



Baker, Jillian G. and Hill, Stephen J. and Summers, Roger J. (2011) Evolution of  $\beta$ -blockers: from anti-anginal drugs to ligand-directed signalling. *Trends in Pharmacological Sciences*, 32 (4). pp. 227-234. ISSN 0165-6147

**Access from the University of Nottingham repository:**

<http://eprints.nottingham.ac.uk/2796/1/HillEvolution.pdf>

**Copyright and reuse:**

The Nottingham ePrints service makes this work by researchers of the University of Nottingham available open access under the following conditions.

- Copyright and all moral rights to the version of the paper presented here belong to the individual author(s) and/or other copyright owners.
- To the extent reasonable and practicable the material made available in Nottingham ePrints has been checked for eligibility before being made available.
- Copies of full items can be used for personal research or study, educational, or not-for-profit purposes without prior permission or charge provided that the authors, title and full bibliographic details are credited, a hyperlink and/or URL is given for the original metadata page and the content is not changed in any way.
- Quotations or similar reproductions must be sufficiently acknowledged.

Please see our full end user licence at:

[http://eprints.nottingham.ac.uk/end\\_user\\_agreement.pdf](http://eprints.nottingham.ac.uk/end_user_agreement.pdf)

**A note on versions:**

The version presented here may differ from the published version or from the version of record. If you wish to cite this item you are advised to consult the publisher's version. Please see the repository url above for details on accessing the published version and note that access may require a subscription.

For more information, please contact [eprints@nottingham.ac.uk](mailto:eprints@nottingham.ac.uk)

# Evolution of $\beta$ -blockers: from anti-anginal drugs to ligand-directed signalling

Jillian G. Baker<sup>1</sup>, Stephen J. Hill<sup>1</sup> and Roger J. Summers<sup>2</sup>

<sup>1</sup>Institute of Cell Signalling, School of Biomedical Sciences, Medical School, Queen's Medical Centre, Nottingham, UK

<sup>2</sup>Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, 399, Royal Parade, Parkville, Vic 3052, Australia

**Sir James Black developed  $\beta$ -blockers, one of the most useful groups of drugs in use today. Not only are they being used for their original purpose to treat angina and cardiac arrhythmias, but they are also effective therapeutics for hypertension, cardiac failure, glaucoma, migraine and anxiety. Recent studies suggest that they might also prove useful in diseases as diverse as osteoporosis, cancer and malaria. They have also provided some of the most useful tools for pharmacological research that have underpinned the development of concepts such as receptor subtype selectivity, agonism and inverse agonism, and ligand-directed signalling bias. This article examines how  $\beta$ -blockers have evolved and indicates how they might be used in the future.**

## Introduction

Sir James Black made a massive contribution to pharmacology by demonstrating that new major classes of drugs could be developed by applying basic knowledge of receptor-driven cell-signalling systems to clinical problems. He developed two new classes of drugs at a time when few specific medical treatments were available:  $\beta$ -blockers\* for cardiovascular disease and H<sub>2</sub>-antihistamines as antacids for gastric and duodenal ulceration [1]. His less well-publicised contribution was to analytical pharmacology, in which many of his pioneering approaches will continue to resonate for many years to come [2]. His first discovery,  $\beta$ -blockers, was stimulated by the sudden death of his father from a myocardial infarction when Sir James was at medical school. He wanted to 'stop the effects of adrenaline on the heart' and therefore improve the chest pain of ischaemic heart disease caused by a lack of oxygen in the heart. Propranolol, which he developed while at ICI pharmaceuticals and still in widespread clinical use today, went on to change medical practice worldwide [3]. In this review we examine how  $\beta$ -blockers have evolved from their origin as treatments for angina and cardiac arrhythmias to be effective therapeutics for hypertension, cardiac failure, glaucoma, migraine and anxiety, and discuss the potential for their future development for the treatment of a variety of conditions.

## Early development and clinical uses of $\beta$ -blockers

In angina, the coronary arteries are partially blocked (usually by atherosclerosis), which reduces blood flow to the heart muscle. Exercise, stress and emotion increase sympathetic drive (which increases the rate and force of contraction), thus requiring increased coronary artery blood flow. If this is not achieved, myocardial ischaemia occurs, resulting in crushing central chest pain typical of angina pectoris. Sir James surmised that blocking the effects of catecholamines would prevent angina by decreasing the workload. Although Ahlquist had proposed in 1948 that widespread adrenaline effects were mediated through two groups of adrenoceptors,  $\alpha$  and  $\beta$  [4], the idea of developing a selective  $\beta$ -adrenoceptor antagonist was still quite radical. After joining ICI Pharmaceuticals in 1958, Sir James used the  $\beta$ -adrenoceptor-selective agonist isoprenaline as a template, but became intrigued that a derivative, dichloroisoprenaline, lowered heart rate [5]. The application of some clever medicinal chemistry by John Stephenson resulted in practolol and pronethanol, the first  $\beta$ -blockers (later withdrawn because of oculomucocutaneous syndrome/sclerosing serositis and carcinogenicity). A safer, more potent derivative soon followed, propranolol, which is still considered the prototypical  $\beta$ -adrenoceptor antagonist (Table 1) [6].

The rationale that Sir James used for the development of  $\beta$ -blockers was that by reducing catecholamine-induced effects of myocardial  $\beta$ -adrenoceptor activation, the pain due to angina would be improved.  $\beta$ -Adrenoceptor blockade leads to an increase in work capacity before pain or ischaemia occurs by reducing oxygen consumption by the heart [7]. Another early clinical observation was that pronethanol and propranolol produced hypotension [8,9]. It was also shown that propranolol has anti-arrhythmic properties. Subsequent work established that the anti-hypertensive and anti-arrhythmic properties, together with the reduction in heart rate and cardiac output, are important class actions of  $\beta$ -adrenoceptor antagonists (Table 1) [10].

## Current cardiovascular uses of $\beta$ -blockers

The number of  $\beta$ -blockers available rapidly increased and became the major first-line therapy for hypertension (atenolol is historically one of the most frequently prescribed of all medicines). Improvements in the symptomatic management of angina were followed by improvements in

Corresponding author: Summers, R.J. (roger.summers@monash.edu)

\*  $\beta$ -Blocker is the commonly used term for  $\beta$ -adrenoceptor antagonist as defined by NC-IUPHAR. The term favoured by Sir James Black was  $\beta$ -receptor antagonist.

**Table 1. Current indications for  $\beta$ -blockers from the British National Formulary September 2010 ([www.bnf.org](http://www.bnf.org))**

Propranolol	Hypertension, ischaemic heart disease (IHD), arrhythmias, portal hypertension, anxiety, essential tremor, migraine, thyrotoxicosis
Acebutolol	Hypertension, IHD, arrhythmias
Atenolol	Hypertension, IHD, arrhythmias, migraine
Bisoprolol	Hypertension, IHD, heart failure
Carvedilol	Hypertension, IHD, heart failure
Celiprolol	Hypertension
Esmolol	Arrhythmias (short-term)
Labetolol	Hypertension
Metoprolol	Hypertension, IHD, arrhythmias, migraine
Nadolol	Hypertension, IHD, arrhythmias, migraine, thyrotoxicosis
Nebivolol	Hypertension, heart failure
Oxprenolol	Hypertension, IHD, arrhythmias, anxiety
Pindolol	Hypertension, IHD
Sotalol	Arrhythmias
Timolol	Glaucoma, hypertension, IHD, migraine
Betaxolol	Glaucoma
Carteolol	Glaucoma
Levobunolol	Glaucoma
Metipranolol	Glaucoma

mortality in acute myocardial infarction (MI) [11] and long-term when given post-MI [12–16].  $\beta$ -Blockers also reduce arrhythmias after both cardiac and non-cardiac surgery [17–21]. Thus,  $\beta$ -blockers now have important role in improving both mortality and symptom control in ischaemic heart disease, arrhythmias and hypertension (Table 1) [22].

Recent studies suggest that the reduction in hypertension following  $\beta$ -blockade has not resulted in as great a reduction in stroke as other (newer) treatments, but other evidence contests this and  $\beta$ -blockers remain an important treatment for hypertension [16,23–25].

### Heart failure and $\beta$ -blockers: paradoxical pharmacology?

Initially  $\beta$ -blockers were contraindicated in heart failure. In 1978, Prichard observed: ‘Two important untoward effects from  $\beta$ -adrenergic receptor blocking drugs that should be avoided with foresight in patient selection: heart failure and asthma. Patients in heart failure, or patients with incipient left ventricular insufficiency . . . are critically dependent on sympathetic activity to the heart to maintain their cardiac output.’ [10]. Indeed, heart failure occurs when the cardiac output is not sufficient to meet the demands of the body so reducing output further with a  $\beta$ -blocker would appear illogical.

However, the healthy human heart also contains functionally well-coupled  $\beta_2$ -adrenoceptors [26], with 80% of cardiac  $\beta$ -adrenoceptors of the  $\beta_1$  and 20% of the  $\beta_2$ -subtype [27,28]. In heart failure, the number of  $\beta_1$ -adrenoceptors decreases such that only 60% are  $\beta_1$  (and 40%  $\beta_2$ ) [27]. The role of  $\beta_3$ -adrenoceptors in human myocardium is still not clear [29]. There is also evidence that long-term activation of  $\beta_1$ -adrenoceptors in animals has more deleterious effects than activation of  $\beta_2$ -adrenoceptors. Transgenic mice with modest overexpression of  $\beta_1$ -adrenoceptors

rapidly develop cardiac failure and die, whereas  $\beta_2$ -adrenoceptor overexpression is better tolerated [30]. In humans, use of  $\beta_1$ -adrenoceptor-selective agonists was associated with an increase in mortality [31] and they are now restricted to short-term maintenance of cardiac output in intensive and coronary care units.

The initial suggestion that  $\beta$ -blockers were beneficial in heart failure was treated with scepticism [32,33], but subsequent large-scale clinical trials confirmed that they prolong longevity. Bisoprolol [34], metoprolol [35], carvedilol [36] and nebivolol [37] (Table 1) are all effective in reducing mortality in patients (see also Kubon *et al.*, this issue). As Prichard noted, heart failure is a state with high catecholamine levels. This is useful in the short term because it increases cardiac drive, but is detrimental in the long term and results in myocardial apoptosis, fibrosis and remodelling. The pharmacological basis for the beneficial effects of  $\beta$ -blockers in heart failure seems to be this reduction in catecholamine-driven detrimental changes [22,38,39]. Furthermore, two  $\beta$ -blockers tested in heart failure patients that were not beneficial, bucindolol [40] and xamoterol [41], both have significant intrinsic sympathomimetic activity (ISA) [39,42,43].

### Other clinical uses of $\beta$ -blockers

$\beta$ -Blockers (e.g. timolol and betaxolol) are also widely used in glaucoma (raised intraocular pressure) (Table 1) [44]. Propranolol is used for prophylactic treatment of migraine and is as effective as many other treatments available [45]. Interestingly, ISA also seems to make  $\beta$ -blockers less effective in the management of migraine [46].  $\beta$ -Blockers are also used as anxiolytics in both acute and generalised anxiety disorders, for which their likely mode of action is blockage of the peripheral effects of high circulating levels of catecholamines [47]. Short-acting drugs such as propranolol are also popular among performers to reduce catecholamine-induced tremor during performances.  $\beta$ -Blockers are also used in portal hypertension [48] and benign essential tremor [49] and propranolol is used for symptomatic management in hyperthyroidism (Table 1) [50].

### Future potential clinical uses of $\beta$ -adrenoceptor antagonists

Osteoporosis is characterised by a decrease in bone mass due to an imbalance between osteoclast bone reabsorption and osteoblast bone formation. This results in bones that are more fragile and easily fractured. Several cross-sectional clinical studies revealed an association between  $\beta$ -adrenoceptor antagonist treatment and reduced risk of bone fracture [51–57]. Some longitudinal studies also suggest that  $\beta$ -adrenoceptor antagonist treatment can have a protective effect [53,58–60]. In rats [61] and mice [62], propranolol treatment increases bone mass, and  $\beta$ -adrenoceptor agonists such as isoprenaline, clenbuterol and salbutamol have the opposite effect [63,64]. The sympathetic nervous system (SNS) is important in the control of bone formation. In conditions associated with reduced SNS activity (e.g. leptin-deficient *ob/ob* mice or dopamine  $\beta$ -hydroxylase-deficient mice) there is high bone density [62]. Removal of the adrenal medulla does not affect bone mass,

which suggests that the effect is neuronally regulated. The effects of  $\beta$ -adrenoceptor ligands on bone density are probably mediated by actions on  $\beta_2$ -adrenoceptors present on osteoblasts [62,65]. However, the role of  $\beta$ -adrenoceptors in bone remodelling is complex and further work is required to reach consensus on the utility of  $\beta$ -adrenoceptor agonists and antagonists for the treatment of osteoporosis [66].

$\beta$ -Adrenoceptors might also be important in cancer metastasis, because metastatic spread can be inhibited by  $\beta$ -adrenoceptor antagonists. Epidemiological studies have revealed a link between the use of  $\beta$ -adrenoceptor antagonists and reduced cancer risk [67,68]. In a mouse model of breast cancer, activation of the SNS by stress causes a 30-fold increase in metastasis mimicked by subcutaneous administration of isoprenaline and blocked by propranolol [69]. In a model of human ovarian cancer, detachment of cells from the extracellular matrix or from neighbouring cells is associated with apoptosis, a process known as anoikis. Treatment of human ovarian cancer cells with catecholamines reduces anoikis, probably by activating protective focal adhesion kinases (FAKs). Noradrenaline treatment causes increased pFAK<sup>Y397</sup> phosphorylation and decreased anoikis, which can be blocked by cell pretreatment with FAK siRNA [70]. Similar blocking effects were produced with propranolol or butoxamine or by pretreating cells with  $\beta_2$ -adrenoceptor siRNA but not with atenolol [70]. The Y397 phosphorylation site on FAK is a high-affinity binding site for the SH2 domain of Src, and phosphorylation of this site following cell exposure to noradrenaline is prevented by the Src inhibitor PP2, but not by the inactive congener PP3 [70]. It will be interesting to determine whether the coupling of  $\beta_2$ -adrenoceptors to Src involves  $\beta$ -arrestin and internalisation, because it is now possible to identify ligands with a ligand-directed signalling bias<sup>†</sup> for the  $\beta$ -arrestin and G-protein-coupled pathways (see below) [71].

$\beta$ -Adrenoceptors also seem to play a role in regulating infection. Merozoite invasion of erythrocytes by the human malaria parasite *Plasmodium falciparum* is enhanced by treatment with  $\beta$ -adrenoceptor agonists and blocked by the antagonist propranolol and inverse agonist ICI118551 (which also reduces the baseline response). The inactive (+)-isomer of propranolol was ineffective against both *in vitro* and *in vivo* infection [72]. In addition to invasion, erythrocyte G<sub>s</sub> signalling is also required for growth and proliferation of malaria parasites [73]. Interestingly, propranolol was more potent in inhibiting growth than in preventing invasion. Used in combination with existing anti-malarial compounds, propranolol reduced the inhibitory concentrations by five- to ten-fold [73]. In studies examining the inhibitory effects of several  $\beta$ -adrenoceptor antagonists (10  $\mu$ M) on maturation of *P. falciparum* in *in vitro* cultures, propranolol, alprenolol and ICI118551 were very effective in reducing viability, whereas other antagonists (e.g. nadolol, butoxamine, acebutalol, atenolol and metoprolol) were less effective [73]. The actions of  $\beta$ -adrenoceptor antagonists on various aspects of the life cycle of *P. falciparum* suggest that there might be potential to develop combination therapies with existing anti-malarial drugs.

<sup>†</sup> Ligand-directed signalling bias is a process whereby different intracellular signalling cascades are differentially stimulated by different ligands via a single receptor subtype.

## $\beta$ -Blockers and airway disease

There are major concerns about the use of  $\beta$ -blockers in patients with respiratory disease, particularly asthma. Blockade of  $\beta_2$ -adrenoceptors in the airways (by  $\beta$ -blockers including atenolol, metoprolol, acebutolol, bevantolol, xamoterol, bisoprolol and betaxolol [74–83]) causes bronchoconstriction and reduces the effectiveness of the main rescue treatment for asthma. Even though some blockers are more  $\beta_1$ -adrenoceptor-selective, this selectivity is poor and escalation of the  $\beta$ -adrenoceptor agonist dose is required to restore lung function. Most studies show a significant decrease in basal lung function in approximately half of patients, with half of patients tolerating a single-dose  $\beta$ -blocker [84]. Similar results were found in studies with concomitant illnesses (e.g. carvedilol in patients with heart failure and asthma) [85]. Several studies reported a rapid decrease in lung function or severe breathlessness following a single dose of  $\beta$ -blocker in some individuals [79,84–86]. Thus, although long-term studies of the effects of  $\beta$ -blockers in asthma patients are lacking,  $\beta$ -blockers are currently absolutely contraindicated in patients with asthma, even for the most  $\beta_1$ -selective antagonists currently available and despite their life-prolonging effects in cardiovascular disease [87]. Studies of long-term administration of low-dose  $\beta$ -blockers (see also Page, this issue) are in progress [88] to determine if the paradoxical pharmacology observed in congestive heart failure is also evident in asthma [89].

## Pharmacological basis for the clinical actions of $\beta$ -adrenoceptor antagonists

Many  $\beta$ -blockers now exist and these differ in physicochemical, pharmacokinetic and pharmacodynamic properties. Current drugs vary in their selectivity for  $\beta_1$ -,  $\beta_2$ - and  $\beta_3$ -adrenoceptors, and some, such as carvedilol and labetalol, are also  $\alpha_1$ -adrenoceptor antagonists. Some have partial agonist activity (ISA), local anaesthetic properties (membrane-stabilising activity), K<sup>+</sup> channel blocking activity or anti-oxidant properties.

## Properties of $\beta$ -adrenoceptor antagonists

### Subtype selectivity

One of the earliest developments of  $\beta$ -adrenoceptor antagonist drugs was subtype selectivity. The prototypical  $\beta$ -blocker propranolol has similar affinity for  $\beta_1$ - and  $\beta_2$ -adrenoceptors and lower affinity for  $\beta_3$ -adrenoceptors [90,91]. However, even the 'cardioselective'  $\beta$ -blockers, a nomenclature based on their selectivity for  $\beta_1$ -adrenoceptors, are not, because none in clinical use are that selective (13-fold at most) [90,92–94]. Given this fact, there might well be a good case for developing a highly selective  $\beta_1$ -adrenoceptor antagonist. Such a compound would be potentially useful in patients with asthma and other respiratory disorders who require inhaled  $\beta_2$ -adrenoceptor agonists to produce life-saving bronchodilatation. Of the six drugs that have been investigated in substantial heart failure trials to date (bisoprolol and nebivolol being the most  $\beta_1$ -selective, carvedilol having slight  $\beta_2$ -selectivity), it is not possible to predict what level of selectivity, if at all, is required for maximum beneficial outcomes in heart failure.

Most currently available  $\beta$ -blockers (including propranolol) have low affinity for the  $\beta_3$ -adrenoceptor. There are,



however, a subset of drugs comprising oxprenolol, carazolol, pindolol, nadolol, tertatolol, carteolol, arotinolol and nebivolol that have agonist effects at the  $\beta_3$ -adrenoceptor that could be responsible for the nitric oxide (NO)-mediated vasodilator properties observed with nebivolol [92,95]. Even SR59230A, claimed to be selective for  $\beta_3$ -adrenoceptors, has a similar potency at all 3 subtypes [95,96]. Other more recent human selective  $\beta_3$ -adrenoceptor ligands (such as L748337) do display greater subtype selectivity [95].

**Intrinsic sympathomimetic activity and partial agonism**  
Some  $\beta$ -blockers are traditionally described as having ISA. These drugs block the stimulatory effects of high-efficacy agonists, such as catecholamines, but stimulate agonist responses of their own. This is evident at both the cellular [95,97–101] and tissue level [42,43,99,102] with acebutolol, carteolol, penbutolol, pindolol, bucindolol and xamoterol, for which it is claimed that bradycardia and bronchoconstriction are less than for other  $\beta$ -adrenoceptor antagonists. However, drugs with ISA are less advantageous in the management of heart failure and migraine.

#### Low-affinity state of the $\beta_1$ -adrenoceptor

Some  $\beta$ -adrenoceptor antagonists stimulate  $\beta_1$ -adrenoceptor function by interacting with a low-affinity state of the  $\beta_1$ -adrenoceptor [103–106] and the  $\beta_3$ -adrenoceptor [100]. Following initial observations [107,108], most detailed observations have been for CGP12177A [109], but  $\beta$ -adrenoceptor antagonists with similar properties include oxprenolol, alprenolol, carazolol, pindolol and carvedilol. These ligands either stimulate agonist responses at concentrations much higher than those required to fully occupy and block the conventional catecholamine  $\beta_1$ -adrenoceptor site, or have biphasic concentration–response curves [92,95,100,105,110,111]. Activation of this low-affinity state of the  $\beta_1$ -adrenoceptor has been demonstrated in model cell systems [99,101,112], cardiomyocytes [113,114], tissues [115], whole animals [116,117] and humans [118]. However, there is currently no therapeutic use for this property of  $\beta$ -adrenoceptor antagonists.

#### Inverse agonism

Many  $\beta$ -adrenoceptor antagonists, at least at the  $\beta_2$ -adrenoceptor, are in fact inverse agonists [119–121] (i.e. rather than just occupying the binding site and thus blocking the actions of agonists, they are associated with conformations of the receptor that turn off signalling [122]).

#### Other properties of $\beta$ -blockers

Local anaesthetic or membrane-stabilising activity is shown by some  $\beta$ -adrenoceptor antagonists, notably propranolol and acebutolol and to a lesser extent pindolol and labetalol. Although this is observed in model systems, it is unlikely to be important in the therapeutic effects of  $\beta$ -blockers because it occurs at much higher concentrations than those normally encountered clinically.

Individual (rather than class effect) properties of certain  $\beta$ -blockers include lipophilicity,  $K^+$  channel blockade and anti-oxidant properties. Propranolol, timolol and metoprolol are somewhat lipophilic. Sotalol can block  $K^+$  channels

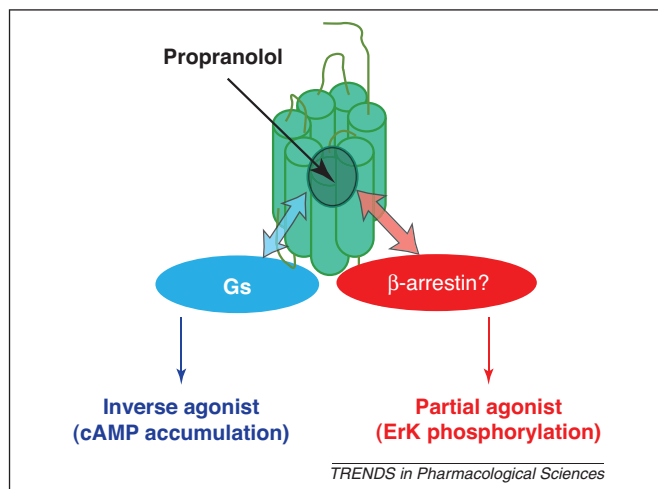
independently of its  $\beta$ -blocking properties. Carvedilol blocks  $\alpha_1$ - and  $\beta$ -adrenoceptors, inhibits apoptosis and possesses antioxidant and free-radical-scavenging actions. Nebivolol causes NO-dependent vasodilation. These properties might contribute to their efficacy in cardiac failure.

#### Emerging properties of $\beta$ -adrenoceptor antagonists: ligand-directed signalling bias

When Sir James developed  $\beta$ -blockers, the idea of  $\alpha$ - and  $\beta$ -adrenoceptor subtypes was quite radical. Three major groups of adrenoceptors are now identified that signal in characteristic patterns.  $\alpha_1$ -Adrenoceptors stimulate phospholipase C, whereas  $\alpha_2$ -adrenoceptors inhibit adenylyl cyclase and  $\beta$ -adrenoceptors activate it. However, in addition to the canonical signalling pathway, the nine adrenoceptor subtypes are now known to couple to other cell signalling mechanisms. It is also clear that linear efficacy (all agonists acting on the receptor in the same manner, with the only variables being affinity and efficacy) is an over-simplification [123]. Abundant evidence has now emerged indicating ligand-directed signalling bias, particularly among  $\beta$ -adrenoceptor antagonists, with the actions of propranolol at the  $\beta_2$ -adrenoceptor being one of the best examples (Figure 1) [120,121].

Many  $\beta$ -blockers are actually weak partial agonists, and others are inverse agonists, at the  $\beta_2$ -adrenoceptor in a variety of functional assays. Propranolol is an inverse agonist at the  $\beta_2$ -adrenoceptor on the canonical cAMP pathway, but in the same cells it also produces a stimulatory Erk1/2 response (Figure 1) [120,121]. This is explained by the existence of several agonist–receptor conformations stabilised by an interaction with a specific signalling protein.

For example, a ligand binds to a transmembrane orthosteric site, which allosterically alters the receptor conformation that then determines the specificity of binding for the intracellular signalling protein (e.g. G protein or  $\beta$ -arrestin). This will occur in a ligand-dependent manner [124], and whether a ligand is considered an agonist,



**Figure 1.** Biased signalling from the  $\beta_2$ -adrenoceptor in response to propranolol in CHO-K1 cells [121]. The presence of different intracellular signalling proteins (e.g.  $G_{\alpha s}$  or  $\beta$ -arrestin) bound to the  $\beta_2$ -adrenoceptor provides an allosteric mechanism for reciprocal interaction between the signalling protein and the orthosteric ligand binding site. However, the extent to which a particular signalling pathway predominates will depend on both the affinity of ligands for particular receptor–effector complexes and the degree of compartmentalisation of these complexes within the cell.

inverse agonist or neutral antagonist (rare) depends on the signalling pathways being examined. After cAMP, the most studied signalling pathways examined for  $\beta$ -adrenoceptor ligands are  $\beta$ -arrestin recruitment [125] and Erk1/2 signalling [120,121], but there is also evidence of tyrosine kinase receptor transactivation and p38 MAPK, PI3 kinase and NO activation, depending on the  $\beta$ -adrenoceptor subtype, level of receptor expression and cell type [123].

Several  $\beta$ -adrenoceptor ligands have complex efficacy profiles for cAMP generation and Erk1/2 activation at both  $\beta_1$ - and  $\beta_2$ -adrenoceptors, which is termed pluridimensional efficacy [110,120,121,126]. For mouse [127] and human [128]  $\beta_3$ -adrenoceptors, SR59230A and L748337 are classical competitive antagonists for cAMP accumulation but agonists for Erk1/2 and p38 MAPK activation. This suggests that compounds selectively activate discrete pathways by interacting with particular receptor conformations. Although many  $\beta$ -blockers express their own spectrum of pharmacological properties, currently there is little to relate clinical efficacy with their ability to activate MAPK or other signalling pathways. Suggestions that the therapeutic benefit of carvedilol in heart failure patients relates to its capacity to activate Erk1/2 signalling by G-protein-independent mechanisms seems premature given that other  $\beta$ -blockers without these properties have similar clinical efficacy [125].

More extensive evaluations of  $\beta$ -blockers have examined their ability to stimulate cAMP production (using Exchange Protein Activated by Cyclic AMP (EPAC)-based biosensors) or Erk1/2 activation in cells expressing the  $\beta_2$ -adrenoceptor [125]. A mutant  $\beta_2$ -adrenoceptor with poor G protein coupling demonstrated that carvedilol remained a partial agonist for Erk1/2 activation, whereas propranolol produced no response [125]. The carvedilol Erk1/2 response was sensitive to siRNA depletion of arrestin-3, but insensitive to pertussis toxin (PTX). Thus, carvedilol (but not propranolol) causes receptor phosphorylation, recruitment of arrestin3-GFP, and receptor internalisation without changes in cAMP [125].

The  $\beta_1$ -adrenoceptor also displays ligand-directed signalling bias. Treatment with isoprenaline caused both  $G_i$ -dependent and G-protein-independent Erk1/2 activation [129]. Bucindolol was a partial agonist and propranolol was an inverse agonist for cAMP, but both caused Erk1/2 activation. The isoprenaline-induced Erk1/2 response was partially blocked by PTX or overexpression of beta Adrenergic Receptor Kinase-carboxy terminus ( $\beta$ ARK-ct) (which sequesters  $G\beta\gamma$  subunits), but responses to bucindolol and propranolol were unaffected [129]. Only receptors treated with isoprenaline produced a Bioluminescence Resonance Energy Transfer (BRET) signal, which suggests that isoprenaline, bucindolol and propranolol promote distinct conformations of the  $\beta_1$ -adrenoceptor [129]. Although these findings suggest that Erk1/2 activation by  $\beta_1$ -adrenoceptor ligands does not involve arrestins [129], it has been shown that carvedilol and alprenolol interact with the  $\beta_1$ -adrenoceptor to promote arrestin-2/3 recruitment, transactivation of the EGF receptor and Erk1/2 activation [130], whereas propranolol does not stimulate arrestin recruitment (bucindolol was not tested) [129]. Thus, different drugs could have distinct modes of action, not only with respect to cAMP and

Erk1/2 signalling, but also in terms of upstream signalling effectors. However, the carvedilol and alprenolol study used the mouse  $\beta_1$ -adrenoceptor [130], whereas the propranolol and bucindolol study used the human  $\beta_1$ -adrenoceptor [129], that have amino-acid differences that could differentially affect phosphorylation or arrestin recruitment.

At the mouse and human  $\beta_3$ -adrenoceptors, drugs that act as antagonists of cAMP responses strongly activate Erk1/2 [127,128]. However, and in contrast to  $\beta_1$ - and  $\beta_2$ -adrenoceptors, the Erk1/2 responses at  $\beta_3$ -adrenoceptors do not involve receptor phosphorylation, arrestin interaction or internalisation because the  $\beta_3$ -adrenoceptor does not undergo any of these processes. In cells expressing modest levels of human  $\beta_3$ -adrenoceptors, L748337 was a competitive antagonist for cAMP accumulation, but had high agonist potency and efficacy for Erk1/2 phosphorylation [128]. Zinterol, by contrast [131], had high efficacy for cAMP accumulation but lower efficacy than L748337 for both Erk1/2 and p38MAPK phosphorylation [128]. Efficacy reversal was also demonstrated for CL316243 and SR59230A at mouse  $\beta_3$ -adrenoceptors [127]. For cAMP accumulation, CL316243 was a full agonist and SR59230A either a partial agonist or antagonist, depending on receptor expression levels. In identical cells but using the extracellular acidification rate as the functional readout, both CL316243 and SR59230A are full agonists at all levels of receptor expression. Analysis of responses using selective MAPK inhibitors and Western blotting showed that p38 MAPK and Erk1/2 signalling are involved and confirmed that SR59230A has much higher efficacy than CL316243 for MAPK signalling. Such examples of reversal of efficacy provide strong support for ligand-directed signalling bias.

At  $\beta_3$ -adrenoceptors, MAPK responses induced by agonist ligands occur via different G proteins from those induced by antagonist ligands. Thus, Erk1/2 activation by L748337 is blocked by PTX, which indicates that  $G_{i/o}$  but not  $G_s$  is involved, whereas activation in response to L75507 is much less affected by PTX, suggestive of coupling predominantly to  $G_s$  [128]. This again highlights differences between the three human  $\beta$ -adrenoceptors because antagonist-stimulated Erk1/2 phosphorylation at the  $\beta_1$ - and  $\beta_2$ -adrenoceptors is not PTX-sensitive [125,129].

Important factors to consider in studying pathway-specific pharmacology include the level of receptor expression and the conditions under which each signalling pathway is measured. Signalling assays used to demonstrate ligand-directed signalling bias are often conducted under different conditions and rarely at equilibrium. cAMP accumulation assays are often conducted with phosphodiesterase inhibitors present. Erk1/2 assays involving  $G\beta\gamma$  or receptor interaction with cSrc tend to peak at 5–8 min, whereas those involving  $\beta$ -arrestin interactions have longer time courses. Concentration–response relationships usually involve picking the response peak for a range of concentrations. Likewise,  $Ca^{2+}$  assays often follow a peak then plateau phase. By contrast,  $\beta$ -arrestin responses and reporter gene assays are followed over minutes/hours. The widely varying conditions under which assays are conducted could play a role in the effects observed. However, comparisons are generally made with a reference agonist, generally the physiological ligand.

### Quantitation of ligand-directed signalling bias

For ligand-directed signalling bias to be useful in drug development, methods for quantitation are required. These have recently become available and, together with the identification of drug profiles associated with therapeutic efficacy, provide a way forward for the development of new classes of selective drugs. Interestingly, the starting point for quantitation of bias is the Black–Leff operational model [2,132]. Bias must encompass both affinity ( $K_A$ ) and efficacy ( $\tau$ ) and the ratio  $\tau/K_A$  is referred to as the transduction ratio [133]. For each bioassay, transduction ratios can be determined and compared to those obtained for a reference, usually an endogenous, agonist. By setting the  $\tau/K_A$  value for the reference agonist to 1, comparisons can be made of the bias factors for other agonists across bioassay systems. This facilitates systematic comparison of ligands acting at target GPCR systems. One advantage of bias factor measurement is that rather than examining systems for extreme behaviour (e.g.  $\beta$ -adrenoceptor antagonists that are Erk1/2 activators), it is possible to examine many of compounds that display a range of different efficacies for different signalling pathways [134].

### Concluding remarks

The development of  $\beta$ -blockers by Sir James Black made a much greater contribution to pharmacology than originally imagined. They have proved much more useful than their original role of improving symptoms and outcome in ischaemic heart disease, a development that both interested and amused him in later years. Not only did he produce a new class of drugs, he also made a major contribution to analytical pharmacology by developing approaches that are now providing the foundations for the analysis of ligand-directed signalling bias. It is possible that the therapeutic applications of  $\beta$ -blockers in cardiac arrhythmias, hypertension and cardiac failure can be further enhanced by improvements in selectivity and exploitation of new properties. Novel applications for  $\beta$ -blockers in the treatment of cancer, osteoporosis, infectious disease and asthma will need further experimental support.

### Acknowledgements

J.G.B. thanks the Wellcome Trust for a Clinician Scientist Fellowship. S.J.H. is funded by the Medical Research Council (G0800006) and BBSRC. R.J.S. is funded by the National Health and Medical Research Council (NHMRC) of Australia Program, Grant 519461.

### References

- Black, J. (2010) A life in new drug research. *Br. J. Pharmacol.* 160 (Suppl. 1), S15–S25
- Black, J.W. *et al.* (1985) An operational model of pharmacological agonism: the effect of E/[A] curve shape on agonist dissociation constant estimation. *Br. J. Pharmacol.* 84, 561–571
- Lyall, J. (2010) James Black: Nobel prize winning pharmacologist who invented  $\beta$  blockers. *Br. Med. J.* 340, c1817
- Ahlquist, R.P. (1948) A study of the adrenotropic receptors. *Am. J. Physiol.* 153, 586–600
- Powell, C.E. and Slater, I.H. (1958) Blocking of inhibitory adrenergic receptors by a dichloro analog of isoproterenol. *J. Pharmacol. Exp. Ther.* 122, 480–488
- Black, J.W. *et al.* (1965) Comparison of some properties of pronethalol and propranolol. *Br. J. Pharmacol. Chemother* 25, 577–591
- Black, J.W. and Prichard, B.N. (1973) Activation and blockade of  $\beta$ -adrenoceptors in common cardiac disorders. *Br. Med. Bull.* 29, 163–167
- Prichard, B.N. (1964) Hypotensive action of pronethalol. *Br. Med. J.* 1, 1227–1228
- Prichard, B.N. and Gillam, P.M. (1964) Use of propranolol (Inderal) in treatment of hypertension. *Br. Med. J.* 2, 725–727
- Prichard, B.N. (1978) The second Lilly Prize Lecture, University of Newcastle, July 1977. ( $\beta$ -Adrenergic receptor blockade in hypertension, past, present and future. *Br. J. Clin. Pharmacol.* 5, 379–399
- (1986) Randomised trial of intravenous atenolol among 16 027 cases of suspected acute myocardial infarction: ISIS-1. First International Study of Infarct Survival Collaborative Group. *Lancet* 2, 57–66.
- (1982) A randomized trial of propranolol in patients with acute myocardial infarction. I. Mortality results. *J Am Med Assoc* 247, 1707–1714.
- Dargie, H.J. (2001) Effect of carvedilol on outcome after myocardial infarction in patients with left-ventricular dysfunction: the CAPRICORN randomised trial. *Lancet* 357, 1385–1390
- Hjalmarson, A. *et al.* (1981) Effect on mortality of metoprolol in acute myocardial infarction A double-blind randomised trial. *Lancet* 2, 823–827
- Pedersen, T.R. (1985) Six-year follow-up of the Norwegian Multicenter Study on Timolol after Acute Myocardial Infarction. *N. Engl. J. Med.* 313, 1055–1058
- Black, H.R. *et al.* (2010) The foundation role of  $\beta$ -blockers across the cardiovascular disease spectrum: a year 2009 update. *Am. J. Med.* 123, S2
- Selzman, C.H. *et al.* (2001) The case for  $\beta$ -adrenergic blockade as prophylaxis against perioperative cardiovascular morbidity and mortality. *Arch. Surg.* 136, 286–290
- Echahidi, N. *et al.* (2008) Mechanisms, prevention, and treatment of atrial fibrillation after cardiac surgery. *J. Am. Coll. Cardiol.* 51, 793–801
- Shantsila, E. *et al.* (2006) Atrial fibrillation post-cardiac surgery: changing perspectives. *Curr. Med. Res. Opin.* 22, 1437–1441
- Crystal, E. *et al.* (2004) Interventions for preventing post-operative atrial fibrillation in patients undergoing heart surgery. *Cochrane Database Syst. Rev.* CD003611
- Auerbach, A.D. and Goldman, L. (2002)  $\beta$ -Blockers and reduction of cardiac events in noncardiac surgery: scientific review. *J. Am. Med. Assoc.* 287, 1435–1444
- Hollenberg, N.K. (2005) The role of  $\beta$ -blockers as a cornerstone of cardiovascular therapy. *Am. J. Hypertens.* 18, 165S–168S
- Aronow, W.S. (2010) Current role of  $\beta$ -blockers in the treatment of hypertension. *Expert Opin. Pharmacother.* 11, 2599–2607
- Bangalore, S. *et al.* (2007) Cardiovascular protection using  $\beta$ -blockers: a critical review of the evidence. *J. Am. Coll. Cardiol.* 50, 563–572
- Basile, J.N. (2010) One size does not fit all: the role of vasodilating  $\beta$ -blockers in controlling hypertension as a means of reducing cardiovascular and stroke risk. *Am. J. Med.* 123, S9–15
- Molenaar, P. *et al.* (2007) ( $\alpha$ )-Adrenaline elicits positive inotropic, lusitropic, and biochemical effects through  $\beta_2$ -adrenoceptors in human atrial myocardium from nonfailing and failing hearts, consistent with  $G_s$  coupling but not with  $G_i$  coupling. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 375, 11–28
- Bristow, M.R. *et al.* (1986)  $\beta_1$ - and  $\beta_2$ -adrenergic-receptor subpopulations in nonfailing and failing human ventricular myocardium: coupling of both receptor subtypes to muscle contraction and selective  $\beta_1$ -receptor down-regulation in heart failure. *Circ. Res.* 59, 297–309
- Buxton, B.F. *et al.* (1987) Characterization and autoradiographic localization of  $\beta$ -adrenoceptor subtypes in human cardiac tissues. *Br. J. Pharmacol.* 92, 299–310
- Michel, M.C. *et al.* (2011) Are there functional  $\beta_3$ -adrenoceptors in the human heart? *Br. J. Pharmacol.* 162, 817–822
- Lee, S. *et al.* (2008) Genetically changed mice with chronic deficiency or overexpression of the  $\beta$ -adrenoceptors – what can we learn for the therapy of heart failure? *Pflugers Arch.* 455, 767–774
- van Veldhuisen, D.J. and Poole-Wilson, P.A. (2001) The underreporting of results and possible mechanisms of ‘negative’ drug trials in patients with chronic heart failure. *Int. J. Cardiol.* 80, 19–27
- Waagstein, F. *et al.* (1975) Effect of chronic  $\beta$ -adrenergic receptor blockade in congestive cardiomyopathy. *Br. Heart J.* 37, 1022–1036



- 33 Swedberg, K. *et al.* (1979) Prolongation of survival in congestive cardiomyopathy by  $\beta$ -receptor blockade. *Lancet* 1, 1374–1376
- 34 (1999) The Cardiac Insufficiency Bisoprolol Study II (CIBIS-II): a randomised trial. *Lancet* 353, 9–13.
- 35 (1999) Effect of metoprolol CR/XL in chronic heart failure: Metoprolol CR/XL Randomised Intervention Trial in Congestive Heart Failure (MERIT-HF). *Lancet* 353, 2001–2007.
- 36 Packer, M. *et al.* (2002) Effect of carvedilol on the morbidity of patients with severe chronic heart failure: results of the carvedilol prospective randomized cumulative survival (COPERNICUS) study. *Circulation* 106, 2194–2199
- 37 Flather, M.D. *et al.* (2005) Randomized trial to determine the effect of nebivolol on mortality and cardiovascular hospital admission in elderly patients with heart failure (SENIORS). *Eur. Heart J.* 26, 215–225
- 38 Ellison, K.E. and Gandhi, G. (2005) Optimising the use of  $\beta$ -adrenoceptor antagonists in coronary artery disease. *Drugs* 65, 787–797
- 39 Cruickshank, J.M. (2007) Are we misunderstanding  $\beta$ -blockers. *Int. J. Cardiol.* 120, 10–27
- 40 The Beta-Blocker Evaluation of Survival Trial Investigators (2001) A trial of the beta-blocker bucindolol in patients with advanced chronic heart failure. *N. Engl. J. Med.* 344, 1659–1667.
- 41 The Xamoterol in Severe Heart Failure Study Group (1990) Xamoterol in severe heart failure. *Lancet* 336, 1–6.
- 42 Maack, C. *et al.* (2000) Different intrinsic activities of bucindolol, carvedilol and metoprolol in human failing myocardium. *Br. J. Pharmacol.* 130, 1131–1139
- 43 Andreka, P. *et al.* (2002) Bucindolol displays intrinsic sympathomimetic activity in human myocardium. *Circulation* 105, 2429–2434
- 44 Stamper, R.L. *et al.* (2002) Primary drug treatment for glaucoma:  $\beta$ -blockers versus other medications. *Surv. Ophthalmol.* 47, 63–73
- 45 Limmroth, V. and Michel, M.C. (2001) The prevention of migraine: a critical review with special emphasis on  $\beta$ -adrenoceptor blockers. *Br. J. Clin. Pharmacol.* 52, 237–243
- 46 Gray, R.N. *et al.* (1999) *Drug Treatments for the Prevention of Migraine Headache*, Agency for Health Care Policy and Research (US)
- 47 Turner, P. (1989) Therapeutic uses of  $\beta$ -adrenoceptor blocking drugs in the central nervous system in man. *Postgrad. Med. J.* 65, 1–6
- 48 Mehta, G. *et al.* (2010) Developments and controversies in the management of oesophageal and gastric varices. *Gut* 59, 701–705
- 49 Uitti, R.J. (1998) Medical treatment of essential tremor and Parkinson's disease. *Geriatrics* 53, 47–53
- 50 Feely, J. and Peden, N. (1984) Use of  $\beta$ -adrenoceptor blocking drugs in hyperthyroidism. *Drugs* 27, 425–446
- 51 Pasco, J.A. *et al.* (2004)  $\beta$ -Adrenergic blockers reduce the risk of fracture partly by increasing bone mineral density: Geelong Osteoporosis Study. *J. Bone Miner. Res.* 19, 19–24
- 52 Schlienger, R.G. *et al.* (2004) Use of  $\beta$ -blockers and risk of fractures. *J. Am. Med. Assoc.* 292, 1326–1332
- 53 Rejnmark, L. *et al.* (2006) Treatment with  $\beta$ -blockers ACE inhibitors, and calcium-channel blockers is associated with a reduced fracture risk: a nationwide case-control study. *J. Hypertens.* 24, 581–589
- 54 Schoo, M. *et al.* (2005) Use of  $\beta$ -blockers is associated with BMD and fracture risk. *Bone* 36, 129–130
- 55 Bonnet, N. *et al.* (2007) Protective effect of  $\beta$ -blockers in postmenopausal women: influence on fractures, bone density, micro and macroarchitecture. *Bone* 40, 1209–1216
- 56 Yang, S. *et al.* (2011) Association between  $\beta$ -blocker use and fracture risk: The Dubbo Osteoporosis Epidemiology Study. *Bone* 48, 451–455
- 57 de Vries, F. *et al.* (2007) Use of  $\beta$ -blockers and the risk of hip/femur fracture in the United Kingdom and The Netherlands. *Calcif. Tissue Int.* 80, 69–75
- 58 Lavasueur, R. *et al.* (2005)  $\beta$ -Blocker use, bone mineral density and fracture risk in older women: results from the Epidemiologie de L'Osteoporose Prospective Study. *J. Am. Geriatr. Soc.* 53, 550–552
- 59 Reid, I.R. *et al.* (2005)  $\beta$ -Blocker use, BMD, and fractures in the study of osteoporotic fractures. *J. Bone Miner. Res.* 20, 613–618
- 60 Meisinger, C. *et al.* (2007)  $\beta$ -Blocker use and risk of fractures in men and women from the general population: the MONICA/KORA Augsburg cohort study. *Osteoporos. Int.* 18, 1189–1195
- 61 Minkowitz, B. *et al.* (1991) Effects of propranolol on bone metabolism in the rat. *J. Orthop. Res.* 9, 869–875
- 62 Takeda, S. *et al.* (2002) Leptin regulates bone formation via the sympathetic nervous system. *Cell* 111, 305–317
- 63 Bonnet, N. *et al.* (2005) Alteration of trabecular bone under chronic  $\beta_2$ -agonists treatment. *Med. Sci. Sports Exerc.* 37, 1493–1501
- 64 Elefteriou, F. (2008) Regulation of bone remodeling by the central and peripheral nervous system. *Arch. Biochem. Biophys.* 473, 231–236
- 65 Huang, H.H. *et al.* (2009) Functional  $\alpha_1$ - and  $\beta_2$ -adrenergic receptors in human osteoblasts. *J. Cell. Physiol.* 220, 267–275
- 66 Bonnet, N. *et al.* (2008) Adrenergic control of bone remodeling and its implications for the treatment of osteoporosis. *J. Musculoskelet Neuronal Interact.* 8, 94–104
- 67 Algazi, M. *et al.* (2004) [Could treatments with  $\beta$ -blockers be associated with a reduction in cancer risk?]. *Rev. Epidemiol. Sante Publique* 52, 53–65
- 68 Perron, L. *et al.* (2004) Antihypertensive drug use and the risk of prostate cancer (Canada). *Cancer Causes Cont.* 15, 535–541
- 69 Sloan, E.K. *et al.* (2010) The sympathetic nervous system induces a metastatic switch in primary breast cancer. *Cancer Res.* 70, 7042–7052
- 70 Sood, A.K. *et al.* (2010) Adrenergic modulation of focal adhesion kinase protects human ovarian cancer cells from anoikis. *J. Clin. Invest.* 120, 1515–1523
- 71 Rajagopal, S. *et al.* (2010) Teaching old receptors new tricks: biasing seven-transmembrane receptors. *Nat. Rev. Drug Discov.* 9, 373–386
- 72 Harrison, T. *et al.* (2003) Erythrocyte G protein-coupled receptor signaling in malarial infection. *Science* 301, 1734–1736
- 73 Murphy, S.C. *et al.* (2006) Erythrocyte G protein as a novel target for malarial chemotherapy. *PLoS Med.* 3, e528
- 74 Decalmer, P.B. *et al.* (1978)  $\beta$ -Blockers, asthma. *Br. Heart J.* 40, 184–189
- 75 van den Bergh, J.H. and van Herwaarden, C.L. (1981) Ventilatory effects of ordinary and slow-release tablets of metoprolol in asthmatic patients. *Eur. J. Respir. Dis.* 62, 168–172
- 76 Greefhorst, A.P. and van Herwaarden, C.L. (1981) Comparative study of the ventilatory effects of three  $\beta_1$ -selective blocking agents in asthmatic patients. *Eur. J. Clin. Pharmacol.* 20, 417–421
- 77 Lofdahl, C.G. and Svedmyr, N. (1981) Cardioselectivity of atenolol and metoprolol A study in asthmatic patients. *Eur. J. Respir. Dis.* 62, 396–404
- 78 Clague, H.W. *et al.* (1984) Influence of cardioselectivity and respiratory disease on pulmonary responsiveness to  $\beta$ -blockade. *Eur. J. Clin. Pharmacol.* 27, 517–523
- 79 Lofdahl, C.G. and Svedmyr, N. (1984) Effects of xamoterol (ICI 118,587) in asthmatic patients. *Br. J. Clin. Pharmacol.* 18, 597–601
- 80 Lammers, J.W. *et al.* (1986) Respiratory tolerance of bisoprolol and metoprolol in asthmatic patients. *J. Cardiovasc. Pharmacol.* 8 (Suppl. 11), S69–S73
- 81 Lammers, J.W. *et al.* (1986) A comparative study on the ventilatory and haemodynamic effects of xamoterol and atenolol in asthmatic patients. *Br. J. Clin. Pharmacol.* 22, 595–602
- 82 Philip-Joet, F. *et al.* (1986) Comparative study of the respiratory effects of two  $\beta_1$ -selective blocking agents atenolol and bevantolol in asthmatic patients. *Eur. J. Clin. Pharmacol.* 30, 13–16
- 83 Miki, A. *et al.* (2003) Betaxolol-induced deterioration of asthma and a pharmacodynamic analysis based on  $\beta$ -receptor occupancy. *Int. J. Clin. Pharmacol. Ther.* 41, 358–364
- 84 Benson, M.K. *et al.* (1978) A comparison of four  $\beta$ -adrenoceptor antagonists in patients with asthma. *Br. J. Clin. Pharmacol.* 5, 415–419
- 85 Kotlyar, E. *et al.* (2002) Tolerability of carvedilol in patients with heart failure and concomitant chronic obstructive pulmonary disease or asthma. *J. Heart Lung Transplant.* 21, 1290–1295
- 86 Devereux, G. *et al.* (1998) Adverse effects of a single dose of (+)-sotalol in patients with mild stable asthma. *Br. J. Clin. Pharmacol.* 46, 79–82
- 87 Chafin, C.C. *et al.* (1999)  $\beta$ -Blockers after myocardial infarction: do benefits ever outweigh risks in asthma? *Cardiology* 92, 99–105
- 88 Hanania, N.A. *et al.* (2008) The safety and effects of the  $\beta$ -blocker, nadolol, in mild asthma: an open-label pilot study. *Pulm. Pharmacol. Ther.* 21, 134–141
- 89 Bond, R.A. *et al.* (2007) Getting to the heart of asthma: can ' $\beta$  blockers' be useful to treat asthma? *Pharmacol. Ther.* 115, 360–374



- 90 Baker, J.G. (2005) The selectivity of  $\beta$ -adrenoceptor antagonists at the human  $\beta_1$   $\beta_2$  and  $\beta_3$  adrenoceptors. *Br. J. Pharmacol.* 144, 317–322
- 91 Hoffmann, C. *et al.* (2004) Comparative pharmacology of human  $\beta$ -adrenergic receptor subtypes – characterization of stably transfected receptors in CHO cells. *Naunyn. Schmiedebergs Arch. Pharmacol.* 369, 151–159
- 92 Arch, J.R.S. (2000)  $\beta_3$ -Adrenoceptor ligands and the pharmacology of the  $\beta_3$ -adrenoceptor. In *The  $\beta_3$ -Adrenoceptor* (Strosberg, A.D., ed.), Taylor & Francis 48–76
- 93 Smith, C. and Teitler, M. (1999)  $\beta$ -Blocker selectivity at cloned human  $\beta_1$ - and  $\beta_2$ -adrenergic receptors. *Cardiovasc. Drugs Ther.* 13, 123–126
- 94 Schnabel, P. *et al.* (2000) Binding properties of  $\beta$ -blockers at recombinant  $\beta_1$ - $\beta_2$ - and  $\beta_3$ -adrenoceptors. *J. Cardiovasc. Pharmacol.* 36, 466–471
- 95 Baker, J.G. (2010) The selectivity of  $\beta$ -adrenoceptor agonists at human  $\beta_1$ - $\beta_2$ - and  $\beta_3$ -adrenoceptors. *Br. J. Pharmacol.* 160, 1048–1061
- 96 Michel, M.C. *et al.* (2010) Tissue functions mediated by  $\beta_3$ -adrenoceptors – findings and challenges. *Naunyn. Schmiedebergs Arch. Pharmacol.* 382, 103–108
- 97 Jasper, J.R. *et al.* (1990) Amplification of cyclic AMP generation reveals agonistic effects of certain  $\beta$ -adrenergic antagonists. *Mol. Pharmacol.* 37, 44–49
- 98 Jasper, J.R. *et al.* (1988) Molecular mechanism of  $\beta$ -adrenergic receptor blockers with intrinsic sympathomimetic activity. *FASEB J.* 2, 2891–2894
- 99 Joseph, S.S. *et al.* (2003) Intrinsic sympathomimetic activity of (–)-pindolol mediated through a (–)-propranolol-resistant site of the  $\beta_1$ -adrenoceptor in human atrium and recombinant receptors. *Naunyn. Schmiedebergs Arch. Pharmacol.* 368, 496–503
- 100 Baker, J.G. *et al.* (2003) Agonist actions of ‘ $\beta$ -blockers’ provide evidence for two agonist activation sites or conformations of the human  $\beta_1$ -adrenoceptor. *Mol. Pharmacol.* 63, 1312–1321
- 101 Baker, J.G. (2005) Sites of action of  $\beta$ -ligands at the human  $\beta_1$ -adrenoceptor. *J. Pharmacol. Exp. Ther.* 313, 1163–1171
- 102 Bundkirchen, A. *et al.* (2002) Bucindolol exerts agonistic activity on the propranolol-insensitive state of  $\beta_1$ -adrenoceptors in human myocardium. *J. Pharmacol. Exp. Ther.* 300, 794–801
- 103 Granneman, J.G. (2001) The putative  $\beta_4$ -adrenergic receptor is a novel state of the  $\beta_1$ -adrenergic receptor. *Am. J. Physiol. Endocrinol. Metab.* 280, E199–202
- 104 Molenaar, P. (2003) The ‘state’ of  $\beta$ -adrenoceptors. *Br. J. Pharmacol.* 140, 1–2
- 105 Kaumann, A.J. (1989) Is there a third heart  $\beta$ -adrenoceptor? *Trends Pharmacol. Sci.* 10, 316–320
- 106 Baker, J.G. (2005) Evidence for a secondary state of the human  $\beta_3$ -adrenoceptor. *Mol. Pharmacol.* 68, 1645–1655
- 107 Arch, J.R. and Kaumann, A.J. (1993)  $\beta_3$  and atypical  $\beta$ -adrenoceptors. *Med. Res. Rev.* 13, 663–729
- 108 Kaumann, A.J. (1983) [Cardiac  $\beta$ -receptors – experimental aspects]. *Z. Kardiol.* 72, 63–82
- 109 Pak, M.D. and Fishman, P.H. (1996) Anomalous behavior of CGP 12177A on (1)-adrenergic receptors. *J. Recept. Signal Transduct. Res.* 16, 1–23
- 110 Walter, M. *et al.* (1984) Stimulant and blocking effects of optical isomers of pindolol on the sinoatrial node and trachea of guinea pig. Role of  $\beta$ -adrenoceptor subtypes in the dissociation between blockade and stimulation. *Naunyn. Schmiedebergs Arch. Pharmacol.* 327, 159–175
- 111 Kaumann, A.J. and Blinks, J.R. (1980)  $\beta$ -Adrenoceptor blocking agents as partial agonists in isolated heart muscle: dissociation of stimulation and blockade. *Naunyn. Schmiedebergs Arch. Pharmacol.* 311, 237–248
- 112 Konkar, A.A. *et al.* (2000) Aryloxypropanolamine and catecholamine ligand interactions with the  $\beta_1$ -adrenergic receptor: evidence for interaction with distinct conformations of  $\beta_1$ -adrenergic receptors. *J. Pharmacol. Exp. Ther.* 294, 923–932
- 113 Lewis, C.J. *et al.* (2004) Overexpression of  $\beta_1$ -adrenoceptors in adult rat ventricular myocytes enhances CGP 12177A cardiostimulation: implications for ‘putative’  $\beta_4$ -adrenoceptor pharmacology. *Br. J. Pharmacol.* 141, 813–824
- 114 Freestone, N.S. *et al.* (1999)  $\beta_4$ -Adrenoceptors are more effective than  $\beta_1$ -adrenoceptors in mediating arrhythmic  $Ca^{2+}$  transients in mouse ventricular myocytes. *Naunyn. Schmiedebergs Arch. Pharmacol.* 360, 445–456
- 115 Kaumann, A.J. and Molenaar, P. (2008) The low-affinity site of the  $\beta_1$ -adrenoceptor and its relevance to cardiovascular pharmacology. *Pharmacol. Ther.* 118, 303–336
- 116 Cohen, M.L. *et al.* (2000)  $\beta_3$ -receptors mediate relaxation in stomach fundus whereas a fourth  $\beta$  receptor mediates tachycardia in atria from transgenic  $\beta_3$ -receptor knockout mice. *Receptors Channels* 7, 17–23
- 117 Kaumann, A.J. *et al.* (2001) Abolition of (–)-CGP 12177-evoked cardiostimulation in double  $\beta_1$ -/ $\beta_2$ -adrenoceptor knockout mice. Obligatory role of  $\beta_1$ -adrenoceptors for putative  $\beta_4$ -adrenoceptor pharmacology. *Naunyn. Schmiedebergs Arch. Pharmacol.* 363, 87–93
- 118 Kaumann, A.J. and Molenaar, P. (1997) Modulation of human cardiac function through 4  $\beta$ -adrenoceptor populations. *Naunyn. Schmiedebergs Arch. Pharmacol.* 355, 667–681
- 119 Chidiac, P. *et al.* (1994) Inverse agonist activity of  $\beta$ -adrenergic antagonists. *Mol. Pharmacol.* 45, 490–499
- 120 Azzi, M. *et al.* (2003)  $\beta$ -Arrestin-mediated activation of MAPK by inverse agonists reveals distinct active conformations for G protein-coupled receptors. *Proc. Natl. Acad. Sci. U.S.A.* 100, 11406–11411
- 121 Baker, J.G. *et al.* (2003) Agonist and inverse agonist actions of  $\beta$ -blockers at the human  $\beta_2$ -adrenoceptor provide evidence for agonist-directed signaling. *Mol. Pharmacol.* 64, 1357–1369
- 122 Swaminath, G. *et al.* (2005) Probing the  $\beta_2$ -adrenoceptor binding site with catechol reveals differences in binding and activation by agonists and partial agonists. *J. Biol. Chem.* 280, 22165–22171
- 123 Evans, B.A. *et al.* (2011) Ligand-directed signalling at  $\beta$ -adrenoceptors. *Br. J. Pharmacol.* 159, 1022–1038
- 124 Kenakin, T. (2010) Functional selectivity and biased receptor signaling. *J. Pharmacol. Exp. Ther.* 336, 296–302
- 125 Wisler, J.W. *et al.* (2007) A unique mechanism of  $\beta$ -blocker action: carvedilol stimulates  $\beta$ -arrestin signaling. *Proc. Natl. Acad. Sci. U.S.A.* 104, 16657–16662
- 126 Galandrin, S. and Bouvier, M. (2006) Distinct signaling profiles of  $\beta_1$ - and  $\beta_2$ -adrenergic receptor ligands toward adenylyl cyclase and mitogen-activated protein kinase reveals the pluridimensionality of efficacy. *Mol. Pharmacol.* 70, 1575–1584
- 127 Sato, M. *et al.* (2007) Ligand-directed signaling at the  $\beta_3$ -adrenoceptor produced by 3-(2-ethylphenoxy)-1-[(1S)-1,2,3,4-tetrahydronaph-1-ylamino]-2S-2-propanol oxalate (SR 59230A) relative to receptor agonists. *Mol. Pharmacol.* 72, 1359–1368
- 128 Sato, M. *et al.* (2008) The  $\beta_3$ -adrenoceptor agonist 4-[(hexylamino)carbonyl]amino-N-[4-[2-[(2S)-2-hydroxy-3-(4-hydroxyphenoxy)propyl]amino]ethyl]-phenyl]-benzenesulfonamide (L755507) and antagonist (S)-N-[4-[2-[(3-[3-(acetamidomethyl)phenoxy]-2-hydroxypropyl]amino)-ethyl]phenyl]benzenesulfonamide (L748337) activate different signaling pathways in Chinese hamster ovary-K1 cells stably expressing the human  $\beta_3$ -adrenoceptor. *Mol. Pharmacol.* 74, 1417–1428
- 129 Galandrin, S. *et al.* (2008) Conformational rearrangements and signaling cascades involved in ligand-biased MAPK signaling through the  $\beta_1$ -adrenergic receptor. *Mol. Pharmacol.* 74, 162–172
- 130 Kim, I.M. *et al.* (2008)  $\beta$ -Blockers alprenolol and carvedilol stimulate  $\beta$ -arrestin-mediated EGFR transactivation. *Proc. Natl. Acad. Sci. U.S.A.* 105, 14555–14560
- 131 Hutchinson, D.S. *et al.* (2006) Agonist effects of zinterol at the mouse and human  $\beta_3$ -adrenoceptor. *Naunyn. Schmiedebergs Arch. Pharmacol.* 373, 158–168
- 132 Black, J.W. and Leff, P. (1983) Operational models of pharmacological agonism. *Proc. R. Soc. Lond. B: Biol. Sci.* 220, 141–162
- 133 Kenakin, T. and Miller, L.J. (2010) Seven transmembrane receptors as shapeshifting proteins: the impact of allosteric modulation and functional selectivity on new drug discovery. *Pharmacol. Rev.* 62, 265–304
- 134 Evans, B.A. *et al.* (2010) Quantification of functional selectivity at the human  $\alpha_{1A}$ -adrenoceptor. *Mol. Pharmacol.* 79, 298–307