



## From inverse agonism to 'Paradoxical Pharmacology'

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### Abstract

The constitutive or spontaneous activity of G protein-coupled receptors (GPCRs) and compounds acting as inverse agonists is a recent but well-established phenomenon. Dozens of receptor subtypes for numerous neurotransmitters and hormones have been shown to possess this property. However, due to the apparently low percentage of receptors in the spontaneously active state, the physiologic relevance of these findings remains questionable. The possibility that the reciprocal nature of the effects of agonists and inverse agonists may extend to cellular signaling is discussed, and that this may account for the beneficial effects of certain  $\beta$ -adrenoceptor inverse agonists in the treatment of heart failure.

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### 1. Brief history of inverse agonism at G protein-coupled receptors

For approximately three-quarters of a century, ligands that interacted with G protein-coupled receptors (GPCRs) were classified either as agonists or antagonists. Receptors were thought to exist in a single quiescent state that could only induce cellular signaling upon agonist binding to the receptor to produce an activated state of the receptor. In this model, antagonists had no cellular signaling ability on their own, but did bind to the receptor and prevented agonists from being able to bind and activate the receptor. Then, in 1989, data were published by Costa and Herz, which demonstrated that receptors could at

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least be manipulated into a constitutively or spontaneously active state, which could produce cellular signaling in the absence of agonist occupation [1]. In the same manuscript, evidence was provided that certain compounds could ‘turn-off’ or inactivate these

Table 1  
Partial list of constitutively active GPCR systems and their inverse agonists

Ligand and receptor subtype	Model	Inverse agonist	Tested for inverse agonism	Reference
<i>Amine</i>				
Acetylcholine (muscarinic)	M2	nat.	pirenzepine	antag. atropine and AF-DX 116 [31]
Adrenoceptor	$\alpha_{2A}$	CAM	rauwolscine, yohimbine, RX821002, MK912	n.d. [32]
Dopamine	$\beta_2$	in vivo mice	ICI 118–551	antag. alprenolol [33]
	D <sub>1A</sub>	nat.	(+)-butaclamol, flupentixol	[34]
	D <sub>1B</sub> (D <sub>5</sub> )			
	D <sub>1A</sub>	nat.	clozapine, fluphenazine	antag. SCH23390 [35]
Histamine	D3	nat.	haloperidol, radopride, (+)-UH-232, (+)-AJ-76	n.d. [36]
	H1	nat.	mepyramine, cetrizine, epinastine, acrivastine	no endog. agonist [37]
	H2	nat.	cimetidine, ranitidine	antag. burimamide [38]
Serotonin	H3	nat.	ciproxifan, thioperamide, FUB 465; clobenpropit, iodophenpropit	antag. proxyfan [39–41]
	5-HT <sub>1A</sub>	nat.	spiperone	antag. WAY100,635 [42]
	5-HT <sub>2C</sub>	nat. ox	SB 206553, clozapine, mianserin	antag. chlorpromazine, thioridazine, thiothixene 5-methoxygramine [43,44]
	5-HT <sub>4A</sub>	nat., CAM	GR 125487, SB 207266	n.d. [45]
<i>Peptide</i>				
Angiotensin	AT <sub>1</sub>	CAM	Losartan, DUP 753, LF 7-0156, LF 8-0129	n.d. [46]
Bradykinin	B2	nat., CAM	NPC17731, HOE140	no endog. agonist [47]
Formyl peptide	FPR	nat.	cyclosporin H	antag. BocPLPLP [48]
CCK-BR		CAM	(R)-L-740,093	antag. YM022 [49]
Neurotensin	NTS1	CAM	SR 48692	n.d. [50]
	NTS2	nat.	JMV 457	antag. neurotensin [51]
Opioid	$\mu$	CAM	naloxone	n.d. [52]
	$\mu$	nat.	naloxone, $\beta$ -chlornaltrexamine	antag. CTAP [53]
	$\delta$	nat.	BNTX, NTB, ICI 174,864, clocinnamox	antag. naltrindole [54]
	$\delta$	nat.	ICI 174864	antag. naloxone [55]
	$\delta_2$	in vivo mice	Leu-Enkephalin	antag. naltriben [56]
<i>Nucleotide-like</i>				
Adenosin	A1	nat.ox	8-cyclopentyl-1,3-dipropylxanthine, WRC 0571	antag. N-0861 [57]

Table 1 (continued)

Ligand and receptor subtype	Model	Inverse agonist	Tested for inverse agonism	Reference	
<i>Cannabis</i>	CB1	nat.	SR 114,716A, AM630	no endog. agonist	[58,59]
	CB2	nat.	SR 144,528	antag. $\Delta$ 9-THC	[60]
<i>PAF</i>	nat ox, CAM	WEB2086, alprazolam, SM10661	antag. BN52021	[61]	

nat., native receptor; nat. ox, native receptor overexpressed; CAM, constitutively active mutant; antag., inverse agonist effect was blocked by neutral antagonist; no endog. agonist, efforts made to rule out contamination with endogenous agonist.

spontaneously active receptors. The authors termed these compounds ‘negative antagonists’ [2]. The term ‘negative antagonist’ has now been largely displaced (in part due to the recommendation of the IUPHAR Nomenclature Committee) by the term ‘inverse agonist’.

In the ensuing 13 years, dozens of manuscripts (if not hundreds) have provided further evidence that GPCRs exist in constitutively or spontaneously active states and that inverse agonists are able to inactivate these spontaneously active receptors (for reviews, see Ref. [3]). Table 1 is a partial list of GPCRs for which evidence has been generated to suggest the existence of spontaneously active states.

However, despite the prevalence of data in support of spontaneously active GPCRs and inverse agonists, their (patho)physiological relevance is still in question. In part this is due to the evidence suggesting that aside from a few diseases produced by receptor mutations, for the overwhelming majority of receptor subtypes, the percentage of the receptors existing in the spontaneously active state(s) appears to be very low. Thus, their physiological contribution seems questionable [3]. Most of the time, some ‘manipulation’ of the system is required before spontaneous activity and inverse agonism can be detected. Three of the more common manipulations are (1) the overexpression of the receptors to amplify the actual number of receptors in the active state, (2) mutating the receptors at specific sites to increase the ratio of active to inactive receptors, and (3) to somehow amplify or ‘prime’ the signaling cascade (for example, using a phosphodiesterase inhibitor or forskolin for Gs-coupled receptors) in order to magnify the signal being generated by the low number of spontaneously active receptors (Table 1).

## 2. Antagonists versus inverse agonists

All inverse agonists identified so far were once classified as antagonists. Because of this history, and because inverse agonists can produce effects qualitatively similar to the ‘effects’ of antagonists, inverse agonists are often viewed as a subset of antagonists. Therefore, it is important to discriminate ‘effects’ of antagonists from the effects of inverse agonists.

In the simple two-state model of receptor theory, receptors exist in an equilibrium between the traditional inactive state (R), and the spontaneously active state (R\*). Agonists preferentially bind to and enrich the number of receptors in the R\* state and decrease the

Table 2  
The reciprocity of agonist and inverse agonist

Agonist	Inverse agonist
<ul style="list-style-type: none"> <li>• Promotes formation of more <i>active</i> receptor (R*)</li> <li>• Increases/decreases 'baseline'</li> <li>• Promotes phosphorylation by GRK</li> <li>• Promotes endocytosis and downregulation of receptor</li> <li>• Promotes conformational changes (decrease fluorescence emission) [62]</li> <li>• Homologous desensitization</li> <li>• Heterologous desensitization</li> <li>• Acutely promotes effect</li> <li>• Chronically inhibits effect blocked by antagonist</li> </ul>	<ul style="list-style-type: none"> <li>• promotes formation of more <i>inactive</i> receptor (R)</li> <li>• decreases/increases 'baseline'</li> <li>• prevents phosphorylation by GRK</li> <li>• promotes up-regulation of cell surface receptors</li> <li>• promotes conformational changes (increase fluorescence emission) [62]</li> <li>• homologous sensitization</li> <li>• heterologous sensitization</li> <li>• acutely inhibits effect</li> <li>• chronically promotes effect</li> </ul>

The effects of agonist and inverse agonist are blocked by an antagonist.

receptors in the R state. Inverse agonists preferentially bind to and enrich the number of receptors in the inactive R state and decrease the amount in the R\* state. Antagonists bind with equal affinity to both R and R\* and do not change their numbers, but do prevent BOTH agonists and inverse agonists from producing their effects. Thus, agonists and inverse agonists appear to modulate cellular activity in a reciprocal manner (Table 2). For example, inverse agonists move 'baseline' in the opposite direction of the agonist. This 'reciprocity' of agonists and inverse agonists seems to hold true at all parameters investigated so far. Furthermore, this reciprocity implies that inverse agonists are 'active' at altering signaling on their own. Their effects are not solely attributable to the prevention of agonist activity as would be true for antagonists. Here we speculate that the reciprocity of agonists and inverse agonists may extend to their ability to induce cellular signaling. That is that while agonists can acutely increase signaling by receptor activation and chronic agonist activation produces desensitization and decreases in signaling, the inverse agonist may be able to chronically increase signaling in addition to the acute effect of decreasing signaling. We further speculate that this characteristic of inverse agonists that is not shared by antagonists may account for therapeutic differences in the disease of heart failure and that these differences may afford utility in other diseases.

### 3. Paradoxical treatment of heart failure

In congestive heart failure, there is a decrease in inotropic function. The contractility of the cardiac muscle is regulated by the  $\beta$ -adrenoceptors. Activation of  $\beta$ -adrenoceptors leads to an increase in cyclic AMP resulting in cardiac muscle contraction [4]. In failing hearts, an elevated level of norepinephrine is present resulting in a constant activation of the  $\beta$ -adrenoceptor system. This eventually leads to a decrease in the  $\beta$ -adrenoceptor density in the heart [5] and desensitization of adenylyl cyclase leading to a reduction in cyclic AMP production [6] ultimately reducing the contractile response.

Since the failing heart has a decrease in contractility, a theoretically logical form of treatment is to administer a positive inotropic agent that increases cyclic AMP levels to

improve the cardiac output. The  $\beta_1$ -adrenoceptor agonist, dobutamine, increases the myocardial contractility [7] and short-term administration of dobutamine in heart failure patients resulted in an improved cardiac performance [8–10]. However, upon continuous administration for 96 h of dobutamine, 32% of the initial increase in cardiac output is lost [11]. Other  $\beta$ -adrenoceptor agonists such as pirbuterol and prenalterol showed promising effects in improving the hemodynamics in heart failure patients when used short-term [12–17]. However, similar to dobutamine, following chronic treatment, the beneficial hemodynamic effects were not maintained [18–20]. In addition, two placebo-controlled clinical trials with intravenous dobutamine [21] and xamoterol [22] reported an increase in mortality among patients treated with these drugs. Therefore, the use of  $\beta$ -adrenoceptor agonists produces initial cardiac improvements, however, the long-term administration results in system tolerance as well as a loss of initial increased cardiac function in failing hearts and an increased risk of mortality. Thus,  $\beta$ -adrenoceptor agonists are useful acutely, yet detrimental if used chronically.

Traditionally, in patients with heart failure, ‘ $\beta$ -blockers’ ( $\beta$ -adrenoceptor antagonists and inverse agonists) have been considered to be negative inotropes producing adverse hemodynamic effects. Therefore, treating a system with decreased contractility, such as heart failure, with these agents was contraindicated. However, studies have suggested that treatment with certain ‘ $\beta$ -blockers’ can improve cardiac function and decrease mortality [23]. The initiation of this treatment may be associated with an initial decrease in hemodynamic effects, yet there is gradual improvement over several months with the patient showing clinical improvement of cardiac function within 2–4 months of continued therapy [24]. Several long-term clinical trials have been conducted using ‘ $\beta$ -blockers’ (bisoprolol [25,26], carvedilol [24] and metoprolol [27]) each resulting in many similar outcomes [28], however, a large-scale trial with the ‘ $\beta$ -blocker’, bucindolol, failed to show a significant beneficial result [29]. In summary, certain ‘ $\beta$ -blockers’, metoprolol and carvedilol, which are the only two with FDA approval in the US, have been shown to decrease mortality and improve cardiac function upon chronic administration.

#### **4. Is inverse agonism the answer?**

A recent manuscript has examined the antagonist or inverse agonist properties of three compounds (metoprolol, carvedilol and bucindolol) in cardiac myocytes from failing human hearts [30]. In cardiac myocytes pretreated with forskolin to amplify any constitutive signaling, metoprolol and carvedilol both functioned as inverse agonists, while bucindolol behaved as an antagonist [30]. Thus, both compounds that have been shown beneficial in the chronic treatment of heart failure, and that have shown initial tolerance problems are inverse agonists. In contrast, the compounds showing no chronic benefits or initial compliance problems, bucindolol, behaved as an antagonist.

In summary, the initial treatment for heart failure was to administer a positive inotropic agent such as  $\beta$ -adrenoceptor agonist that improved the hemodynamic function of the heart in the short-term. However, these effects were not maintained over the long-term. In addition, long-term administration of these agents increased mortality. The present treatments of heart failure include administration of  $\beta$ -adrenoceptor inverse agonists that were

originally contraindicated due to the negative inotropic characteristics. Although patients experience some initial undesirable hemodynamic effects, chronic treatment with two  $\beta$ -adrenoceptor inverse agonists have reduced the risk of mortality and increased the quality of life for patients with heart failure.

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### Discussion 3

M. Lohse

I don't know what happens in the US, but at least in Germany, people are being chronically treated also with  $\beta$ -agonists orally in severe asthma. Do you know what the study basis is for this?

R. Bond

No, I don't know. Certainly you have long-acting  $\beta_2$ -agonists that don't appear to compromise response, although a lot of human data show that you do lose the bronchoprotective effect. They don't get worse, but they don't work as well. In New Zealand, there's a very controversial study that has pulled a  $\beta_2$ -agonist off the market. I think the main advantage there is that these are horrible drugs — steroids and  $\beta$ -agonists would kill people except that we have a unique access to the lungs. If you chronically treat people with a systemic  $\beta$ -agonist, they would die. If you give some steroids systemically, it would have all kinds of side effects. However, we can get away with it because of the unique access delivery to the lungs. Therefore, I don't want to use that to stop me from doing the experiments. How bad is chronic agonism, but it's a valid point. There's a whole list that we could all generate in here about chronic experiments that I think someone should run: what about insulin receptor and inverse agonist in type 2 diabetes? What about M1 inverse agonist in Alzheimer's?

W. Clarke

You did present some data about the  $\beta$ -adrenoceptor looking like it increased cardiac function after prolonged treatment with carvedilol. I was wondering if you'd care to comment on what you might expect the mechanism to be given that carvedilol being present onboard should be blocking the receptor and preventing agonist activation of the receptor? In the face of increased receptor density, there's a potential mechanism.

R. Bond

My question would be, what good is increasing receptor number if they're occupied by an inverse agonist? However, if you put a person with heart failure, who's been on a  $\beta$ -blocker for months, on a treadmill, they respond with an increase in heart rate. It's not an all or none response. Their receptor's left unoccupied, and may be having 20% of well coupled receptors, efficiently working, when the demand comes you have a good response. The synapse can have micromolar concentrations of agonists and maybe that's a lot better than having 100% of the receptors running at 10%. Maybe it just recouples and allows you to have that reserve.

W. Clarke

I actually was thinking about more on the idea that there can be changes intracellularly that may occur as well as changes in the number of receptors. There may be changes in the signalling molecules in the cells that allow for increased activity of those receptors that are

still available to interact with their signalling molecules. We've done some of the work on sensitisation that takes place even without a change in receptor density.

C. Maack

I'm not so much concerned about the occupation of receptors by  $\beta$ -blockers, since there is still competitive antagonism of the agonist that gets on the receptor. Even if there is a  $\beta$ -blocker, there's always a balance between the sensitisation state and occupation with the inverse agonists. It rather surprised me that you really see  $\beta$ -adrenergic receptor ( $\beta$ -AR) up-regulation by carvedilol treatment. As far as I know, this is contrasting to observations in heart failure, where you have a lack of up-regulation by carvedilol treatment in heart failure patients concerning cardiac ventricular  $\beta$ -AR density. There are even studies that indicate that carvedilol itself down-regulates  $\beta$ -AR density.

R. Bond

How do they do those studies? And what can you say about getting carvedilol off the receptor? I think that you can't label that site with carvedilol there.

C. Maack

They do those experiments by  $\beta$ -AR binding studies. It appears that carvedilol does not readily dissociate from  $\beta$ -ARs. Thus, it is important to consider whether binding experiments are carried out as "one-point determinations", using only one concentration of the radioligand, or if you perform a Scatchard plot analysis from multiple radioligand concentrations. When you treat cardiac tissue with carvedilol and attempt to wash it from the receptor by high-speed centrifugation and resuspension of membranes, you still achieve the maximum binding sites ( $B_{max}$ ) by Scatchard plot analysis. However, the  $K_D$  remains shifted to the right, i.e. to higher radioligand concentrations. This is not the case in similar experiments with metoprolol, which is easily washed from the  $\beta$ -AR by this procedure. This means that carvedilol still binds to the receptor without reducing  $\beta$ -AR density (e.g. by irreversible binding kinetics). If you perform only one-point determination (e.g. when the amount of tissue you gain from small cardiac biopsies is not sufficient for more than one experiment), the estimated  $B_{max}$  may be artificially reduced. However this is due to the rightward shift of the  $K_D$ . However, the authors who performed the studies with carvedilol in heart failure patients indicate that they used Scatchard plot analysis. One possible explanation for  $\beta$ -AR down-regulation (or lack of up-regulation in the setting of heart failure) could be the agonist-like binding characteristics of carvedilol, i.e. GppNHp-modulatable binding.

W. Clarke

I think that there are certainly different tissues that will respond differently to the same ligands. The lung  $\beta$ -adrenoceptor may go up after carvedilol treatment, and they may not in the heart. There can be secondary mechanisms - enhanced sensitivity to Gpp(NH)p shift - implying that maybe there were changes in G proteins in the cells. Agonists can down-regulate G protein levels, and maybe certainly inverse agonists in some systems can up-regulate them. It may be just the different mechanisms. C. Maack I think the  $\beta$ -adrenoceptor kinetics is even down-regulated by carvedilol. This is even in favour of the receptor sensitisation by this drug, though you may not see it by receptor density.

R. Bond

And let's not forget that that is at least a mixed  $\beta_1$ - $\beta_2$  versus a predominant  $\beta_2$ . I very carefully put ligand efficacy; I never thought I would give a talk where I didn't care about the receptor subtype. And I don't care, whether it's  $\beta_1$  or  $\beta_2$ .

G. Milligan

We discussed an issue that came back to a point that had already been made about likely occupancy curves. I know you are in the process of doing a series of experiments at tenfold lower concentrations of the drugs, to see if this might actually allow a degree of agonism, maybe to get a signal even when the receptor is maybe being up-regulated. I don't know if you want to add any comment on that.

R. Bond

I think a part of the problem is what if the  $\beta$ -blockers decrease the incidence of asthma attacks, what are you going to do if the patient has an asthma attack? In this situation, is the agonist going to work? It might well be possible to lower the dose and derive the benefit of the up-regulation, yet still be enough to administer the short-acting  $\beta_2$ -agonist? In the idyllic visual you would see that you could give the inverse agonist systemically, which would block cardiac  $\beta$ -receptors, which are the main side effects, and you'd still give the  $\beta$ -agonist by inhalation. So presumably, side effects would be minimal.