REVIEW

Inverse agonism: the classic concept of GPCRs revisited

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Abstract. In the classical two-state model, G protein-coupled receptors (GPCRs) are considered to exist in equilibrium between an active and an inactive conformation. Thus, even at the resting state, some subpopulation of GPCRs is in the active state, which underlies the basal activity of the GPCRs. In this review, we discuss inverse agonists, which are defined as GPCR ligands that shift the equilibrium toward the inactive state and thereby suppress the basal activity. Theoretically, if constitutive activation plays an essential role in the pathogenesis of a disease, only inverse agonists, and not neutral antagonists, can reverse this pathophysiological activation. Although many pharmacological examples of inverse agonism have been identified, its clinical importance is still unclear and debated. Thus, even though inverse agonism of angiotensin receptor blockers (ARBs) has been discussed for more than 10 years, its clinical relevance remains to be completely clarified.

Key words: G protein-coupled receptor (GPCR), Inverse agonist, Antagonist, Angiotensin II type 1 receptor (AT1), Angiotensin receptor blocker (ARB)

G PROTEIN-COUPLED RECEPTORS (GPCRs) transduce extracellular signals into cells by activating trimeric G proteins [1-3]. About 800 GPCRs have been identified in mammals, each responding to a hormone, neurotransmitter, or sensory stimulus and activating one or more $\alpha\beta\gamma$ trimeric G proteins (composed of one of each of 16 α , 5 β , and 12 γ subunits). Agonist-occupied GPCRs activate G proteins by promoting the exchange of GTP for GDP bound to the α subunit of the G protein ($G\alpha$), generating two potential signaling molecules, α -GTP and free $\beta\gamma$, and activating effector enzymes and ion channels [4-6]. GPCR ligands (such as agonists, antagonists, and inverse agonists) are classically considered to regulate the conformation of GPCRs. In addition, the conformation of GPCRs is allosterically regulated by interaction with G protein; G proteins were the first allosteric modulators of GPCRs

to be identified. Interaction with G protein produces high-affinity binding of GPCR agonists. Agonists bind the active form of GPCRs (R 'on'), which interacts with G proteins, with higher affinity than the inactive form of GPCRs (R 'off'), whereas antagonists bind R 'on' and R 'off' equally.

GPCR activation model: classic vs. novel

In the classical two-state model of GPCR activation, GPCRs are considered to exist in equilibrium between an inactive state and an active state [1, 7] (Fig. 1). This model explains why GPCRs show basal activity, that is, "signal leak", at the resting state in the absence of agonist stimulation. In addition, it is postulated that agonists shift the GPCR equilibrium toward the active form, whereas inverse agonists do the opposite, and that antagonists, especially neutral antagonists, competitively inhibit agonists without affecting the equilibrium, interacting with R 'on' and R 'off' equally (Figs. 1 and 2). However, several lines of evidence lend support to a multi-state model for GPCRs, in which these receptors can spontaneously adopt multiple active and inactive conformational states [7-11]. In this new model, each ligand is hypothesized to recognize and stabilize a specific conformation of each type of GPCR, leading to

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Fig. 1 The classical two-state model of GPCR activation
In the classical two-state model, the GPCR exists in equilibrium between an active state and an inactive state, explaining why GPCRs show basal activity in the resting state (please also see also Fig. 2). Agonists shift the equilibrium toward the active form, whereas inverse agonists do the opposite. Neutral antagonists are defined as GPCR ligands that competitively inhibit agonist action without affecting the equilibrium. Recently, however, accumulating evidence support an alternative multi-state model for GPCRs. Based on this new model, the presence of neutral antagonists is being challenged.

Fig. 2 The classification of GPCR ligands
GPCRs show basal activity at the resting state (please also see Fig. 1), the extent of which is different among GPCRs. Full and partial agonists show full and partial efficacy (GPCR activation), respectively. Inverse agonists stabilize the inactive form of GPCRs, thereby suppressing the GPCR activity below the basal levels. In the classical model, antagonists are defined as ligands that competitively inhibit agonist binding/action, unaffected the GPCR equilibrium by themselves, and thus inhibiting the GPCR signal maximally to the basal levels.

a set of unique and specific biological effects. Based on this model, the presence of neutral antagonists is being challenged. Essentially all antagonists, as defined classically, may, strictly speaking, be either partial agonists or inverse agonists (Figs. 1 and 2).

Inverse agonism: why it has not been featured

Although many examples of inverse agonism are well known pharmacologically [12-14], this issue has largely been ignored clinically. This may be at least partly due to the dogma that, as therapeutic drugs, neutral antagonists must be better than partial or inverse agonists because they do not change the resting state existing as a “gifted harmony” and inhibit only agonist-dependent signals. Logically, inverse agonists may shift and thus perturb the equilibrium between the inactive and active states of the GPCRs. If its basal activity in the resting state plays an important physiological role, the expression of the GPCR in question may be upregulated by a certain compensatory mechanism, causing the signal to be suppressed until it can over-rebound after the cessation of the inverse agonist. For example, histamine H₂ antagonist, which decreases the production of gastric acid from parietal cells by blocking the action of histamine, is a representative inverse agonist [15]. It has been reported that cimetidine and ranitidine induce spontaneous agonist-independent H₂ receptor activity by inducing H₂ receptor upregulation in cultured cell lines [16, 17]. Thus, the development of tolerance after prolonged clinical use and the recurrence of ulcer after sudden withdrawal of treatment may be caused, at least in part, by overactivation of H₂ receptor signaling. However, their inverse agonist characteristics have not been emphasized, at least somewhat, because the main and most important role of H₂ blocker is to block the action of histamine against H₂ receptor as an antagonist. Similarly, in agonist-deficient knockout mice, the expression of the GPCR activated by the agonist may be upregulated [18].

Spontaneous activation of GPCRs vs. inverse agonism

In the setting of spontaneous activation of GPCRs, especially when this activation is accompanied by negative feedback suppression of their respective agonists,

antagonists no longer work and only inverse agonists can inhibit the activation (Fig. 3) [12-14]. A constitutively active mutation of a GPCR [13, 19, 20] is one of the representative situations that induce spontaneous GPCR activation. For example, congenital hyperthyroidism, neonatal inappropriate antidiuresis, and autosomal dominant hypocalcemia, caused by constitutively active mutations of the TSH receptor [21], V2 receptor [22, 23], and CaSR [24], respectively, can be treated not by neutral antagonists, but by inverse agonists. Besides constitutively active mutations, increased expression of GPCRs, allosteric activation, or existence of interacting proteins including GPCR dimerization might lead to a spontaneous (autonomous) activation of GPCRs (Fig. 4).

Heart failure and AT1 signaling

The past two decades have witnessed marvelous advances in the understanding of the pathophysiology of chronic heart failure, leading to significant improvements in therapy [25-27]. Recognition of the deleterious effects of overactivation of the renin-angiotensin system (RAS) and AT1-Gq/G13 signaling [28-30], in addition to desensitization of cardioprotective β AR-Gs signaling [31-33], has made RAS a major therapeutic target, whether systemically or regionally [34, 35]. In many randomized, controlled trials, ACE inhibitor (ACEi) reduced morbidity and mortality more than comparators in patients with congestive heart failure and coronary heart disease [36-40]. After the cardioprotective effect of ACEi was established, angiotensin receptor blocker (ARB) was originally suggested as a candidate alternative or more promising drug for blocking the RAS because ACEi sometimes also causes cough and angioedema or loses effectiveness over time, called the escape phenomenon. As expected, the LIFE trial added further evidence that blockade of the RAS by ARB, as well as by ACEi, may provide cardiovascular protection beyond the blood-pressure lowering effect [41]. Moreover, the CHARM-Added trial revealed that the addition of candesartan to ACEi and other treatments leads to a further clinically important reduction in relevant cardiovascular events in patients with chronic heart failure and a reduced left ventricular ejection fraction [42]. This result was interpreted to be because ARB blocked the harmful effect of angiotensin II (AII) produced by the redundant pathway ACEi cannot inhibit.

Fig. 3 Inverse agonist vs. antagonist

Inverse agonists shift the GPCR equilibrium toward the inactive form, whereas antagonists do not affect the equilibrium, competitively inhibiting the agonist action (please also see Fig. 1). Thus, both inverse agonists and antagonists inhibit agonist-dependent activation of GPCRs, whereas only inverse agonists, but not antagonists, inhibit autonomous activation of GPCRs, that is, basal activity of wild type GPCRs and increased basal activity of constitutively active mutant GPCRs.

Fig. 4 Mechanisms causing constitutive GPCR activation

Constitutive GPCR activation can be caused by increased expression of GPCRs, constitutively active mutant GPCRs, allosteric activation, existing of interacting proteins including GPCR dimerization that facilitate activation, or mechanical stretch (please also see Fig. 1).

Spontaneous activation of AT1 and ARBs

If spontaneous activation of the AT1 receptor plays a major role in the overactivation of AT1-Gq/G13 signaling, the effect of ARBs may be explained by their inverse agonist activity, which we discovered for the AT1 receptor [43, 44] (Fig. 5). If this is the case, it may be expected that ARBs with inverse agonist activity would show a unique cardiovascular protective effect not shown by other antihypertensive drugs, including ACEi, and that ARBs with stronger inverse agonist activity would show a greater cardioprotective effect. This would be in addition to the fact that add-on therapy of ARBs with inverse agonist activity to ACEi would show a greater cardioprotective effect. If these effects were proven in clinical trials, it would support the belief that the inverse agonist activity of ARB has vital effects beyond merely blocking the harmful effect of AII.

Although the initial data appeared to be positive [42], the subsequent clinical studies appeared to show negative results in chronic heart failure and cardiovascular diseases [45, 46]. These findings may suggest that spontaneous activation of AT1 might not play as much of a role in the pathophysiology of chronic heart failure as initially speculated. Alternatively, we may have to consider the relative potencies of ARBs as inverse agonists when evaluating the results of clinical trials because inverse agonist activities vary among ARBs [43, 44] (Fig. 6). The question then arises as to how we can explain the data clearly showing that cardiac hypertrophy caused by mechanical stress in angiotensinogen knockout mice was inhibited by ARB treatment [43]. The artificial condition in which AT1 receptor was remarkably overexpressed as a result of a feedback mechanism caused by a lack of AII [18] might have led to this result because only an inverse agonist could inhibit such agonist-independent overactivity of the receptor. We may not then be able to directly apply the results of angiotensinogen knockout mice to the clinical settings, in which an agonist, AII, is not suppressed. Even when spontaneous activation of AT1 receptor occurs, AII-dependent AT1 activation may be dominant and ARB may act mainly as an antagonist to block the action by AII.

The role of spontaneous AT1 activation

The physiological role of mechanical stretch-dependent spontaneous activation of AT1 [43, 47, 48] is a

Fig. 5 ARBs: inverse agonist vs. antagonist
When overexpressed in COS-7 cells, wild type (WT) and constitutively active (CA) AT1 receptor show basal activity and increased basal activity of Gq-dependent PI turnover signal, respectively (white). Candesartan (CV: 100 nM) acts as an inverse agonist, suppressing these activities below the basal levels (red), whereas losartan (LO: 100 nM) acts as an antagonist, unaffected these activities (blue). (For details, please see the references [10, 43, 44])

principal question, especially when this activation is not accompanied by feedback inhibition of AII. We speculate that AII-dependent and spontaneous activation of AT1 may operate in a synergistic manner, that is to say, spontaneous activation of AT1 may be the machinery that potentiates AII-dependent AT1 activation in a cell-specific manner. Although these speculations have not been discussed nor clearly proven, interestingly, it has been reported that in vascular smooth muscle cells, mechanical stretch synergistically augments AII-dependent AT1 activation [49]. That report showed that the synergy is at least partly caused by augmented AT1 receptor expression and is inhibited by ARB. Even in that case, the main action of ARB may be due to its antagonist activity, although inverse agonist activity would have some effect against the overactivity of AT1 receptor due to augmented expression [49, 50].

Clinical use of ARBs: inverse agonism and biased agonism

How can we interpret the results of some clinical studies showing the benefits of ARB add-on, as in the CHARM-added study [42]? If ARBs show biased

Fig. 6 ARBs: inverse agonist activity and anti-inflammation

(A) ARBs and their metabolites are different in their inverse agonist activity and anti-inflammation effect (beyond AT1 effect). This anti-inflammation effect is likely to be *via* PPAR γ activation and evaluated by the inhibition of MCP-1 and IL-6 secreted from cultured adipocytes. (B) Inverse agonist activity is quantitatively shown by the ARB (1-1,000 nM)-dependent inhibition of constitutively active mutant AT1 signals (Please also see Fig. 5 and its references). Data with olmesartan (OL), candesartan (CV), losartan (LO), EXP3174 (EX: an active metabolite of losartan), telmisartan (TE), valsartan (VA), and irbesartan (IR) are shown.

action, similar to that of beta-blockers [51, 52], which inhibit one signaling pathway but do not inhibit the other, the ARB add-on effect might be explained. Indeed, a biased AT1 agonist and allosteric activation of AT1 have been reported [53, 54]. However, when attention first started being paid to the concept of biased agonism around 2007, it was rigorously examined whether ARB displays biased action. This hypothesis could not be proven however (Lefkowitz RJ, personal communication) [52]. Overall, accumulating data likely have failed to show the superiority of ARB against ACEi and the benefit of the ARB add-on effect to ACEi in cardiovascular protection [45]. Rather, some other metaanalysis reports have suggested that ACEi, but not ARB, may have the beyond blood pressure-lowering effect [46, 55, 56]. In addition, we might have to consider the differences among ARBs [57], including their inverse agonist activities (Fig. 6).

We can also speculate about some potential problems of ARBs if they do act as inverse agonists in the clinical settings. First, RAS over-rebound may occur when the action of ARBs is suddenly stopped. Second, problems might occur in pregnant patients. It has been reported that augmented AT1 signaling due to dimerization of AT1 and bradykinin receptor causes preeclampsia [58], although it is still being debated whether meaningful dimerization occurs

[59]. If ARBs were allowed to be prescribed during late pregnancy when teratogenicity is no longer a problem, they would be a useful treatment option for preeclampsia. In that case, the less inverse agonistic activity ARB shows, the more useful it might be, because such ARBs would hardly affect the basal equilibrium of AT1 during pregnancy.

Conclusion

In summary, now that we have extensive evidence of the benefits of ARBs in cardiovascular disease, it is time to reconsider the *in vitro* and *in vivo* data thus far obtained from artificial settings. This topic can be considered a good example of the importance of bidirectional reflection, not only from the bench to the bed, but also from the bed to the bench.

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Disclosure

None of the authors have any potential conflicts of interests associated with this research.

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