I
General Concepts

1
Historical Background and Introduction

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Paul Ehrlich (1854–1915) and John Newport Langley (1854–1936) are generally credited with the introduction of the concept of receptors or receptive substances to describe the interaction of drugs with cells. A few years later, Alfred J. Clarke (1885–1941) began the process of applying mathematical modeling to the ligand/receptor interaction, and could thus be said to be the father of modern receptor theory. Receptor theory was then modified and expanded by others: Ariens' concept of partial agonists, Stephenson's seminal paper on efficacy, and Furchgott's modification of Stephenson's theory to produce the system-independent concept of intrinsic efficacy. (For a detailed account of the evolution of receptor theory and references see Kenakin, 2004 [1].) These developments, along with other contributions such as the Schild regression analysis, had receptor theory firmly established by the 1960s.

However, the theory was still very much based on the 'black box' concept; many scientists were still highly dubious that receptors existed as distinct proteins or entities. By the 1980s, new discoveries had begun to change the 'black box' concept. One was the cloning of receptors and another was the clear separation of ion channel receptors from receptors coupled to the newly discovered G proteins (initially also referred to as N-proteins, as an acronym for nucleotide-binding proteins). The discovery of G proteins also produced a modification of receptor theory to include precoupled receptors in what is now termed the ternary complex model. Thanks to technological advantages, scientists working on ion channels were able to record the activity of a single ion channel and realized it had a probability of being in the open state irrespective of the presence of ligand. Ligands simply altered the probability of it being in the open state. Because of this, it was easier to comprehend that 'baseline' activity could be accounted for by the probability of the channel being in the open state. Accordingly, it appeared possible to find ligands for receptors modulating these channels not only to increase their probability of being in the open state, but also to decrease this probability. Indeed, it was in the context of the GABA–benzodiazepine–receptor complex (GABA = γ-aminobutyric acid) that the term "inverse agonist" was first used to describe the allosteric modulation of the receptor complex by the benzodiazepines in a manner opposite to the modulation produced by GABA.

For G protein-coupled receptors (GPCRs), the skepticism about their existence vanished with the cloning of the first members of the superfamily, such as the β_2-adre-

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noceptor (β_2AR) [2, 3], but as far as receptor theory for GPCRs was concerned, there was no apparent need to postulate spontaneously active GPCRs. Receptor theory appeared to work quite nicely with only two classes of ligands, agonists and antagonists, and one quiescent state of the receptor.

However, evidence slowly began accumulating that, at least in reconstituted systems, empty or unliganded receptors could couple to G proteins [4]. This was followed by several studies providing more functional evidence in cell lines and membranes for direct activation or inhibition of second messenger assays by empty receptors and the notion that certain antagonists could prevent this basal activation [5–10]. Though a lot of the evidence came from studies using βARs, other GPCRs were shown to exhibit constitutive activity as well. In 1989, for example, Costa and Herz described the constitutive activity of δ-opioid receptors (DOP(δ)Rs) natively expressed in NG-108 neuroblastoma cells [5]. By substitution of potassium for sodium in the assay medium, they were able to enhance 'baseline' (unstimulated) high-affinity GTP hydrolysis used as an index of receptor–G protein coupling (see Chapter 8), thus demonstrating the constitutive activity of DOP(δ)Rs. This study also demonstrated that certain compounds termed 'negative antagonists' could decrease this raised baseline. These 'negative antagonists' had previously been classified as DOP(δ)R antagonists. However, not all opioid receptor antagonists were able to produce the decrease in baseline; some had little effect on baseline and were therefore called 'neutral antagonists'. The term 'negative antagonist' has now been largely replaced with the term 'inverse agonist', in part because of the IUPHAR Receptor Nomenclature Committee's recommendation. In 1993 and 1994, additional papers were published showing constitutive activity of other GPCRs, and compounds that behaved as inverse agonists at the receptors, most notably for 5-hydroxytryptamine receptors (5-HTRs) [11], bradykinin B2-receptors (B_2Rs) [12], and α_2-adrenoceptors (α_2Rs) [13].

At the same time, evidence started accumulating that GPCRs could also be mutated (usually in the third intracellular loop) to reveal a more robust constitutive activity, and that this spontaneous activity could again be modified by certain ligands. In fact, constitutively active mutant GPCRs were discovered serendipitously. Susanna Cotecchia, working in the Lefkowitz laboratory, had created a chimeric α_{1B}-adrenoceptor (α_{1B}AR), in which a short homologous stretch of amino acid residues from the α_{1B}AR was exchanged into the C-terminal portion of the third cytoplasmic loop of the β_2AR [14]. It was expected that this would decrease α_{1B}AR coupling to its cognate G protein, G_q. Instead, it unexpectedly led to agonist-independent – that is, constitutive – activity. Subsequently, it was demonstrated that virtually any amino acid replacement at a specific site in this region (alanine 293) resulted in graded levels of constitutive activity (see Chapter 11). This suggested that only the naturally occurring residue at this position was compatible with a completely constrained or inactive conformation of the receptor [15]. Subsequently, similar findings were published for the ß$_2$AR [16] and then the α_2AR [13]. As discussed below, these findings necessitated a rethinking of classical receptor theories, such as the ternary complex model. An extended ternary complex model (ETC model), which explains these findings, adds an explicit isomerization step regulating the formation of the so-called active or R* receptor from R, the inactive form (see Chapters 2 and 3) [20]. In this model, the elevated constitutive ac-

tivity of mutant receptors is due to an increase in the isomerization (i.e., mutant receptors are more prone to adopt the active or R* conformation spontaneously in the absence of agonists). The model also predicts the experimentally verifiable findings that both agonists and partial agonists have higher affinity for the constitutively active mutant receptors in proportion to their efficacy [16].

One of the key questions that had to be addressed in these studies was whether it truly was constitutive activity or simply contamination with endogenous hormone or neurotransmitter that was producing the activated receptors. To address this issue, it became necessary to block the effects of the inverse agonist by use of a 'neutral' antagonist. Such experiments were performed in all of the studies above except the bradykinin B_2R study, which relied on showing the absence of bradykinin in the system. The field had now generated enough interest and a review has been published [17]. However, as the title of that review – "Inverse agonism: Pharmacological curiosity or potential therapeutic strategy?" – suggested, there was still a great deal of skepticism as to the physiological relevance of the constitutive activity and inverse agonism.

Much of the skepticism about the phenomena appeared to involve the lack of physiological data in support of constitutive activity and inverse agonism. The data generated so far had all been obtained in cell lines and membranes often manipulated to include substantial overexpression of the receptors or mutated receptors. One of the first studies in a physiological system to imply constitutive activity and inverse agonism was performed in isolated guinea pig and human cardiac myocytes (see Chapter 10) [18]. In 1995, the first physiological report of the use of transgenic mice cardiac-specifically overexpressing the β_2AR was published [19]. This study showed that whether one used membranes and measured cAMP formation, or used the isolated atria and measured isometric tension, or measured an index of cardiac contractility *in vivo*, it was possible to restore the elevated levels of all three indices back to normal with the β_2AR inverse agonist ICI 118 551 ((±)-1-[(7-methyl-2,3-dihydro-1*H*-inden-4-yl)oxy]-3-[(1-methylethyl)amino]-2-butanol), but not with the neutral antagonist alprenolol. Furthermore, alprenolol could be used to block the inverse agonist effects of ICI 118 551.

With regard to receptor theory, the most obvious consequence of spontaneously active receptors was the need for at least two states of the receptor, and the inclusion of inverse agonists in addition to agonists and antagonists as a class of ligands. The modeling began to change because of the necessity to extend the ternary complex model. Several models were proposed, ranging in complexity from the two-state model to the ternary cubic (and extended ternary cubic) model (see Chapters 2 and 3) [16, 20, 21]. While the two-state model remains very useful in its predictive ability for many conditions, there is now considerable evidence for multiple receptor states and for the ability of ligands to enrich different states preferentially (see Chapter 9) [22–24]. Indeed, the existence of multiple receptor states produced a subtle but important shift in the concept of ligand efficacy. The notion of efficacy as being the ligand's ability to *induce* a conformational change of the receptor, through which it now gained affinity for the signaling component (usually the G protein), was replaced by the notion that the ligand simply *selected* or stabilized an already existing conformational state and thereby produced its enrichment [33].

Thus, constitutively active GPCRs and inverse agonism have evolved along the same path as most discoveries: skepticism about isolated reports, criticisms about methodologies and systems, and the physiological relevance of the findings. Many of the authors of the pioneering articles discussed so far are contributing authors to this volume. Now the focus has turned to the question of whether there is any therapeutic relevance to the difference between antagonists and inverse agonists (see Chapters 7, 12, 13, and 14). This issue is complicated by the fact that many of the drugs on the market labeled as blockers or antagonists are in fact inverse agonists. A recent publication surveyed the literature for the percentage of antagonists and inverse agonists, and out of several hundred compounds tested, the overwhelming majority are actually inverse agonists [1].

There is no doubt that, at least in theory, inverse agonists would have a distinct advantage over antagonists in treating diseases produced by constitutively active mutants (CAMs). For example, inverse agonists directed at constitutively active thyroid stimulating hormone (TSH) receptors might be used to treat 90% or more of sporadic hyperfunctioning thyroid nodules, which are due to activating mutations in the TSH receptor. Diseases caused by constitutively activating mutations have also been reported in the case of receptors for luteinizing hormone, parathyroid hormone, and a growing list of other ligands [25]. In all of these cases, inverse agonists might theoretically be of therapeutic value.

Some data can be interpreted in support of therapeutic differences between antagonists (or very weak partial agonists) and true inverse agonists in non-CAM diseases. Specifically, a study using cardiac myocytes from congestive heart failure patients revealed that both carvedilol and metoprolol behaved as inverse agonists, while bucindolol was an antagonist (on average) (see Chapter 7) [26]. This correlates with their clinical efficacy in chronic heart failure: carvedilol and metoprolol are beneficial at reducing mortality, while bucindolol is not [27–30]. However, the mechanism of the beneficial effect can also be explained by ligand-directed trafficking of the receptor [23–24]; the issue is also applicable to other disease states such as schizophrenia (see Chapter 14) [32]. Similar data have been obtained in a murine model of asthma in which chronic treatment with the inverse agonists carvedilol and nadolol produces a reduction in peak airway resistance, while the weak partial agonist alprenolol does not [31]. This study tested the hypothesis that the opposing effects of agonists and inverse agonists may also extend to their effects over time: agonists acutely increase signaling, but when given chronically may decrease signaling due to desensitization mechanisms, while inverse agonists may cause the exact opposite [31].

Over the past dozen years or so, the intimately linked concepts of constitutive activity of GPCRs and the existence of inverse agonists have been validated and have become part of the mainstream of thinking in receptor biology. As this volume demonstrates, these ideas have provided fruitful avenues for experimentation and theory in numerous areas of GPCR receptor biology. It seems likely that in the years ahead this body of experimentation and theory will ultimately give rise to novel therapeutics. All these topics will be discussed in this volume. Table 1.1 provides an overview of the various GPCRs discussed in the individual chapters so that the reader interested in a specific GPCR can easily find the desired information.

Table 1.1 Classification of the G protein coupled receptors discussed in this book. Numbers in bold indicate chapters specifically dealing with these receptors.

GPCR	IUPHAR nomenclature	Appearance in Chapter
Class A Receptors		
5-Hydroxytryptamine (serotonin) receptors	5-HTR	**14**, 1, 4
Subtype 1	5-HT_1R	**14**
	5-HT_{1A}R	**14**, 6, 7
	5-HT_{1B}R	**14**, 7
	5-HT_{1D}R	**14**, 7
Subtype 2	5-HT_2R	**14**
	5-HT_{2A}R	**14**
	5-HT_{2C}R	**14**, 5, 7
Subtype 3	5-HT_3R	**14**, 13
Subtype 4	5-HT_4R	**14**, 4, 5
	5-HT_{4A}R	4
	5-HT_{4B}R	4
	5-$HT_{4E,F}$R	4
Subtype 6	5-HT_6R	**14**
Subtype 7	5-HT_7R	**14**, 4
	5-HT_{7A}R	**14**, 4
	5-HT_{7B}R	4
	5-HT_{7D}R	4
Adrenoceptors	AR	**11**
α-Adrenoceptors	αAR	12
Subtype1	α_1AR	**11**, 7
	α_{1A}AR	**11**
	α_{1B}AR	**11**, 1, 2, 3, 9, 12, 13, 14
	α_{1D}AR	**11**
Subtype 2	α_2AR	**11**, 1, 2, 7, 13
	α_{2A}AR	6
	α_{2B}AR	**11**
	α_{2C}AR	**11**
β-Adrenoceptors	βAR	**9**, **10**, 2, 3, 4, 11, 12, 13
Subtype 1	β_1AR	**9**, **10**, 6, 7,11
Subtype 2	β_2AR	**9**, **10**, 1, 2, 3, 4, 6, 7, 11, 13, 14, 15
Subtype 3	β_3AR	**9**, **10**, 11
Angiotensin II receptors	AT_1R	3
Bradykinin receptors	BR	2
Subtype 2	B_2R	1, 2
Cannabinoid receptors	CBR	
Subtype 1	CB_1R	2, 9
Chemokine receptors	CXCR1	**15**
	CXCR2	**15**
	CXCR4	**15**, 7
	CCR1	**15**
	CCR5	7

Table 1.1 continued.

GPCR	IUPHAR nomenclature	Appearance in Chapter
Class A Receptors		
	CCR6	**15**
	CX_3CR1	**15**
	XCR1	**15**
Cholecystokinin receptors	CCKR	3
Subtype 2	CCK_2R	5
Complement C5a receptor	C5aR	8
Dopamine receptor	DR	
Subtype 1	D_1R	7
Subtype 2	D_2R	3, 7, 14
Subtype 3	D_3R	7
Subtype 4	D_4R	7
Subtype 5	D_5R	5
Formyl peptide receptors		
Subtype 1	FPR1	**8**, 5
Glycoprotein hormone receptors		
Follicle-stimulating hormone receptors	FSHR	5, 7
Lutenizing hormone receptors	LHR	5, 7, 14
Thyroid-stimulating hormone receptors	TSHR	1, 5, 7, 14
Gonadotrophin-releasing hormone receptors	GnRHR	3
Histamine receptors	HR	
Subtype 1	H_1R	**13**, 7
Subtype 2	H_2R	**13**, 2, 3, 7, 9, 11, 15
Subtype 3	H_3R	**13**, 7
Subtype 4	H_4R	**13**
Leukotriene B4 receptors	BLTR	**8**
M-cholinoceptors	MR	**12**, 7, 13, 14
Subtype 1	M_1R	**12**, 3, 11
Subtype 2	M_2R	**12**
Subtype 3	M_3R	**12**, 3
Subtype 4	M_4R	**12**
Subtype 5	M_5R	**12**
Melanocortin receptors	MCR	7
Subtype 1	MC_1R	5, 7
Subtype 3	MC_3R	7
Subtype 4	MC_4R	5, 7
Neuropeptide Y receptors	YR	
Subtype 1	Y_1R	2
Subtype 2	Y_2R	2
Subtype 4	Y_4R	2
Opioid receptors	OPR	
δ-Opioid receptors	DOP(δ)R	1, 2, 6, 7, 12, 13
μ-Opioid receptors	MOP(μ)R	2, 7

Table 1.1 continued.

GPCR	IUPHAR nomenclature	Appearance in Chapter
Class A Receptors		
Platelet-activating factor receptor	PAFR	8
Prostanoid receptors		4
Prostaglandin E_2 subtype 3 receptors	EP_3R	4, 5
	$EP_{3\alpha}R$	4
	$EP_{3\beta}R$	4
	$EP_{3\gamma}R$	4, 5
Prostaglandin $F_{2\alpha}$ receptors	FPR	4
	FP_AR	4
	FP_BR	4
Thromboxan receptors	TP R	4
	$TP_\alpha R$	4
	$TP_\beta R$	4
Rhodopsin		3, 4, 5, 7, 11, 12, 14, 15
Somatostatin receptors	SRIFR	2
Thyrotropin-releasing hormone receptors	TRHR	4, 3
Vasopressin (oxytocin) receptors	VR	11
Subtype 2	V_2R	
Class B receptors		
PTH receptor family		
PTH/PTH-related peptide receptors	PTH1R	5, 7, 14
Class C receptors		
γ-Aminobutyric acid receptors	GABAR	
Subtype B1	$GABA_{B1}R$	1, 3, 13
Subtype B2	$GABA_{B2}R$	3, 13
Metabotrotropic glutamate receptors	mGluR	3, 4, 11
Subtype 1	$mGlu_1R$	4, 5
	$mGlu_{1A}R$	4
	$mGlu_{1B}R$	4
	$mGlu_{1C}R$	4
	$mGlu_{1D}R$	4
Subtype 5	$mGlu_5R$	3, 4
	$mGlu_{5A}$ R	4
	$mGlu_{5B}R$	4
Calcium sensor receptor	CaSR	5, 7

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