



# Different inverse agonist activities of $\beta$ -adrenergic receptor antagonists—pharmacological characterization and therapeutical implications in the treatment of chronic heart failure

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## Abstract

The treatment of chronic heart failure with most  $\beta$ -adrenergic receptor ( $\beta$ -AR) antagonists leads to an improvement of symptoms and left ventricular function. However, only metoprolol, bisoprolol and carvedilol have been shown to reduce mortality in these patients. Bucindolol did not reduce mortality and xamoterol even increased it. These differences may be related to different inverse agonist or partial agonist activity of  $\beta$ -AR antagonists. This review focusses on the determination of different intrinsic activity of the mentioned  $\beta$ -AR antagonists in the human myocardium. Furthermore, the clinical impact of these differences is examined. In this regard, the effect of the different  $\beta$ -AR antagonists on  $\beta$ -AR regulation, minimum heart rate and exercise tolerance, as well as prognosis, is highlighted. It is concluded that the degree of inverse agonism of a  $\beta$ -AR antagonist determines the degree of  $\beta$ -AR resensitization, reduction of minimum heart rate, improvement of exercise tolerance and possibly also improvement of prognosis of patients with chronic heart failure.

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## 1. Introduction

Activation of several neurohormonal systems occurs in patients with chronic heart failure. One important component of neurohormonal dysregulation is the activation of the sympathetic nervous system. In patients with chronic heart failure, plasma norepinephrine levels are substantially elevated and correlate with the poor prognosis of this syndrome [1]. As a consequence of chronic sympathetic activation, the  $\beta$ -adrenergic system is desensitized to agonist stimulation [2,3] due to a reduction of myocardial  $\beta$ -adrenergic receptor ( $\beta$ -AR) density [4], uncoupling of  $\beta$ -ARs from the stimulatory G-protein ( $G_s$ ) [5] and up-regulation of the inhibitory G-protein [2].  $\beta$ -AR desensitization and down-regulation is facilitated by increased expression of  $\beta$ -AR kinase [6]. Furthermore, studies on transgenic animals overexpressing different components of the  $\beta$ -adrenergic signaling cascade ( $\beta_1$ -AR [7],  $\beta_2$ -AR [8],  $G_s$ [9]) suggest that chronic sympathetic activation induces cardiac hypertrophy, fibrosis, necrosis and apoptosis. While at the early stages of hypertrophy, cardiac function is maintained, continuous  $\beta$ -adrenergic stimulation leads to progressive loss of ventricular function. This continuum termed “cardiac remodeling” results in ventricular dilation with reduced left ventricular (LV) ejection fraction and increased LV end-diastolic dimensions and pressure.

While desensitization of the  $\beta$ -adrenergic system may affect cardiac performance during exercise, progressive ventricular remodeling may be the critical mechanism leading to an impairment of ventricular function already at rest. Worsening of cardiac contractile function by both processes leads to a decrease of peripheral perfusion. As a response, activation of the sympathetic nervous system initiates a vicious cycle with progressive deterioration of cardiac function resulting in heart failure (Fig. 1). Thus, therapeutic intervention of this vicious cycle by inhibiting the effects of sympathetic activation by the application of a  $\beta$ -AR antagonist is important for the treatment of chronic heart failure.

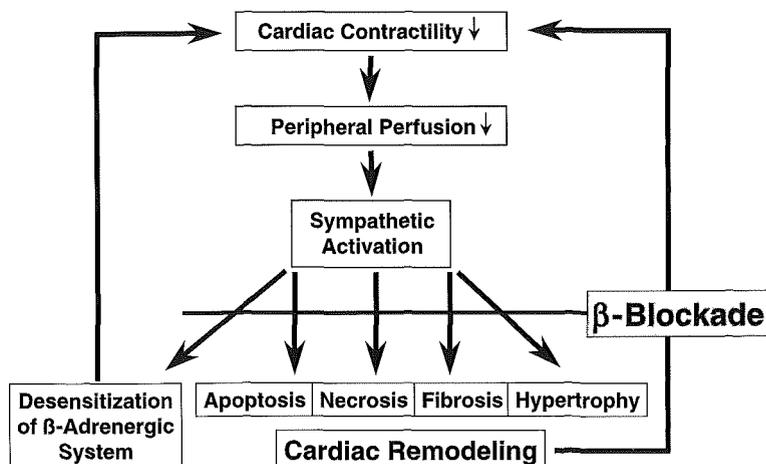


Fig. 1. Vicious cycle of sympathetic activation in chronic heart failure. Pharmacological intervention by  $\beta$ -blockade is a tool to interrupt this vicious cycle.

Treatment of patients with  $\beta$ -AR antagonists leads to an improvement of symptoms and left ventricular (LV) function. However, while treatment with metoprolol [10], carvedilol [11,12] and bisoprolol [13] improved prognosis, the partial agonist xamoterol led to an increase of mortality in patients with heart failure [14]. Recently, the  $\beta$ -blocker bucindolol failed to reduce mortality in patients of the New York Heart Association (NYHA) classes III–IV in the BEST trial [15]. These different results in outcome suggest that among  $\beta$ -AR antagonists; different pharmacological properties may have an important impact on prognosis of heart failure patients.

In recent years, the discovery of inverse agonist activity of  $\beta$ -AR antagonists, based on a modification of the ternary complex model of receptor activation, provided important information concerning different interaction of antagonists with  $\beta$ -ARs and their effects on downstream signaling molecules. This review focusses on different inverse agonist/partial agonist activities of  $\beta$ -AR antagonists used in the treatment of chronic heart failure. Furthermore, the clinical relevance of different intrinsic activity of  $\beta$ -AR antagonists is discussed.

## 2. Agonist and inverse agonist activity of $\beta$ -AR antagonists

According to the ternary complex model of receptor activation established by De Lean et al. [16], the binding of an agonist to the  $\beta$ -AR leads to an activation of the receptor and allows the coupling to the stimulatory G-protein ( $G_s$ ) and adenylate cyclase. In this context, the binding of a partial agonist leads to partial activation, whereas the binding of an antagonist merely inhibits agonist-induced activation of the receptor. The development of transgenic mouse models with overexpression of the  $\beta_2$ -AR provided further insight to the mechanisms of receptor action in response to antagonists. In mice with 200-fold overexpression of the  $\beta_2$ -AR, baseline left atrial tension was comparable to maximum isoprenaline-induced tension in wild-type animals [17]. In contrast to wild-type animals, no further increase of atrial tension could be induced by isoprenaline in these animals [17]. The authors concluded that unoccupied  $\beta$ -ARs consist of a certain amount of receptors in an activated state ( $R^*$ ) that maintain a baseline degree of adenylate cyclase activation. When overexpressing  $\beta_2$ -ARs, the fraction of receptors in the activated state is sufficient to account for complete activation of adenylate cyclase because further downstream, molecules of the signal transduction cascade ( $G_s$ , adenylate cyclase) were not overexpressed. In this mouse model (TG4), application of different  $\beta$ -AR antagonists led to different degrees of inhibition of contractility [18]. These data indicate that unoccupied  $\beta$ -ARs already exert a basal amount of intrinsic activity. The ability of  $\beta$ -AR antagonists to reduce this basal  $\beta$ -AR activity is termed “inverse agonist activity”. The action of a strong inverse agonist (e.g., ICI 118.551) on  $\beta_2$ -AR activity can be antagonized by a weak inverse agonist (e.g., alprenolol) [18]. According to these new findings, the ternary complex model had to be modified by establishing the allosteric ternary complex model of receptor activation (Fig. 2), in which the  $\beta$ -AR exists in an equilibrium between the inactivated ( $R$ ) and the activated state ( $R^*$ ). The binding of an agonist leads to a conformational change of the receptor, allowing it to interact with  $G_s$  and adenylate

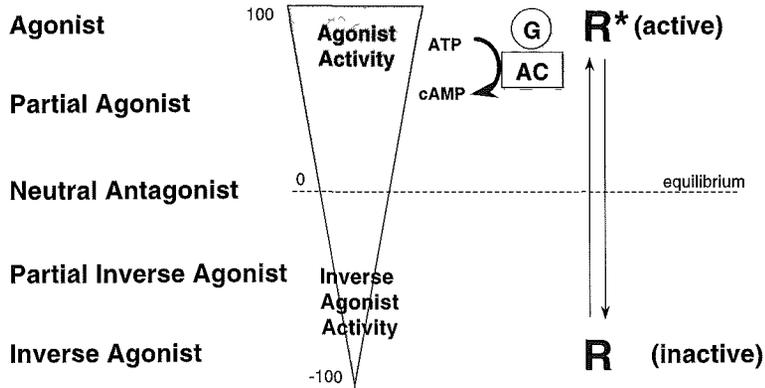


Fig. 2. Allosteric ternary complex model of receptor activation. In contrast to the model of De Lean et al. [16], a “second dimension” of *inverse* agonist activity has to be considered.

cyclase. In contrast, the binding of an inverse agonist stabilizes the  $\beta$ -AR in its inactive conformation, thereby maximally inhibiting interaction of the receptor with  $G_s$ . The binding of a neutral antagonist leaves the equilibrium more or less unchanged.

### 3. Impact of $\beta$ -AR signaling on receptor regulation

The activation state of the  $\beta$ -AR is of importance not only for contractility, but also for receptor regulation. Only receptors in the activated state ( $R^*$ ) are a substrate for phosphorylation by the  $\beta$ -AR kinase and, thus, desensitization and down-regulation. The intrinsic activity of several partial and full agonists correlates with the degree of receptor phosphorylation and in turn desensitization and down-regulation [19]. Accordingly, also inverse agonism has an impact on receptor regulation. When transfecting mice with a constitutively active mutant of the  $\beta_2$ -AR (CAM  $\beta_2$ ), no overexpression of this receptor could be achieved in these animals [20]. Only when treating these animals with the inverse agonist ICI 118.551 could robust levels of CAM  $\beta_2$ -AR density be achieved. These results indicate that inverse agonist-induced inactivation of receptors leads to inhibition of phosphorylation of receptors by  $\beta$ -ARK and, thus, to sensitization and up-regulation of receptor density, while agonist-induced activation facilitates phosphorylation and, thus, desensitization and down-regulation of receptors.

### 4. Inverse agonism of $\beta$ -AR antagonists in human myocardium

In order to determine intrinsic activity (inverse agonist or partial agonist activity) of  $\beta$ -blockers in human myocardium, we performed radioligand binding experiments with [ $^{125}$ I]iodocyanopindolol in the absence and presence of Gpp(NH)p in order to estimate  $\beta$ -

AR/G-protein interaction [21]. Furthermore, contraction experiments on isolated ventricular muscle strip preparations from failing myocardium were performed [22,23].

Binding curves of bucindolol and carvedilol, but not of bisoprolol, nebivolol or metoprolol, were affected by the presence of Gpp(NH)p, a nonhydrolyzable GTP analogue [22,23] (Fig. 3). Carvedilol and bucindolol exerted biphasic binding curves with the identification of a high- and a low-affinity receptor binding site (Fig. 3B and C). The high-affinity binding site was eliminated in the presence of Gpp(NH)p, indicating that the

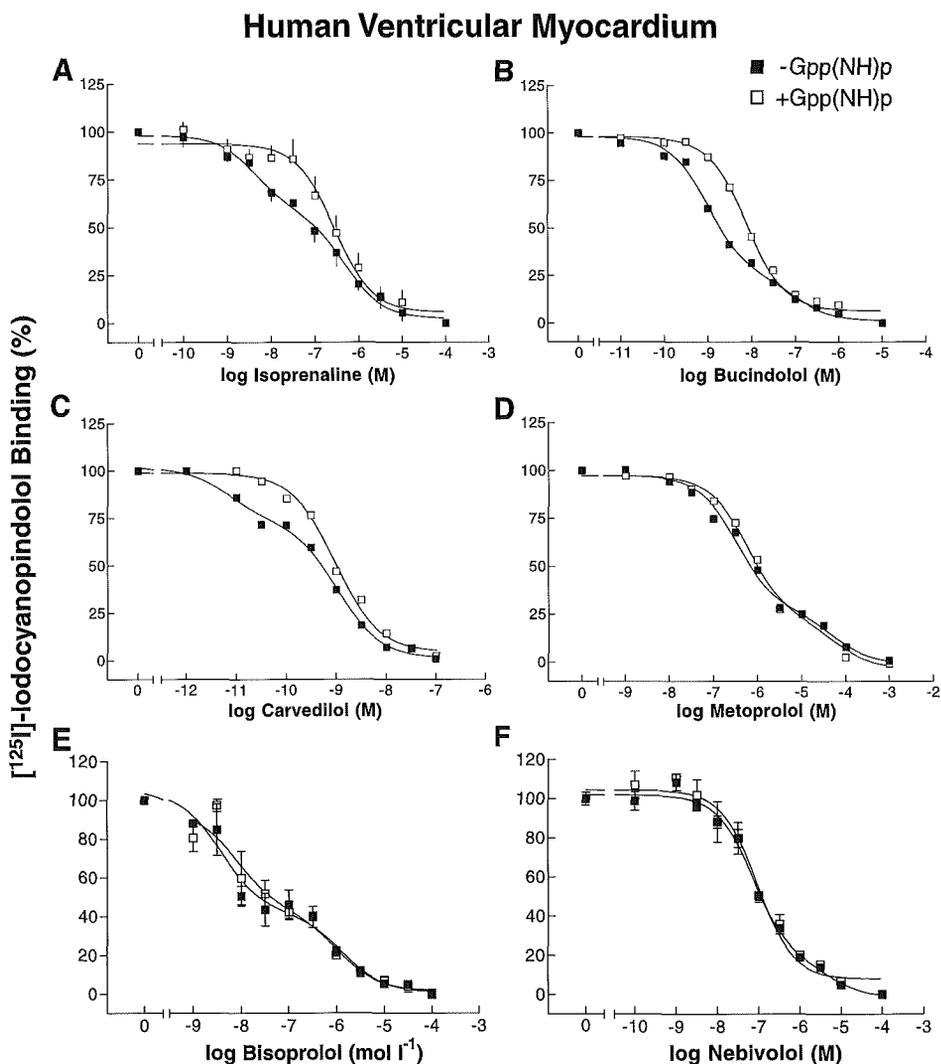


Fig. 3. Radioligand binding experiments on human ventricular myocardium.  $[\text{125I}]\text{iodocyanopindolol}$  was used as the radioligand. Competition curves were performed with various  $\beta\text{-AR}$  ligands in the absence and presence of Gpp(NH)p (100  $\mu\text{M}$ ). Note that Gpp(NH)p eliminates the high-affinity binding site of isoprenaline, bucindolol and carvedilol (data from Refs. [21,22]).

binding of these agents to the  $\beta$ -AR induces a dissociation of the  $\alpha$ -subunit from the  $\beta\gamma$ -subunits of  $G_s$ . This dissociation is a prerequisite for adenylyl cyclase activation and usually a sign for (partial) agonist activity of a ligand [21], as can be seen from binding characteristics of the full agonist isoprenaline (3A). Bisoprolol [23] and metoprolol [22] also identified two different binding sites; however, the high-affinity site could not be eliminated in the presence of Gpp(NH)p, but in the presence of CGP207.12A, a highly selective  $\beta_1$ -AR antagonist (not shown). This indicates that the biphasic binding characteristics are due to  $\beta_1$ -selectivity of metoprolol and bisoprolol because human ventricular membranes contain both  $\beta_1$ - and  $\beta_2$ -ARs.

To evaluate the functional relevance of these biochemical findings, contraction experiments were performed in the presence of forskolin, a diterpene that facilitates the coupling of the  $\alpha$ -subunit of the stimulatory G-protein ( $G_{S\alpha}$ ) to the catalytic unit of adenylyl cyclase [24]. All  $\beta$ -blockers except bucindolol significantly reduced force of contraction [22,23] (Fig. 4). However, the degree of the negative inotropic effects was different. At 100%  $\beta$ -AR occupation, metoprolol reduced force by about 85%, an effect that could be antagonized by co-incubation with bucindolol [22] (Fig. 5). This indicates that the negative inotropic effect of this agent is mediated by the  $\beta$ -AR and, thus, can be attributed to inverse agonist activity. The rank order of inverse agonist activity was metoprolol>bisoprolol=neбиволol>carvedilol (Fig. 4).

The application of bucindolol led to no significant change of cardiac contractility. This was due to the fact that bucindolol had a positive inotropic effect in one part and a weak negative inotropic effect in the other part of experiments [22] (Fig. 6). This may be due to different initial activation states of  $\beta$ -ARs in the different tissue samples. In  $\beta_2$ -AR-expressing Sf9 cells, dichloroisoproterenol (DCI) was shown to act as either a partial

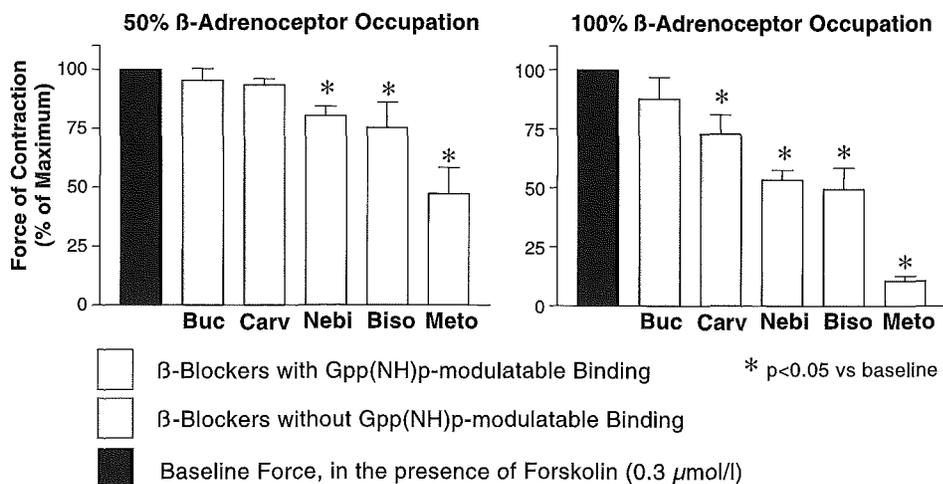


Fig. 4. Different inverse agonist activity of different  $\beta$ -AR antagonists in human ventricular myocardium.  $\beta$ -AR antagonists that displayed Gpp(NH)p-modulatable binding (Fig. 3), which indicates  $\beta$ -AR/G-protein interaction, have less inverse agonist activity compared to agents that do not have this property. Buc, bucindolol; Carv, carvedilol; Biso, bisoprolol; Nebi, nebivolol; Meto, metoprolol. Data are from Refs. [21,22].

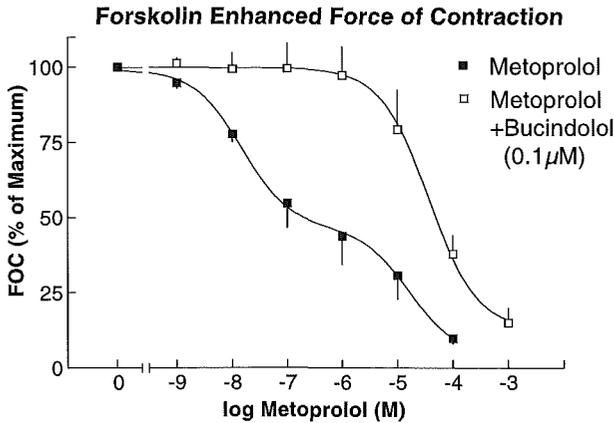


Fig. 5. Inverse agonist activity of metoprolol in human ventricular myocardium. The negative inotropic effect of metoprolol can be antagonized in the presence of bucindolol (from Ref. [21]).

agonist or inverse agonist, depending on the degree of isoprenaline-induced desensitization of the system [25]. In Fig. 7, the effects of 100%  $\beta$ -AR occupation by bucindolol on forskolin-enhanced force of contraction is compared to the effects of receptor occupation by carvedilol (Fig. 7A) and metoprolol (Fig. 7B) in the hearts from the same patients, respectively. Assuming that the initial state of  $\beta$ -AR activation is similar in samples that come from the same tissue, these plots indicate that the activation state of the  $\beta$ -ARs has an influence on the functional response to the respective ligands. In the study of Chidiac et al. [25], desensitization of  $\beta$ -ARs by isoprenaline treatment turned *partial* agonist activity of DCI to *inverse* agonist activity. Since contraction experiments were performed on human failing myocardium, desensitization of  $\beta$ -ARs by endogenous catecholamines in

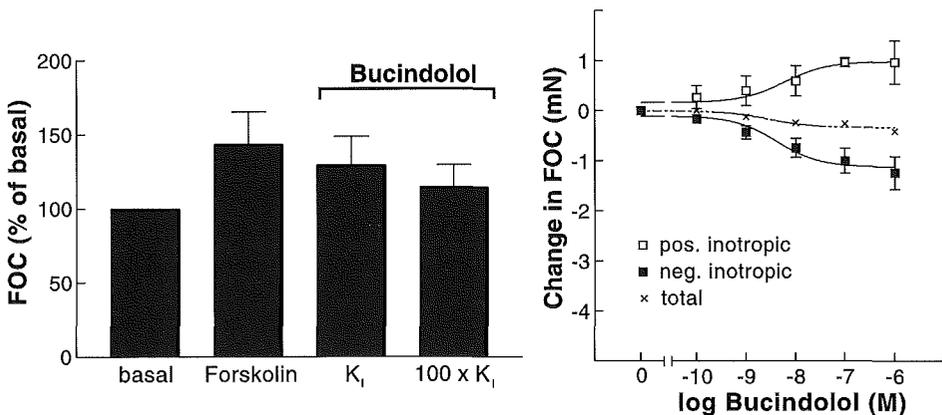


Fig. 6. Bucindolol does not reduce force of contraction in human ventricular myocardium. This is due to the fact that it had a slight negative inotropic effect in one part of experiments and a positive inotropic effect in the other part (from Ref. [21]). Coupling of  $G_{sa}$  to adenylate cyclase was facilitated by the addition of forskolin.  $K_1$ ,  $100 \times K_1$ ; concentration at which 50% ( $K_1$ ) and 100%  $\beta$ -AR ( $100 \times K_1$ ) occupation by  $\beta$ -AR antagonist occurs.

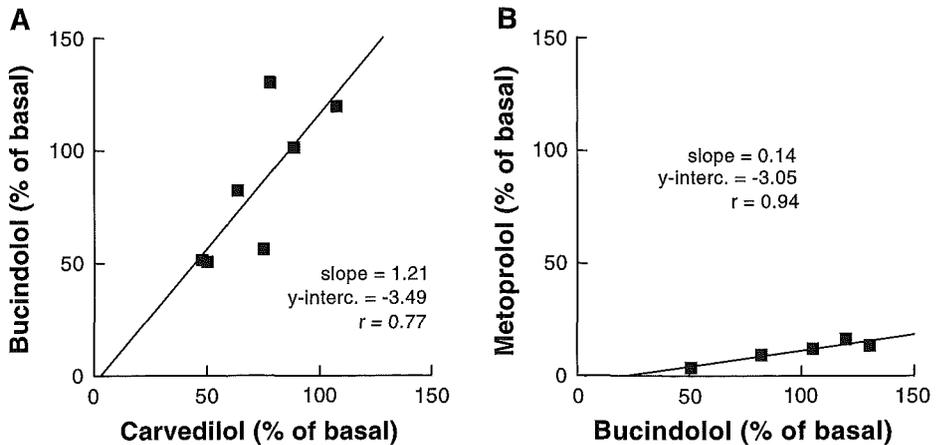


Fig. 7. Comparison of intrinsic activity of bucindolol, carvedilol and metoprolol. Panel A, force of contraction (FOC) in % of basal values after  $100 \times K_i$  of carvedilol (abscissa) is plotted against FOC in percentage of basal values after  $100 \times K_i$  of bucindolol (ordinate) in preparations from the same hearts, after forskolin ( $0.3 \mu\text{M}$ ) prestimulation of left ventricular myocardium from patients with heart failure. Panel B, bucindolol (abscissa) against metoprolol (ordinate), the same conditions as in panel A. Data are from Ref. [21].

the *in vivo* situation could have masked partial agonist activity of bucindolol. Preliminary data indicate that *in vitro* pretreatment of failing myocardium with the inverse agonist metoprolol (with subsequent wash-out) restores the positive inotropic effect of bucindolol (unpublished data). This indicates that intrinsic activity especially of weak partial agonists is dependent on the initial activation (or sensitization) state of  $\beta$ -ARs.

## 5. Clinical implications of inverse agonist activity of $\beta$ -AR antagonists

In chronic heart failure, plasma catecholamine levels are elevated [1]. The question is whether in this situation, different inverse agonist activity of  $\beta$ -AR antagonists may lose importance since at agonist-occupied receptors, any  $\beta$ -blocker primarily antagonizes agonist-induced activation of receptors. Nevertheless, even at agonist-occupied receptors, the degree of inactivation by  $\beta$ -blockers is variable. While bucindolol lowers contractility to baseline values, metoprolol reduces contractility beyond baseline [22] (Fig. 8). Thus, any  $\beta$ -AR ligand may stabilize the receptor at a certain conformation and, thus, activity, which is independent of the initial activation state of the receptor. This implicates that in the clinical situation, net  $\beta$ -adrenergic signaling may be dependent on the intrinsic activity of the  $\beta$ -blocker that is applied.

Which clinical parameter is an indicator of  $\beta$ -adrenergic signaling? A characteristic hallmark of increased  $\beta$ -adrenergic signaling is an elevation of heart rate at rest. In animal models, overexpression of  $\beta_1$ - and  $\beta_2$ -ARs, as well as  $G_{s\alpha}$ , leads to elevated baseline heart rates in these animals [7,18,9]. In addition, in patients with heart failure, heart rate is increased due to sympathetic activation. Thus, the effect of a  $\beta$ -blocker on heart rate could

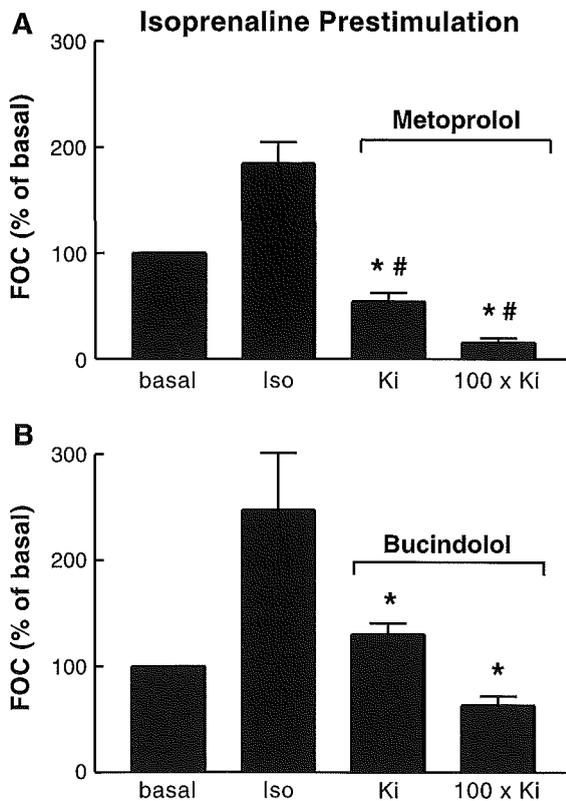


Fig. 8. Both metoprolol and bucindolol antagonize the positive inotropic effect of isoprenaline (Iso) in human ventricular myocardium. Metoprolol, but not bucindolol decreases force below baseline values.  $K_i$ ,  $100 \times K_i$ : concentration at which 50% ( $K_i$ ) and 100%  $\beta$ -AR ( $100 \times K_i$ ) occupation by  $\beta$ -AR antagonist occurs. Data are from Ref. [21].  $p < 0.05$  vs. Iso; #  $p < 0.05$  vs. basal.

be considered as its effect on  $\beta$ -adrenergic signaling. Especially in situations of decreased adrenergic stimulation, i.e., during the night, heart rate mirrors intrinsic activity of the respective  $\beta$ -blocker. In patients with heart failure, heart rate at night is increased by the partial agonist xamoterol [14], decreased by the inverse agonist metoprolol and unchanged by bucindolol [26,27]. Interestingly, mortality is affected in a similar way by these three agents. While xamoterol treatment of heart failure patients increased mortality [14], metoprolol reduced it [10] and bucindolol neither increased nor decreased it significantly [15]. Of course, one has to be cautious in drawing such direct conclusions. However, increased heart rate is associated with excess mortality in patients with arterial hypertension [28], coronary artery disease [29] and chronic heart failure. A meta-analysis of trials in patients after myocardial infarction indicates that there is a close correlation between the degree of heart rate reduction and mortality reduction [30]. It is of note that agents displaying small amounts of partial agonist activity (e.g., pindolol or oxprenolol) have lesser effects on mortality compared to inverse agonists (e.g., metoprolol). These authors even postulate that a reduction of heart rate by 1 beat/min reduces mortality by 2%

[30]. Similar results were obtained in patients with chronic heart failure, where in large pharmacological intervention trials on heart failure patients, a correlation between heart rate reduction and mortality reduction exists [31]. These data suggest that (1) reduction of heart rate in patients with cardiovascular disorders is associated with reduced mortality, and (2) inverse agonists, neutral antagonists and partial agonists affect heart rate and, thus,  $\beta$ -adrenergic signalling in different ways.

Furthermore, intrinsic activity of  $\beta$ -AR antagonists is of importance for receptor regulation. Activated  $\beta$ -ARs ( $R^*$ ) are phosphorylated, uncoupled from  $G_s$  and down-regulated. In contrast, inactivated receptors are dephosphorylated, resensitized and up-regulated. Accordingly, in patients with chronic heart failure,  $\beta$ -AR density was upregulated by the strong inverse agonist metoprolol but not by the weak inverse agonist carvedilol [32]. In patients with heart failure, ventricular  $\beta$ -AR density is positively correlated with the maximum increase of heart rate, as well as maximum oxygen consumption at exertion [33]. This may explain why in clinical trials with  $\beta$ -blockers, maximum exercise tolerance was improved in most studies with metoprolol but not carvedilol [34].

## 6. Conclusions

These data illustrate that different inverse agonist activities of  $\beta$ -AR antagonists have an impact on  $\beta$ -AR regulation, exercise tolerance, minimum heart rate and possibly on prognosis in patients with chronic heart failure.  $\beta$ -ARs that lack inverse agonist activity or exert partial agonist activity have a neutral effect (bucindolol) or even a negative effect (xamoterol) on prognosis of patients with chronic heart failure. Thus, inverse agonism should be considered as an important property of a  $\beta$ -AR antagonist in the treatment of cardiovascular diseases.

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## Discussion 4

### G. Milligan

I suppose one of the issues about the protocols you've been following, compared to the discussion that Richard Bond started, clearly is the aspect of the chronic treatment that presumably the patient will be receiving vs. some of the relatively short-term periods that you were studying. How do you think the balance between those things lie, if you took some of the pretreatments to more extensive periods?

### C. Maack

I think that on the level of the  $\beta$ -adrenergic system, there will be no big qualitative difference between short- and long-term treatment concerning the effects that we observed. I believe so because after 1 h of pretreatment, we already observed sensitization or desensitization of the  $\beta$ -adrenergic system. This means that apparently, changes of the  $\beta$ -adrenergic signaling cascade take place within minutes to hours. However, these effects of  $\beta$ -blockers on the receptor level may not necessarily be responsible for the beneficial long-term effects of these agents. It could be assumed that reducing  $\beta$ -adrenergic signaling in the long term improves the biology of the heart by reducing the triggers that induce hypertrophy, fibrosis and even apoptosis or necrosis.

The important observation we made in the short-term setting is that when we are removing a ligand from the receptor, the activation state of the receptor doesn't really relax back to the original condition within 1–2 h. This holds true for receptors activated by agonists or inactivated by inverse agonists. This may really be the difficulty when measuring intrinsic activity of ligands in human myocardium. Different patients will have been treated with different  $\beta$ -blockers, different doses of  $\beta$ -blockers and agonist drugs, and may have different degrees of endogenous release of catecholamines. In this situation, this "cocktail" of  $\beta$ -adrenoceptor active agents is probably putting the receptors into a certain state of activation that we cannot fully reverse to its native condition by washing ligands off the receptors before starting the experiment.

T. Costa

I am curious to know what you mean by this sort of equilibrium. If the receptor has a “memory” of the ligand for a long time, perhaps you’re not only talking about an equilibrium, but something else, some sort of long term stabilization. Do you think it might be membrane localisation, crowding around some specialised parts of the membrane, segregation, or some sort of other scenery? Or do you really think that this phenomenon is something related to the receptor molecule itself?

C. Maack

I imagine when the conformation of the receptors is changed into an unrelaxed condition by an agonist, it looks like the dissociation of the ligand from the receptor doesn’t occur with a complete relaxation of the receptor back to its original condition. It seems to be some other factor, another equilibrium that predicts the return to the original situation. From our results, I do not know which factors are involved in this process. Maybe it has to do with the proposal of Chidiac. This would mean that the desensitization state of receptors persists longer than the receptor is occupied by the agonist.

W. Clarke

I think you suggested in some of your diagrams that perhaps posttranslational modification of the receptor alters the ability of the receptor to adopt active or inactive confirmation (i.e., the allosteric constant). Maybe phosphorylation as a result of GRK, or protein kinase A, can change the capacity of the receptor to flip back and forth between conformations. And one test of that could be to investigate with dominant negative GRKs in the system, or with other ways of removing the phosphate groups, or mutating the sites on the receptor. I think that’s consistent with Chidiac’s data and it’s certainly consistent with your hypotheses as well.

M. Lohse

You mentioned that you thought the equilibrium between the R\* and R was not the same as the sensitization state. In my mind, it had always been the same. So could you try to explain this again, why you think these are two different things?

C. Maack

We obtained our data from human myocardium, in order to test what happens if you incubate muscle strips with agonists or inverse agonists, and then washing out these ligands. In these experiments, we observed that after metoprolol treatment there was a consistent shift to the right in the forskolin dose–response. At the same time, isoprenaline dose–response curves were shifted to the left, probably because  $\beta$ -adrenergic receptors in that tissue were desensitized.

M. Lohse

I think we may be talking about two different things. One is the desensitization of the receptor by the mechanisms that we all know and that William Clarke was alluding to, like phosphorylation, arrestin binding, etc. And for the desensitization of the receptor, you would expect exactly what you saw: you pretreat with an antagonist or inverse agonist, desensitization disappears and the response is greater than before. And I think you also have a second mechanism where I don’t know the biochemistry but I see the mechanism. That’s coupling between  $G_s$  and cyclase. And that appears to behave differently because you see different responses and different shifts than you would expect from receptor desensitization.

C. Maack

The question is why after agonist treatment, the forskolin dose–response curve shifts to the left whereas the isoprenaline dose–response curve shifts to the right. Is there any explanation for these diverging directions that the forskolin response is sensitized and the isoprenaline response is desensitized? When the  $\beta$ -adrenergic receptor is activated,  $G_{s\alpha}$  dissociates from  $\beta\gamma$  subunits and activates the adenylyl cyclase and increases cAMP production. Consequently, the receptor may be uncoupled from  $G_s$  by cAMP-dependent kinases and GRKs. However, despite uncoupling of the  $\beta$ -AR from  $G_s$ , coupling between  $G_{s\alpha}$  and adenylyl cyclase appears to be persistent. This may indicate that an activated, but partially uncoupled receptor, yields a higher degree of  $G_s$  interaction with subsequent adenylyl cyclase activation than a sensitized but inactivated receptor. The divergence of isoprenaline and forskolin sensitivity may thus reflect the negative feedback mechanism that balances increased  $\beta$ -AR activation by uncoupling of the  $\beta$ -AR from  $G_s$ .

A. IJzerman

You carefully avoided naming subtypes of  $\beta$ -adrenoceptors, and you just talked about  $\beta$ -adrenoceptor ligands. In failing heart and in the tissues that you've used, I can imagine that there is more than just the  $\beta_1$ -adrenoceptor. May the different ligands that you used affect other than  $\beta_1$ -adrenoceptors?

C. Maack

We did not discriminate between the effects on  $\beta_1$  and  $\beta_2$ -adrenoceptors because it is really difficult in such an in vitro system. We know that in our set of experiments using isoprenaline we act on both receptors. We are also aware of the fact that in heart failure, especially  $\beta_1$ -adrenoceptors are down-regulated, and  $\beta_2$ -adrenoceptors are rather uncoupled. Bucindolol binds nonselectively to both  $\beta_1$ - and  $\beta_2$ -adrenoceptors. Furthermore, we see Gpp(NH)p-induced shifting of bucindolol binding, which indicates agonist-like binding properties on both  $\beta_1$ - and  $\beta_2$ -adrenoceptors. We could try to discriminate the effects of bucindolol by pretreating muscles with ICI 118,551—a  $\beta_2$ -adrenoceptor inverse agonist—compared to metoprolol, which is predominantly a  $\beta_1$ -inverse agonist. However, in the concentrations we applied metoprolol, it occupies  $\beta_2$ -adrenoceptors as well.

R. Bond

I think it's a difficult question to get to because of the approved ones, metoprolol has very poor selectivity. There is a head-to-head trial going on between metoprolol and carvedilol, which might give some clues. And then you also have the added confounding in the sense that inotropy is bad. I don't care what receptor does it, it is exactly the opposite of what we used to think. But you've also got prejunctional facilitatory  $\beta_2$ -receptors that increase the release of noradrenaline and adrenaline. So blocking them might also be useful, and we don't know what part is due to that.

M. Lohse

All the compounds, in high enough concentrations, which act as inverse agonists at the  $\beta_1$ -adrenoceptors, are also inverse agonists at the  $\beta_2$ -adrenoceptors. Then probably the response would be the same whether you look at  $\beta_1$ - or at  $\beta_2$ -adrenoceptors. With respect to the inotropic response resulting in the damage, I'm not so sure whether that's really always the case. In your studies in mouse, the  $\beta_2$ -adrenoceptor induced increase in contractility is actually quite well tolerated compared to the  $\beta_1$ -adrenoceptors or other transgenic mice. That's something that puzzles me, why is one receptor well-tolerated,

even though it drives the heart for months and months, and another receptor is not well-tolerated? In my mind there, is some mysterious protective signalling pathway that makes sure that the  $\beta_2$ -adrenoceptor doesn't kill the heart and the  $\beta_1$ -adrenoceptor does.

R. Bond

In the paper that you pointed out to me, celiprolol, which is a  $\beta_1$  antagonist and a  $\beta_2$  agonist, failed in heart failure. In another study, I found it was detrimental. In conclusion, blocking the  $\beta_1$ -adrenoceptor and activating the  $\beta_2$ -adrenoceptors by this fortuitous combination was detrimental or had no effect.

T. Schwartz

I can get a long discussion on  $\beta_1$  and  $\beta_2$ -adrenoceptors and also relating to  $R^*$  and  $R$ , and so forth. But it is necessary to know that this topic becomes really complicated because several different subtypes of receptors may be involved, and it also depends on how the tissue and the cells have been treated. One of the most seminal and important papers on inverse agonism was published by Michel Bouvier. A ligand is something on a receptor under some given circumstances, so it means you are calling ligands—being inverse agonists or partial agonists, and so forth—but it depends so extraordinarily much on which tissue the receptor is in and in what circumstances you see it in. Partial agonists can become inverse agonists just by a little pretreatment with one or another drug. What happens if we go into a human being? Is there any way we can find out where the ligands really are under those circumstances? Probably the best thing, or not, is give it to 200 patients and see what happens.