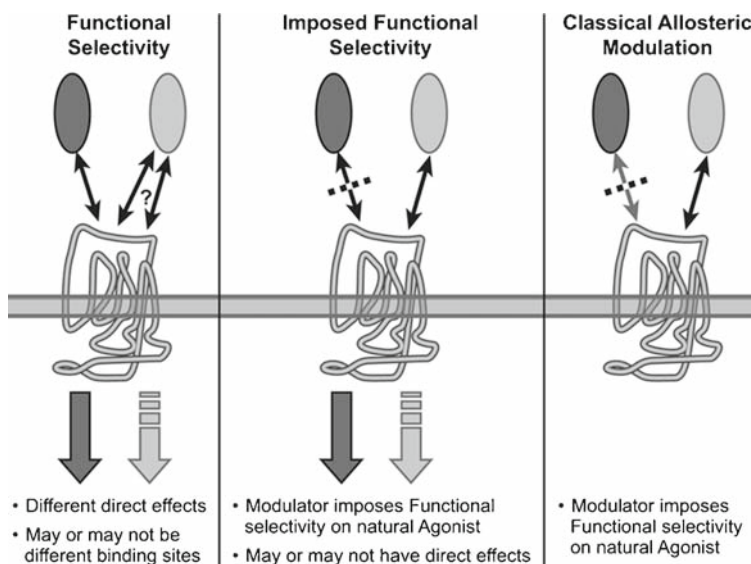


Fig. 2.5 Relationship of functional selectivity (stimulus trafficking) to conventional ligand-induced allosterism. The production of different receptor active states (*left panel*) may or may not involve a binding site for the selective agonism separate from the endogenous binding site. Conventional models of allosterism (*right panel*) describe a modulator binding to a site separate from the endogenous site to modify the interaction of the receptor with the endogenous agonist. There is no formal difference between this model and one that describes direct consequences of modulator binding (i.e., agonism) or modification of endogenous signaling by a modulator (*middle panel*)

2.4 Beyond G-Proteins and Application to Therapeutics

At this point in time, there is little doubt that ligands are able to exhibit functional selectivity and the question now becomes, is it physiologically relevant and can pharmacology harness such a potentially powerful mechanism to therapeutic advantage? Some of the earliest work in this area, originating from work closely associated with therapeutics (namely dopamine treatment of CNS disorders; for review see (35)), suggests that direct therapeutic advantages may be derived from functional selectivity. Functional selectivity also has been associated with CNS behavior patterns for serotonin ligands through the 5-HT_{2A} receptor providing further links to the molecular mechanism and therapeutic events (36). For future work in this field, two general ideas may be relevant. The first is that the efficacy of any given ligand is defined by the assay used to detect it, e.g., the ERK stimulating activity of propranolol was not detected for 40 years until propranolol was tested in an ERK assay (37–39). A related idea is that binding of ligands to receptors is an active, not passive, process and that ensembles of receptor conformations are changed by the binding of ligands (i.e., see (40)). Therefore, the most generic

screening assay available may be the most efficient since this would detect all compounds that bind to the receptor with no reference to predefined efficacy. Functional efficacies then could be detected in various other assays on a smaller scale. Thus, a generic screen (i.e., bioluminescence resonance energy transfer (BRET) and fluorescence resonance energy transfer (FRET)) of a million compounds might detect 300 that bind and then these could be tested in 5–10 therapeutically oriented assays to determine possible useful activity (Fig. 2.6). This may be preferable to arbitrarily choosing a therapeutically oriented assay to start with and risk not seeing ligand interaction with molecules that bind to the target but do not elicit that particular observed effect. In this sense, any ligand that binds to the receptor should be considered a potentially efficacious drug in a variety of settings.

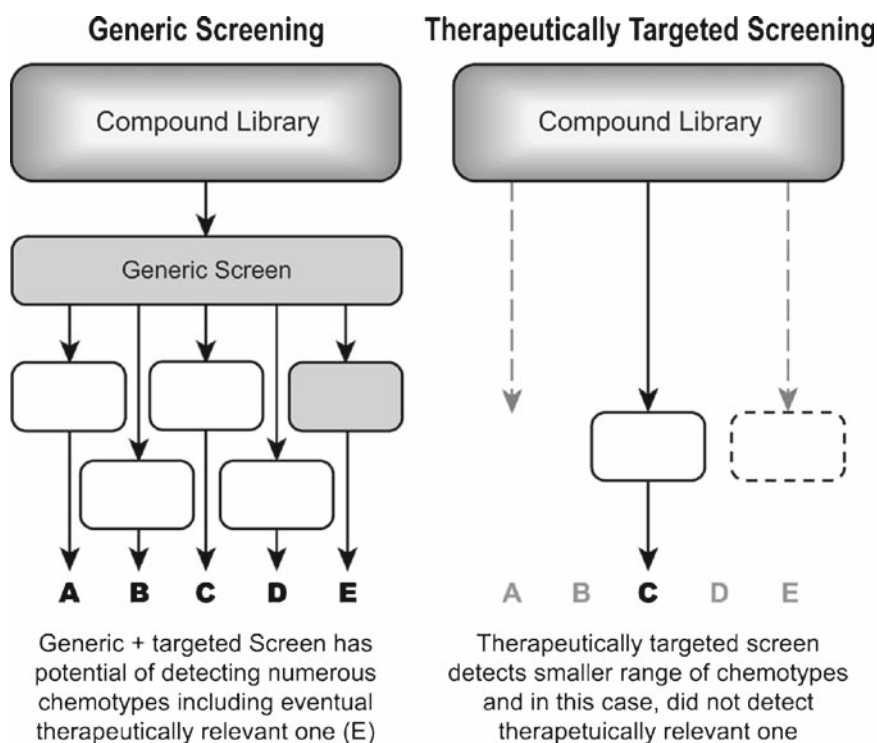


Fig. 2.6 Two modes of screening. In generic screening (i.e., BRET or FRET detection of ligand-receptor interaction), the fact that the ligand-bound receptor is thermodynamically different from the unliganded receptor predicts that all molecules that bind to the receptor will be detected. Secondary testing of the subset of binding molecules (much smaller set than the original library) can then bind compounds with respect to function. On the *right* is shown a therapeutically relevant screen where a specific receptor coupling pathway is chosen for detection. This may shorten the process if evidence is strong that the pathway is all that is required for therapeutic activity. On the other hand, ligands with unknown potential will be missed and the approach will not work if the chosen pathway is the incorrect one

It may be useful to speculate on where functional selectivity of natural system might be a useful physiological control. One such area might be in systems with pleiotropic receptor coupling. For example, associations between selective coupling and physiology have been made for the thyrotropin receptor, which couples to Gs and Gq protein; the Gs protein coupling may be associated with thyroid growth and differentiation, while the Gq coupling may be more associated with thyroid hormone synthesis (41). Another case may be the orexin receptor, where selective agonism may have significance with respect to differences in adrenal steroid production and release (Gs protein) and catecholamine release (Gq protein) (42,43).

A second area where functional selectivity may be important is in redundant systems, and it raises the question whether or not natural systems make use of this potentially powerful mechanism. There are suggestions that this may be the case. Thus, studies show that ligand-bound receptor active states (some with natural ligands such as catecholamines, dopamine, and natural enkephalins) differ from spontaneously formed constitutive active states (44–46). This would be a way to achieve fine control of signaling through the same receptor in response to hormonal input vs cellular constitutive setpoints.

Another setting where functional selectivity may be important is systems where the chemical input to the receptor is redundant. Perhaps the most redundant and pleiotropic receptor system of all is the chemokine system, where multiple natural agonists are known to activate a range of receptors (Fig. 2.7). It might be expected that this redundancy could naturally be exploited to yield subtle differences in signaling for physiological benefit. Evidence of such functional selectivity is emerging;

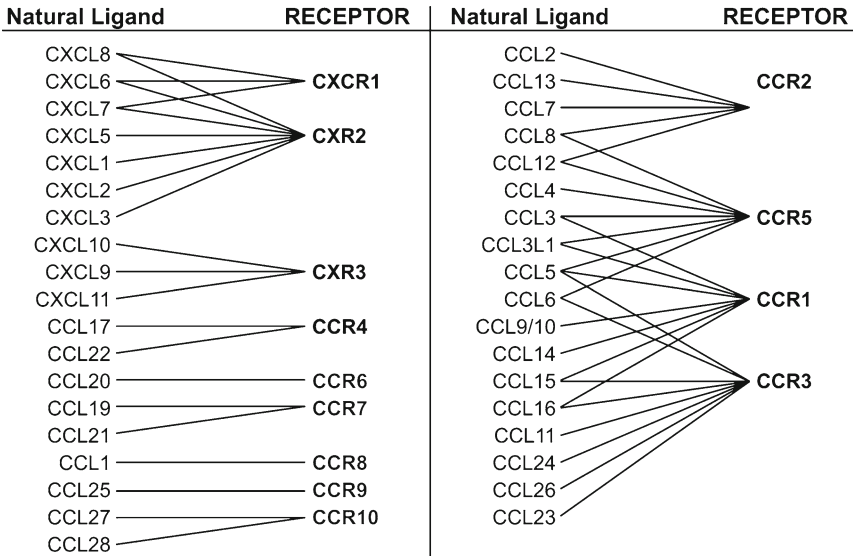


Fig. 2.7 Redundancy of chemical input to chemokine receptors. Taken from (47)

for example, two natural agonists for the CCR7 chemokine receptor, namely CCL19 and CCL21, differ in the type of pathway stimulation they elicit through this receptor. Specifically, only CCL19, not CCL21, causes the receptor to undergo agonist-dependent phosphorylation and recruitment of β -arrestin as a means of terminating the G-protein stimulus (48).

A therapeutic application of functional selectivity theoretically can be found in the treatment of AIDS through CCR5-mediated blockade of HIV-1 entry. Specifically, a number of allosteric CCR5 HIV-1 entry inhibitors have been described (see 49,50 for review) and these function through prevention of the virus binding to and utilizing CCR5 for infection. Interestingly, separate data indicate that the natural chemokine system can be beneficial in the delay of AIDS from HIV-1 infection (51–56). Although suggestive, these studies are difficult to interpret since measurement of elevated chemokines is technically difficult as chemokines are produced and utilized at the site of action. A novel way around this limitation has been reported in a large clinical trial (1,064 patients) where the gene copy number for a variable chemokine ligand for CCR5 (CCL3L1) is strongly correlated with AIDS survival (57). Specifically, patients with high gene copy numbers for CCL3L1 have a much greater rate of survival and slowed progression to AIDS than patients with a low gene copy number for this chemokine. At present, on the one hand, all clinically tested CCR5 HIV-1 inhibitors block chemokine function as well as HIV-1 entry; theoretically, an allosteric modulator that prevents the utilization of CCR5 by HIV-1 but otherwise allows the natural chemokine system to function through this receptor (i.e., exhibits functional selectivity) could increase the efficacy for treatment of AIDS. On the other hand, it may be simplistic to suggest that preservation of chemokine function could uniformly be beneficial since CCR5 receptor activation through natural chemokines is known to produce a variety of effects, some not conducive to protection against AIDS (Table 2.1). Thus, although activation of AKT and increased neuronal survival in AIDS dementia have been suggested to be useful effects of chemokine receptor stimulation in HIV-infected patients, other signals such as activation of P38 leading to immunocompetent cell death, Gi-protein-mediated increased replication of HIV virus, and nonspecific inflammation have negative ramifications (58–61). This makes it incumbent upon pharmacologists to understand the pathophysiology of the target to define which functions mediated by the receptor would be therapeutically beneficial. One protective action of CCR5 activation that has been identified as beneficial is the internalization of the CCR5 receptor since this would block HIV-1 entry and also preclude viral resistance through mutation (51–56).

Table 2.1 Physiological consequences of CCR5 receptor activation

| Adverse effects | Beneficial effects |
|--|---|
| <ul style="list-style-type: none"> • Immunocompetent cell death (\uparrowP38) • \uparrowHIV replication • \uparrowInflammation | <ul style="list-style-type: none"> • Immune preservation (\uparrowAKT) • \uparrowCCR5 internalization • \uparrowNeuronal survival (AIDS Dementia) |

Data from (58–61)

2.5 Conclusions and Perspective

The ability of different ligands to direct signals to different cellular interactants to produce texture in signaling (a general phenomenon referred to as “functional selectivity”) is well established in mechanistic, theoretical, thermodynamic, and experimental terms. It would be tempting to believe that such a versatile physiological system would not be redundant in normal biology but rather would be employed for fine control of chemical signaling to cells. However, to date, sound data to associate this pharmacological mechanism with normal physiology or pathophysiology is still lacking. However, this gap should be eliminated in the near future as new classified functional ligands are introduced into clinical therapy; it would be hoped that translational medicine will connect functional selectivity with therapeutic phenotypic behavior. The supply end of this process still requires functionally selective ligands and the key to finding these is the appropriate assay system (both in screening and lead optimization of new chemical entities). At the very least, the concept of receptors as “on-off” switches (as first described by John Newport Langley (1852–1926) – (62)) is laid to rest with the appreciation of the complexity of seven transmembrane signal processing capability.

References

1. Ariens EJ. Affinity and intrinsic activity in the theory of competitive inhibition. *Arch Int Pharmacodyn Ther* 1954; 99:32–49.
2. Stephenson RP. A modification of receptor theory. *Br J Pharmacol* 1956; 11:379–93.
3. Furchgott RF. The use of β -haloalkylamines in the differentiation of receptors and in the determination of dissociation constants of receptor-agonist complexes. In: Harper NJ, Simmonds AB, eds. *Advances in Drug Research*, Vol. 3, Academic Press, New York; 1966: 21–55
4. Black JW, Leff P. Operational models of pharmacological agonism. *Proc R Soc Lond [Biol]* 1983; 220:141–62.
5. Kenakin TP, Ambrose JR, Irving PE. The relative efficiency of β -adrenoceptor coupling to myocardial inotropy and diastolic relaxation: Organ selective treatment for diastolic dysfunction. *J Pharmacol Exp Ther* 1991; 257:1189–97.
6. Kenakin TP, Morgan PH. The theoretical effects of single and multiple transducer receptor coupling proteins on estimates of the relative potency of agonists. *Mol Pharmacol* 1989; 35:214–22.
7. Fraunfelder H, Parak F, Young RD. Conformational substrates in proteins. *Annu Rev Biophys Biophys Chem* 1988; 17: 451–79.
8. Fraunfelder H, Sligar SG, Wolynes PG. The energy landscapes and motions of proteins. *Science* 1991; 254:1598–603.
9. Hilser J, Freire E. Predicting the equilibrium protein folding pathway: structure-based analysis of staphylococcal nuclease. *Protein Struct Funct Genet* 1997; 27:171–83.
10. Hilser J, Dowdy D, Oas TG, Freire E. The structural distribution of cooperative interactions in proteins: Analysis of the native state ensemble. *Proc Natl Acad Sci USA* 1998; 95:9903–8.
11. Onaran HO, Costa T. Agonist efficacy and allosteric models of receptor action. *Ann NY Acad Sci* 1997; 812:98–115.

12. Onaran HO, Scheer A, Cotecchia S, Costa T. A look at receptor efficacy. From the signaling network of the cell to the intramolecular motion of the receptor. In: Kenakin TP, Angus JA, eds. *The Pharmacology of Functional, Biochemical, and Recombinant Systems Handbook of Experimental Pharmacology*, Vol. 148, Springer, Heidelberg; 2000; 217–80.
13. Burgen ASV. Conformational changes and drug action. *Fed Proc* 1966; 40:2723–8.
14. Kenakin TP. Efficacy at G protein coupled receptors. *Nat Rev (Drug Discovery)* 2002; 1:103–9.
15. Kenakin, TP. Collateral efficacy as pharmacological problem applied to new drug discovery. *Expert Opin Drug Disc* 2006; 1:635–52.
16. Kenakin TP. Collateral efficacy in drug discovery: taking advantage of the good (allosteric) nature of 7TM receptors. *Trends Pharmacol Sci* 2007; 28:407–15.
17. Kenakin TP. Agonist-receptor efficacy II: Agonist-trafficking of receptor signals. *Trends Pharmacol Sci* 1995; 16:232–8.
18. Spengler D, Waerber C, Pantaloni C, Holsboer F, Bockaert J, Seeburg PH et al. Differential signal transduction by five splice variants of the PACAP receptor. *Nature (London)* 1993; 365:170–5.
19. Kenakin TP. Efficacy at G Protein Coupled Receptors. *Annu Rev Pharmacol Toxicol* 2002; 42:349–79.
20. Kenakin TP. Efficacy in drug receptor theory: Outdated concept or under-valued tool? *Trends Pharmacol Sci* 1999; 20:400–5.
21. Urban JD, Clarke WP, von Zastrow M, Nichols DE, Kobilka B, Weinstein H et al. Functional selectivity and classical concepts of quantitative pharmacology. *J Pharmacol Exp Ther* 2007; 320:1–13.
22. Hermans E. Biochemical and pharmacological control of the multiplicity of coupling at G-protein receptors. *Pharmacol Ther* 2003; 99:25–44.
23. Perez DM, Karnick SS. Multiple signaling states of G-protein coupled receptors *Pharmacol Rev* 2005; 57:147–61.
24. Kukkonen JP. Regulation of receptor-coupling to (multiple) G proteins: A challenge for basic research and drug discovery. *Recept Channels* 2004; 10:167–83.
25. Kenakin TP. Collateral efficacy in drug discovery: Taking advantage of the good (allosteric) nature of 7TM receptors (Special Issue on Allosterism and Collateral Efficacy in Drug Discovery). *Trends Pharmacol Sci* 2007; 28:407–15.
26. Gether U, Lin S, Kobilka BK. Fluorescent labeling of purified β 2-adrenergic receptor: evidence for ligand specific conformational changes. *J Biol Chem* 1995; 270:28268–75.
27. Ghanouni P, Gryczynski Z, Steenhuis JJ, Lee TW, Farrens DL, Lakowicz JR et al. Functionally different agonists produce distinct conformations in G-protein coupling domains of the β 2-adrenergic receptor. *J Biol Chem* 2001; 276:24433–6.
28. Palanche T, Ilien B, Zoffmann S, Reck MP, Nucher B, Edelstein SJ et al. The neurokinin A receptor activates calcium and cAMP responses through distinct conformational states. *J Biol Chem* 2001; 276:34853–61.
29. Swaminath G, Xiang Y, Lee TW, Steenhuis J, Parnot C, Kobilka BK et al. Sequential binding of agonists to the β 2 adrenoceptor: Kinetic evidence for intermediate conformational states. *J Biol Chem* 2004; 279:686–91.
30. Hruby VJ, Tollin G. Plasmon-waveguide resonance (PWR) spectroscopy for directly viewing rates of GPCR/G-protein interactions and quantifying affinities *Curr Opin Pharmacol* 2007; 7:507–14.
31. Jakubic J, Bacakova L, Lisá V, El-Fakahany EE. Tucek of muscarinic acetylcholine receptors via their allosteric binding sites *Proc Natl Acad Sci USA* 1996; 93:8705–9.
32. Mathiesen JM, Ulven T, Martini L, Gerlach LO, Heineman A, Kostenis E. Identification of indole derivatives exclusively interfering with a G protein-independent signaling pathway of the prostaglandin D2 receptor CRTH2. *Mol Pharmacol* 2005; 68:393–402.
33. Sachpatzidis A, Benton BK, Manfredi JP, Wang H, Hamilton A, Dohlman HG, Loliset E. Identification of allosteric peptide agonists. *J Biol Chem* 2003; 278:896–907.

34. Maillet EL, Pellegrini N, Valant C, Bucher B, Hibert M, Bourguignon J-J, Galzi J-L. A novel, conformation-specific allosteric inhibitor of the tachykinin NK2 receptor (NK2R) with functionally selective properties. *FASEB J* 2007; 21:2124–34.
35. Mailman RB, GPCR functional selectivity has therapeutic impact. *Trends Pharmacol Sci* 2007; 28:390–7.
36. Schmid CL, Raehal KM, Bohn LM. Agonist-directed signaling of the serotonin 2A receptor depends on β -arrestin2 interactions in vivo. *Proc Natl Acad Sci USA* 2008; 105:1079–84.
37. Galandrin S, Bouvier M. Distinct signaling profiles of β 1 and β 2 adrenergic receptor ligands toward adenylyl cyclase and mitogen-activated protein kinase reveals the pluridimensionality of efficacy. *Mol Pharmacol* 2006; 70:1575–84.
38. Azzi M, Charest PG, Angers S, Rousseau G, Kohout M. β -arrestin-mediated activation of MAPK by inverse agonists reveals distinct active conformations for G-protein-coupled receptors. *Proc Natl Acad Sci USA* 2003; 100:11406–11.
39. Baker JG, Hall IP, Hill SJ. Agonist and inverse agonist actions of β -blockers at the human β 2-adrenoceptor provide evidence for agonist-directed signaling. *Mol Pharmacol* 2003; 64:1357–69.
40. Kenakin TP, Onaran O. The ligand paradox between affinity and efficacy: Can you be there and not make a difference? *Trends Pharmacol Sci* 2002; 23:275–80.
41. Vassart G, Dumont D. The thyrotropin receptor and the regulation of thyrocyte function and growth *Endocr Rev* 1992; 13:596–611.
42. Mazzocchi G, Malendowicz LK, Aragona F, Nussdorfer GG. Human pheochromocytomas express orexin receptor type 2 gene and display an in vitro secretory response to orexins A and B. *J Clin Endocrinol Metab* 2001; 86:4818–21.
43. Mazzocchi G, Malendowicz LK, Gottardo AF, Nussdorfer GG. Orexin A stimulates cortisol secretion from human adrenocortical cells through activation of the adenylyl cyclase-dependent signaling cascade. *J Clin Endocrinol Metab* 2001; 86:778–82.
44. Zhou Y-Y, Cheng H, Song L-S, Wang D, Lakatta EG, Xiao R-P. Spontaneous β 2-adrenergic signaling fails to modulate L-type Ca^{2+} current in mouse ventricular myocytes. *Mol Pharmacol* 1999; 56:485–93.
45. Wiens BL, Nelson CS, Neve KA. Contribution of serine residues to constitutive and agonist-induced signaling via the D2s dopamine receptor: evidence for multiple, agonist-specific active conformations. *Mol Pharmacol* 1998; 54:435–44.
46. Liu JG, Ruckle MB, Prather PL. Constitutively active μ -opioid receptors inhibit adenylyl cyclase activity in intact cells and active G-proteins differently than the agonist [D-Ala2,N-MePhe4, Gly-ol5]enkephalin. *J Biol Chem* 2001; 276:37779–86.
47. Wells TNC, Power CA, Shaw JP, Proudfoot AEI. Chemokine blockers – therapeutics in the making? *Trends Pharmacol Sci* 2004; 27:41–47.
48. Kohout TA, Nicholas SL, Perry SJ, Reinhart G, Junger S, Struthers RS. Differential desensitization, receptor phosphorylation, β -arrestin recruitment, and ERK1/2 activation by the two endogenous ligands for the CC chemokine receptor 7. *J Biol Chem* 2004; 279:23214–22.
49. Kazmierski W, Peckham JP, Duan M, Kenakin TP, Jenkinson S, Gudmundsson KS et al. Recent progress in discovery of new CCR5 and CXCR4 chemokine receptor antagonists as inhibitors of HIV-1 entry. Part 2. *Curr Med Chem Anti-Infective Agents* 2005; 4:2456–72.
50. Kazmierski WM, Kenakin TP, Gudmundsson KS. Peptide, peptidomimetic and small-molecule drug discovery targeting HIV-1 host-cell attachment and entry through gp120, gp41, CCR5 and CXCR4. *Chem Biol Drug Dis* 2006; 67:13–26.
51. Xiang J, George SL, Wünschmann S, Chang Q, Klinzman D, Stapleton JT. Inhibition of HIV-1 replication by GB virus C infection through increases in RANTES, MIP-1 α , MIP-1 β , and SDF-1 *Lancet* 2004; 363:2040–6.
52. Garzino-Demo A, Moss RB, Margolick JB, Cleghorn F, Sill A, Blattner WA et al. Spontaneous and antigen-induced production of HIV-inhibitory β -chemokines are associated with AIDS-free status. *Proc Natl Acad Sci USA* 1999; 96:11986–91.

53. Heredia A, Davis C, Amoroso A, Dominique JK, Le N, Klingebiel E et al. Induction of G1 cycle arrest in T lymphocytes results in increased extracellular levels of β -chemokines: A strategy to inhibit R5 HIV-1. *Proc Natl Acad Sci* 2003; 100:4179–84
54. Ullum H, Cozzi A, Victor J, Aladdin H, Phillips AN, Gerstoft J et al. Production of β -chemokines in human immunodeficiency virus (HIV) infection: Evidence that high levels of macrophage inflammatory protein-1 β are associated with a decreased risk of HIV disease progression. *J Infect Dis* 1998; 177:331–7.
55. Lori F, Jessen H, Folli A, Lisiewicz J, Matteo PS. Long-term suppression of HIV-1 by hydroxyurea and didanosine. *JAMA* 1997; 277:1437–8
56. Rogez C, Martin M, Dereuddre-Bosquet N, Martal J, Dormont D, Clayette P. Human immunodeficiency virus activity of tau Interferon in human macrophages: involvement of cellular factors and β -chemokines. *J Virol* 2003; 77:12914–20
57. Gonzalez E, Kulkarni H, Bolivar H, Mangano A, Sanchez R, Catano G et al. The influence of *CCL3L1* gene-containing segmental duplications on HIV-1/AIDS susceptibility. *Science* 2005; 307:1434–40.
58. Choi W-T, Kaul M, Kumar S, Wang J, Kumar IMK, Dong CZ et al. Neuronal apoptotic signaling pathways probed and intervened by synthetically and modularly modified (SMM) chemokines. *J Biol Chem* 2007; 282:7154–63.
59. Kaul M, Lipton SA. Chemokines and activated macrophages in HIV gp120-induced neuronal apoptosis. *Proc Natl Acad Sci USA* 1999; 96:8212–6.
60. Tyner JW, Uchida O, Kajiwarra N, Kim EY, Patel AC, O'Sullivan MP et al. CCL5-CCR5 interaction provides antiapoptotic signals for macrophage survival during viral infection. *Nat Med* 2005; 11:1180–7.
61. Vlahakis SR, Villasis-Keever A, Gomez T, Vanegas M, Vlahakis N, Payaet CV. G protein-coupled chemokine receptors induce both survival and apoptotic signaling pathways. *J Immunol* 2002; 169:5546–54.
62. Holmstedt B, Liljestrand G. *Readings in Pharmacology*, Raven Press, New York, 1981.

Functional Selectivity of G Protein-Coupled Receptor
Ligands

New Opportunities for Drug Discovery

Neve, K. (Ed.)

2009, X, 292 p., Hardcover

ISBN: 978-1-60327-334-3

A product of Humana Press