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Adrenoceptors

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Preface

Adrenoceptors mediate the physiological effects of the endogenous catecholamines adrenaline and noradrenaline. They consist of nine subtypes grouped into three subfamilies, termed α_1-, α_2-, and β-adrenoceptors, each of which has three subtypes (α_{1A}, α_{1B}, α_{1D}, α_{2A}, α_{2B}, α_{2C}, β_1, β_2, and β_3). Adrenaline was isolated more than 120 years ago, and adrenoceptors have been targeted by drugs for clinical benefit ever since. Adrenoceptors and their ligands therefore have one of the richest histories, in terms of understanding, drug development, diverse ligand availability, and widespread clinical uses of agonist and antagonist drugs of any of the G protein-coupled receptors (GPCRs). As such, they have been, and remain, prototype GPCRs for new discoveries enabling a better understanding of the concept of G protein-coupled receptors, their function, structure, signal transduction, and regulation. Accordingly, adrenoceptor research has led to several Nobel Prizes including the 1971 prize to Earl E. Sutherland Jr, the 1988 prize to Sir James Black, the 1994 prize to Alfred G. Gilman and Martin Rodbell, and the 2012 prize to Brian Kobilka and Robert J. Lefkowitz.

Today, adrenoceptors remain the molecular targets for worldwide guideline-recommended drugs for the treatment of a wide variety of conditions, and many of these adrenoceptor drugs are the standard of care for their indications. β-antagonists (β-blockers, 4th most commonly prescribed class of drugs) are used for cardiovascular disease (e.g., bisoprolol, carvedilol, metoprolol), glaucoma (e.g., timolol), and migraine (e.g., propranolol). β-agonists (11th most commonly prescribed class of drugs) are used for asthma and COPD (e.g., formoterol, salbutamol, salmeterol, vilanterol). Adrenaline can be lifesaving in anaphylaxis and shock. All of these medications are named on the WHO List of Essential Medicines 2023. α-antagonists are also commonly used (25th most commonly prescribed class of drugs) for hypertension (e.g., doxazosin) and benign prostatic hyperplasia (e.g., alfuzosin, tamsulosin), and α-agonists, e.g. dexmedetomidine, are increasingly used for their sedative properties in intensive care settings. At the other end of the spectrum, highly efficacious inhaled β-agonists (no longer used as inhaled medications – adrenaline, isoprenaline, orciprenaline, and fenoterol) were linked with epidemics of deaths in those with asthma in several areas of the world in the 1960s and 1970s, whilst other β-agonists (clenbuterol) have caused human harm when entering the food chain and

are on the World Anti-Doping Association (WADA) and International Olympic Committee (IOC) list of prohibited drugs.

This volume of the Handbook of Experimental Pharmacology covers a full range of information from in vitro and in vivo studies and understanding to human clinical studies, and on to current and potential future clinical uses, many within each chapter. A short history of adrenoceptor research is provided by Martin C. Michel (Mainz, Germany), and more topic-specific historical aspects are included in the other chapters. Lukas Helfinger and Christopher G. Tate (Cambridge, UK) discuss structures of adrenoceptors identifying details of how ligands bind to orthosteric and allosteric sites to influence receptor activity and transducer coupling, and Andrea Nahles and Stefan Engelhardt (Munich, Germany) review genetic variants of adrenoceptors with in-depth discussion of those posing as significant risk factors. Jillian G. Baker (Nottingham, UK) and Roger J. Summers (Parkville, Australia) provide an in-depth discussion of adrenoceptor ligands, with an overview of their clinical uses, molecular pharmacology, and the assays available to study them. Chantel Mastos, Xiaomeng Xu, Alastair C. Keen, and Michelle L. Halls (Parkville, Australia) review the canonical pathways, new paradigms, and the importance of spatial and temporal control in the signal transduction mechanisms of adrenoceptor subtypes.

Other chapters discuss the roles of adrenoceptors and their subtypes in specific organ systems. Bela Szabo (Freiburg, Germany) reviews the role of neurotransmitter release-modifying adrenoceptors. Three chapters cover cardiovascular aspects, an area that was instrumental in adrenoceptor discovery and has many adrenoceptor-targeted drugs in clinical use. Yee W. Wong, Haris Haqqani, and Peter Molenaar (Chermside, Australia) discuss the role of the three β-adrenoceptor subtypes and drugs acting at β-adrenoceptors in heart failure, tachyarrhythmias, and other cardiovascular disorders. Spoorthy Kulkarni and Ian B. Wilkinson (Cambridge, UK) review the role of adrenoceptors in the pathophysiology and treatment of various forms of arterial hypertension. Erica Langnas and Mervyn Maze (San Francisco, USA) summarize the clinical uses of adrenergic receptor ligands in acute care settings with particular emphasis on the use of α_2-adrenoceptor agonists including dexmedetomidine.

Martin Hennenberg (Munich, Germany) and Martin C. Michel (Mainz, Germany) comprehensively review the role of adrenoceptors, their signal transduction mechanisms, and the use of α_1-adrenoceptor antagonists and β_3-adrenoceptor agonists for the treatment of diseases of the lower urinary tract. Jillian G. Baker and Dominick E. Shaw (Nottingham, UK) discuss the development of drugs for the treatment of asthma and chronic obstructive airways disease with particular emphasis on the role of β_2-adrenoceptors and their agonists and how these could be improved in the future. Yue Ruan, Francesco Buonfiglio, and Adrian Gericke (Mainz, Germany) present an examination of the expression, distribution, and functional roles of α_1-, α_2-, and β-adrenoceptors within various components of the eye and associated structures and how individual receptor subtypes can be targeted to treat ocular conditions including glaucoma.

Two chapters focus on the role of adrenoceptors in the central nervous system. S. Clare Stanford and David J. Heal (London, Nottingham and Bath, UK) review the role of adrenoceptors in psychiatric disorders and their treatments emphasizing the challenges associated with a lack of animal models that recapitulate the human condition as well as a lack of causal links in clinical studies. Actions of drugs used to treat psychiatric disorders on adrenoceptors may contribute to the therapeutic effect or be responsible for side effects. Rachel A. Matt, Renee S. Martin, Andrew K. Evans, Joel R. Gever, Gabrial A. Vargas, Mehrdad Shamloo, and Anthony P. Ford (San Carlos and Palo Alto, USA) discuss the role of noradrenergic pharmacology in the locus coeruleus and identify promising targets for the treatment of neurodegenerative disease.

Finally, Haneen Dwaib (Bethlehem, Palestine) and Martin C. Michel (Mainz, Germany) discuss the role of adrenoceptors in metabolic control, focusing on the role of α_{2A}-adrenoceptors and β-adrenoceptors in the regulation of insulin release from the pancreas. Rosario Amato, Martina Lucchesi, Silvia Marracci, Luca Filippi, and Massimo Dal Monte (Pisa, Italy) examine the role of β-adrenoceptor subtypes in cancer with the β_2-adrenoceptor emerging as important in tumour development and β_1- and β_3-adrenoceptors involved in certain types of cancer.

Taken together, these 16 chapters provide a comprehensive overview of the current state of play for adrenoceptors, their physiological and pathophysiological role, and their ligands as drug treatments for a wide variety of diseases. We trust that this will prove to be a valuable resource to basic science and clinical researchers in both academia and industry and will attract additional investigators to this well-established but still highly active field.

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Introduction: A Short History of Adrenoceptor Research

Martin C. Michel

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Abstract

This chapter provides a short history of adrenoceptor research starting from the initial discovery of adrenaline. It covers the evolving classification of adrenoceptor subtypes, the cloning of these subtypes from multiple species, and factors such as adrenoceptor regulation, inverse agonism and biased agonism. More details on many of these aspects are provided in other chapters of this volume of Handbook of Experimental Pharmacology.

Keywords

Adrenoceptor · History · Receptor classification

Various reports in the nineteenth century indicated that adrenal glands included a bioactive principle that can increase blood pressure and could be useful in the treatment of asthma. Following experiments with crude extracts by George Oliver

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& E. Schäfer (University College London) (Oliver and Schäfer 1895) and a partial purification by John J. Abel (Johns Hopkins University) that he named epinephrine, Jokichi Takamine (Parke, Davis & Co.) was the first to prepare a pure substance from the adrenals and named it adrenaline (Takami 1902). In the beautiful prose of the time, he wrote "Last summer I began experiments with the object of isolating the active principle, and am pleased to be able to announce that I have succeeded in obtaining a stable crystalline body of constant composition from the gland … The fact that a fraction of a drop of a 1 in 50,000 solution of this body when dropped into the eye blanches the conjunctiva, leaves little doubt that it is the active principle of the gland. The injection of 1 c.c. of a 0.001 per cent. solution of adrenaline into the vein of an 8 kg dog causes the blood-pressure to rise 30 mmHg … The isolation of the active principle of the gland seems to point towards the fact that the wonderful physiological action of the various glands may depend upon the effects of apparently simple chemical substances, and such isolation would naturally give an impetus for the search of active principles of the various organs concerning which we know but little."

Various lines of evidence indicated that certain nerves release an active principle that can increase heart rate and blood pressure. While these effects were mimicked by adrenaline, it was more than 40 years after the discovery of adrenaline that Ulf von Euler (Karolinska Institute) isolated noradrenaline (norepinephrine in American English) as the active principle (von Euler 1945). To this date, American English and the INN classification prefer the epinephrine/norepinephrine terminology, whereas British English and IUPHAR terminology prefer adrenaline/noradrenaline; we will use the latter for reasons discussed elsewhere (Aronson 2000). Similarly, we will use the British English and IUPHAR preferred term adrenoceptor and not its American English synonym of adrenergic receptors. Field-specific aspects of adrenoceptor research will be covered in many corresponding sections of this book.

1 Early Receptor Research

The concept that adrenaline and noradrenaline act on some type of receptor was pioneered by the work of Sir Henry Dale (Wellcome Physiological Research Laboratory) who reported that some ergot alkaloids could reverse the pressor effect of adrenaline (Dale 1906). Testifying to the scientific rigor of Dale, he included control experiments in which the pressor and uterine contraction effects of pituitary extracts were not antagonized by the ergot alkaloids. Various catecholamine derivatives and additional blocking agents including tolazoline and phenoxybenzamine were developed thereafter (see chapter on "Adrenoceptors: receptors, ligands and their clinical uses, molecular pharmacology and assays" in this volume). However, they blocked only the smooth muscle excitatory receptors.

This led to the idea of excitatory and inhibitory adrenoceptors, a concept rejected by Raymond P. Ahlquist (University of Georgia) (Ahlquist 1948). Based on a rank order of potency of various catecholamine derivatives in a large panel of preparations, he found that the same rank order found for most contractile responses

also applied to some relaxant responses. Therefore, he concluded that "because of the opposite effects associated with each type of receptor, the customary signs, E (excitatory) and I (inhibitory), cannot be applied. Therefore, for convenience they have been designated as *alpha* adrenotropic receptors and the *beta* receptors." Realizing the overall complexity of the data, Ahlquist already emphasized that this dual classification was likely to have only interim value (Ahlquist 1967).

Indeed, investigators including Saul Z. Langer (Babraham Institute) and Klaus Starke (University of Essen, later University of Freiburg) proposed in the early 1970s that stimulation of α-adrenoceptors had excitatory effects on smooth muscle but inhibitory effects on transmitter release from neurons. The two receptors appeared to differ in location (post- vs. pre-synaptic) and in ligand recognition profile, leading to the subclassification into postsynaptic α_1- and presynaptic α_2-adrenoceptors, thereby creating a trichotomous classification into α_1-, α_2-, and β-adrenoceptors (Langer 1974; Starke 1987). We meanwhile know that transmitter release modifying adrenoceptors can exist not only on neurons synthesizing and releasing noradrenaline (and adrenaline) but also on those using other neurotransmitters; these can be therefore referred to as presynaptic autoreceptors (modulating the release of noradrenaline and adrenaline) and heteroreceptors (modulating that of other neurotransmitters), respectively (Bennett 1999). Interestingly, presynaptic β-adrenoceptors also exist that in most but not all cases promote neurotransmitter release (Okeke et al. 2017). While imperfect, this classification enabled the discovery of many important drugs that remain in clinical use today. A pioneer in this field was Sir James Black, who discovered among other things propranolol, a discovery rewarded with the 1988 Nobel Prize (Black 1989).

Lands (Sterling Winthrop Research Institute) identified that the effects of adrenaline, noradrenaline, isoprenaline, and several derivatives thereof on the heart and on various smooth muscle preparations could not be reconciled with a homogeneous population of β-adrenoceptors; accordingly, he proposed a further subdivision into β_1- and β_2-adrenoceptors (Lands et al. 1967a, b). Thus, the general agreement in the 1970s became that there were two families of adrenoceptors, i.e., α- and β-adrenoceptors with two subtypes each (α_1, α_2, β_1, and β_2; Fig. 1).

The classification efforts in the 1940s to 1960s occurred with the postulated receptors being a black box. However, it emerged at a similar time to the classification of α- and β-adrenoceptors, that at least some of these receptors conveyed the extracellular signals coming from the catecholamines to intracellular effects via mediators such as cAMP (Rall and Sutherland 1959). The importance of this work was highlighted by the award of the 1971 Nobel Prize to Earl W. Sutherland (Western Reserve University). However, concepts at that time did not yet appreciate that the formation of cAMP resulted from a protein complex, not just the receptor. Subsequently, it became clear that α_2-adrenoceptors primarily signal via inhibition of cAMP formation (Pettinger et al. 1987). Also much later, investigators such as Michael J. Berridge (University of Cambridge) discovered a role for phospholipase C as an effector enzyme in signal transduction including that of phosphatidylinositol hydrolysis (Berridge 1993), that is now considered the primary signaling mechanism for α_1-adrenoceptors (Bylund et al. 1994). Concomitantly it became clear that G

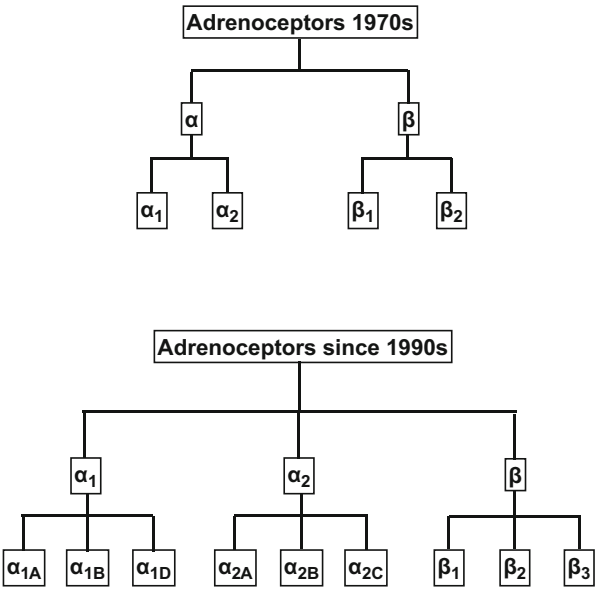

Fig. 1 Classification of adrenoceptors over time

proteins were involved in the signal transduction pathway of adrenoceptors as pioneered by Alfred G. Gilman (University of Texas) (Gilman 1987). Gilman was also pivotal in the discovery of adenylyl cyclase as the effector enzyme of both β- and α_2-adrenoceptors (Tang and Gilman 1992). These discoveries were honored with the 1994 Nobel Prize to Al Gilman and Martin Rodbell (Lefkowitz 1994). They also led to the concept that α_1-adrenoceptors prototypically couple to G proteins of the $G_{q/11}$ family, α_2-adrenoceptors those of the $G_{i/o}$ family, and β-adrenoceptors to G_s proteins (Bylund et al. 1994).

2 Pharmacological Discovery of Additional Adrenoceptor Subtypes

Based on techniques such as radioligand binding and the availability of a greater selection of compounds, the dichotomous classification of adrenoceptors became increasingly challenged in the 1980s. Based on tools such as WB 4101, Leslie Morrow and Ian Creese (University of California San Diego) proposed a further division of α_1-adrenoceptors into the subtypes α_{1A} and α_{1B} (Morrow and Creese 1986). Using a different approach utilizing the alkylating agent chloroethylclonidine, the group of Kenneth Minneman (Emory University) supported this subdivision and expanded the evidence to functional data at the tissue level (Johnson and Minneman 1987). In this scheme, the α_{1A}-adrenoceptor had high affinity for WB 4101 and was less sensitive to inactivation by chloroethylclonidine, whereas the α_{1B}-adrenoceptor had low affinity for WB 4101 and was more sensitive to inactivation by chloroethylclonidine. Other key compounds used to differentiate

these proposed α_1-adrenoceptor subtypes included 5-methyl-urapidil and the stereoisomers of the Ca^{2+}-channel inhibitor niguldipine (Michel et al. 1990). However, even this subdivision could not explain the ligand recognition profile of some α_1-adrenoceptors such as those mediating contraction of rat aorta (Eltze et al. 2001; Oriowo and Ruffolo 1992). Finally, based on a surprisingly low potency of prazosin at some α_1-adrenoceptors, an α_{1L}-subtype was proposed (Kava et al. 1998), although it eventually became clear that this is not a distinct subtype but rather a phenotype of the α_{1A}-adrenoceptor that becomes detectable in some cellular contexts and/or under some experimental conditions (White et al. 2019). The discovery of α_1-adrenoceptor subtypes led to the development of α_{1A}-selective antagonists such as tamsulosin and silodosin that proved useful in the treatment of lower urinary tract symptoms suggestive of benign prostatic hyperplasia and displayed fewer cardiovascular effects compared to those originally introduced for the treatment of arterial hypertension such as doxazosin and terazosin (Michel et al. 2001).

Evidence for heterogeneity of α_2-adrenoceptors emerged in the same period. This included the observations of differences in ligand recognition profiles between tissues and species as pioneered by the groups of Stefan R. Nahorski (University of Leicester) (Cheung et al. 1982; Summers et al. 1983) and David Bylund with the latter being first to formally propose a subdivision into α_{2A}- and α_{2B}-adrenoceptors (Bylund 1985). Interestingly, the pharmacological characterization of these subtypes was based on several ligands that preferentially bind to α_1-adrenoceptors including prazosin and WB 4101.

Soon after the division of β-adrenoceptors into the β_1 and β_2 subtypes, it emerged that the ligand recognition profile in some models was not sufficiently explained by these two subtypes (Furchgott 1972). Specifically, this included lipolysis in rodent adipose tissue as pioneered by the group of Johan Zaagsma (Vrije Universiteit Amsterdam, later Rijksuniversiteit Groningen) (Harms et al. 1974) and relaxation of urinary bladder in some species (Nergardh et al. 1977). While a β_3-adrenoceptor subtype was proposed, this did not find general agreement because these atypical adrenoceptors differed in ligand recognition profile among models and laboratories. Nonetheless, this proposal was sufficient to launch a drug discovery program aimed at the treatment of obesity and type 2 diabetes in various companies (Dwaib and Michel 2023) as pioneered by Jon R. Arch and Mike Cawthorne (Beecham Pharmaceuticals) (Arch et al. 1984). Originally based on experiments with CGP 12177 in the heart, a β_4-adrenoceptor was proposed by the groups of Alberto J. Kaumann (Babraham Institute) and Peter Molenaar (Melbourne University, later University of Queensland) (Kaumann and Molenaar 1997). However, it later become clear that this represents an heterotopic site on the β_1-adrenoceptor (Molenaar 2003).

3 Cloning of Adrenoceptor Subtypes

Many of the above controversies did not get resolved until the various adrenoceptor subtypes were cloned. The first adrenoceptor to be cloned, the β_2-adrenoceptor, was isolated from a hamster smooth muscle cell line by the group of Robert J. Lefkowitz

(Duke University) (Dixon et al. 1986). This was followed in rapid succession by its human ortholog (Kobilka et al. 1987) and by various other adrenoceptor subtypes from humans and other mammalian and non-mammalian species (Table 1). While initial cloning efforts were based on purified protein, later ones were based on homology screening. While most of the encoding genes are intronless, some have introns and/or splice variants in at least some species.

Based on the combined evidence from the pharmacological studies and the receptor cloning, a trichotomous nomenclature of the adrenoceptors was agreed upon in 1994 (Bylund et al. 1994) and slightly updated in 1995 (Hieble et al. 1995), which has stood the test of time (Fig. 1).

Major progress has been made in our understanding of how the adrenoceptors work at the molecular level following their cloning. An important part of this was elucidating their crystal structures. Following that of the human β_2-adrenoceptor (Rasmussen et al. 2007), crystal structures have been determined for other adrenoceptor subtypes including the human α_{1B}-adrenoceptor (Deluigi et al. 2022), the human α_{1D}-adrenoceptor (Janezic et al. 2019), and the turkey β_1-adrenoceptor (Huang et al. 2013). Particularly for the β_2-adrenoceptor, crystal structures have been determined from multiple species and in multiple conformations, i.e., bound to an agonist or an antagonist. Honoring these achievements, the 2012 Nobel Prize in chemistry was awarded to Brian Kobilka (Stanford University) and Robert J. Lefkowitz (Duke University).

4 Additional Adrenoceptor Features

With the advent of radioligand binding studies enabling quantification of adrenoceptor protein density in tissues and cell lines in the 1970s, it became possible to directly determine the regulation of adrenoceptor expression at the protein level. Many groups made major contributions to this research including those of Robert J. Lefkowitz (Duke University), T. Kendall Harden (University of North Carolina), Paul A. Insel (University of California San Diego), and Otto-Erich Brodde (University of Essen). An early review of this field was provided in 1983 (Harden 1983). Following the cloning of the adrenoceptor cDNA, additional investigation of the regulation of adrenoceptor expression at the mRNA level became possible. Thus, prolonged agonist exposure can cause desensitization, whereas prolonged antagonist exposure (although documented in fewer settings) can cause sensitization. The discovery of additional players in the signaling of adrenoceptors revealed that the regulation of adrenoceptor expression and function is complex and differs not only between specific receptor subtypes but also to some degree between the cells expressing them (Gurevich and Gurevich 2008; Kohout and Lefkowitz 2003; Moo et al. 2021). Thus, mechanisms involved in agonist-induced desensitization include a rapid phosphorylation of the receptor by a G protein receptor kinase and uncoupling of the receptor from the G protein and internalization of the receptor; later events include the down-regulation of the mRNA expression, which in turn may include reduced transcription and/or reduced mRNA stability, that leads to decreased

Table 1 Adrenoceptor genes in humans, rats, and mice. aa: number of amino acids; cM: centi-Morgan

	Human			Mouse			Rat		
Subtype	Gene symbol	Chromosome	aa	Gene symbol	Chromosome	aa	Gene symbol	Chromosome	aa
α_{1A}	ADRA1A	8p21.2	466	Adra1a	14D1	466	Adra1a	15p12	466
α_{1B}	ADRA1B	5q33.3	520	Adra1b	11 26.81 cM	514	Adra1b	10q21	515
α_{1D}	ADRA1D	20p13	572	Adra1d	2 63.5 cM	562	Adra1d	3q36	561
α_{2A}	ADRA2A	10q25.2	465	Adra2a	19 49.04 cM	465	Adra2a	1q55	465
α_{2B}	ADRA2B	2q11.2	450	Adra2b	2 61.95 cM	450	Adra2b	3q36	453
α_{2C}	ADRA2C	4p16.3	462	Adra2c	5 18.09 cM	458	Adra2c	14-q21	458
β_1	ADRB1	10q25.3	477	Adrb1	19 51.96 cM	466	Adrb1	1q55	466
β_2	ADRB2	5q32	413	Adrb2	18 35.1 cM	418	Adrb2	18q12.1	418
β_3	ADRB3	8p11.23	408	Adrb3	8 15.94 cM	400	Adrb3	16q12.3	400

expression at the protein level. Moreover, it can include an altered expression of the G proteins a receptor couples to and of the post-receptor events such as the effector enzymes. Many of these regulatory pathways are believed to involve phosphorylation of the receptor, but one of the nine subtypes, the β_3-adrenoceptor lacks the required phosphorylation sites; accordingly, this subtype is less sensitive to agonist-induced regulation but can nonetheless be regulated by other mechanisms in some settings (Okeke et al. 2019).

While the original classification of adrenoceptor ligands included agonists and antagonists that activated the receptor and prevented that, respectively, it emerged early that the efficacy of β-adrenoceptor ligands relative to a reference compound such as isoprenaline covers a wide range from full agonist (i.e., efficacy similar to isoprenaline), partial agonism, and antagonism. This can partly be attributed to the expression density and the cell type in which the receptor is expressed, but also in part to the intrinsic efficacy of the ligand, leading to partial agonism (Jasper and Insel 1992). Historical concepts of adrenoceptor agonism or receptor agonism in general had assumed that an antagonist is a compound that blocks the effects of agonists but lacks direct effects on receptor activity. This was challenged by findings where antagonists could reduce receptor signaling in the absence of agonists. While this was initially found largely in systems with overexpressed and/or constitutively active receptors, it meanwhile has also been observed with natively expressed adrenoceptors and is referred to as inverse agonism (Michel et al. 2020; Schütz and Freissmuth 1992). Whether a ligand exhibits inverse agonism or partial agonism is at least partly dependent on the cell type under investigation.

Moreover, classic concepts of molecular adrenoceptor pharmacology had assumed a single binding pocket for the endogenous catecholamines that is used also by xenobiotic agonists and antagonists. However, most adrenoceptor subtypes and other G protein-coupled receptors exhibit additional (heterotopic) sites outside the pocket that can be used by the xenobiotic ligands. Binding to such heterotopic sites could have direct effects and/or could positively or negatively modulate receptor activation by orthosteric ligands, a phenomenon called allosteric modulation as pioneered, for instance, by Nigel Birdsall (National Institute for Medical Research) or Arthur Christopoulos (Monash University) (Christopoulos and Kenakin 2002; Lazareno et al. 2000).

Finally, investigators such as Terry P. Kenakin (Glaxo Research Laboratories, later University of North Carolina) or Roger Summers (Monash University) found that the rank order of potencies to activate receptors may differ between the cellular responses being measured (Evans et al. 2010, 2013; Kenakin and Morgan 1989). While this phenomenon originally was referred to by many terms, it is now generally called biased agonism. However, similar to partial and inverse agonism, this is strongly affected by the cell type under investigation (tissue/cell type bias) and not only by intrinsic features of the compound. Additional factors such as disease state or prior treatment may also affect how biased agonism can be observed at least quantitatively (Michel et al. 2014). Biased agonism is attractive therapeutically because at least in theory it may allow the discovery of ligands that elicit a desired response while having less potential to cause an adverse reaction mediated by the

same receptor but a different signaling pathway. Whether this is a realistic route in drug discovery and development is being debated (Kenakin 2018; Michel and Charlton 2018).

5 Conclusions on Clinical Implications

This volume of the Handbook of Experimental Pharmacology will discuss the ligands, the signal transduction, and the physiological and therapeutic role of various adrenoceptor subtypes. Overall adrenoceptor research and drug discovery played a major role in our biological understanding and in advances in clinical medicine. Thus, particularly approaches and techniques developed related to the β_2-adrenoceptor have been the prototypes for understanding the function, regulation, and structure of G protein-coupled receptors in general. Adrenoceptors have become the target of many drugs in clinical use. For instance, β-adrenoceptor antagonists alone have 20 FDA-approved indications plus another 11 generally accepted off-label indications (Table 2). Thus, adrenoceptors arguably have shaped modern

Table 2 Indications of β-adrenoceptor antagonists approved by the US Food and Drug Administration (FDA) or established off-label uses. Reproduced with permission from Bond et al. (2022)

FDA-approved indications	Off-label uses
1. Angina	1. Anxiety
2. Hypertension	2. Public speaking
3. Congestive heart failure	3. Post-traumatic stress
4. Myocardial infarction prophylaxis	4. Hypotension induction
5. Atrial fibrillation	5. Portal hypertension
6. Open-angle glaucoma	6. Ethanol withdrawal
7. Migraine prophylaxis	7. Esophageal varices
8. Tremor	8. Hypertensive emergency
9. Thyrotoxicosis	9. Variceal bleeding prophylaxis
10. Atrial flutter	10. Perioperative hypertension+
11. Ventricular arrhythmias (ventricular premature beats)	11. Infantile hemangiomas
12. Myocardial infarction	
13. Pheochromocytoma	
14. Ocular hypertension	
15. Paroxysmal supraventricular tachycardia	
16. Idiopathic hypertrophic subaortic stenosis	
17. Scleroderma renal crisis	
18. Hypertrophic subaortic stenosis	
19. Supraventricular tachycardia or non-compensatory sinus tachycardia	
20. Intraoperative and postoperative tachycardia and hypertension	

medicine more than any other drug target family. Four adrenoceptor-related Nobel Prizes further testify to the groundbreaking role of adrenoceptor research and its impact on biology and human well-being.

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Structures of Adrenoceptors

Lukas Helfinger and Christopher G. Tate

Contents

Abstract

The first structure of an adrenoceptor (AR), the human β_2-adrenoceptor (hβ_2AR) was published in 2007 and since then a total of 78 structures (up to June 2022) have been determined by X-ray crystallography and electron cryo-microscopy (cryo-EM) of all three βARs (β_1, β_2 and β_3) and four out of six αARs (α_{1B}, α_{2A}, α_{2B}, α_{2C}). The structures are in a number of different conformational states, including the inactive state bound to an antagonist, an intermediate state bound to agonist and active states bound to agonist and an intracellular transducer (G protein or arrestin) or transducer mimetic (nanobody). The structures identify molecular details of how ligands bind in the orthosteric binding pocket (OBP; 19 antagonists, 18 agonists) and also how three different small molecule allosteric modulators bind. The structures have been used to define the molecular details of receptor activation and also the molecular determinants for transducer coupling. This chapter will give a brief overview of the structures, receptor activation, a comparison across the different subfamilies and commonalities of ligand–receptor interactions.

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Keywords

Cryo-EM · Structure · X-ray

Abbreviations

AR	Adrenoceptor
ECL	Extracellular loop
EM	Electron microscopy
GPCR	G-protein coupled receptor
H	Helix
hβ_1AR	Human β_1-adrenoceptor
ICL	Intracellular loop
MD	Molecular dynamics
OBP	Orthosteric binding pocket
RMSD	Root mean square deviation
TM	Transmembrane
tβ_1AR	Turkey β_1-adrenoceptor

1 Structure Determination

G protein-coupled receptors are integral membrane proteins that span biological membranes, thus providing a physical link between the extracellular environment and the cytoplasm of the cell (Pierce et al. 2002; Kobilka and Deupi 2007; Oldham and Hamm 2008). The portion of the receptor embedded in the membrane is highly hydrophobic, which necessitates the use of detergents to extract them from the membrane for subsequent purification and structure determination. X-ray crystallography was the predominant technique for determining protein structures at the end of the twentieth century, but unfortunately the small detergents most suitable for crystallising membrane proteins are very harsh and inevitably inactivated receptors even before they could be purified (Tate 2010). The exception was the light-sensing GPCR, rhodopsin, that in its inactive state in the dark is extremely stable (for a GPCR) and also has the advantage of having extremely low basal activity. The structure of rhodopsin purified from bovine retinas was published in the year 2000 (Palczewski et al. 2000) and showed the canonical GPCR fold of seven transmembrane helices (H1-H7) with a short amphipathic helix (H8) on the intracellular surface (Fig. 1).

Despite the success of determining the structure of rhodopsin, another 7 years elapsed before the first GPCR structure was determined of a receptor that bound a diffusible ligand, the human β_2AR (Cherezov et al. 2007; Rasmussen et al. 2007), with the turkey β_1AR (tβ_1AR) structure published the following year (Warne et al. 2008). The tβ_1AR was used for X-ray structure determination rather than hβ_1AR because it is considerably more stable upon detergent solubilisation than the human

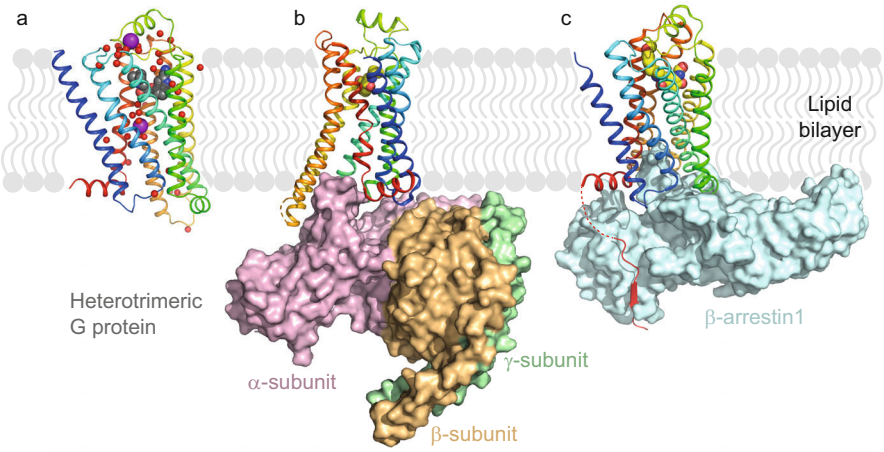
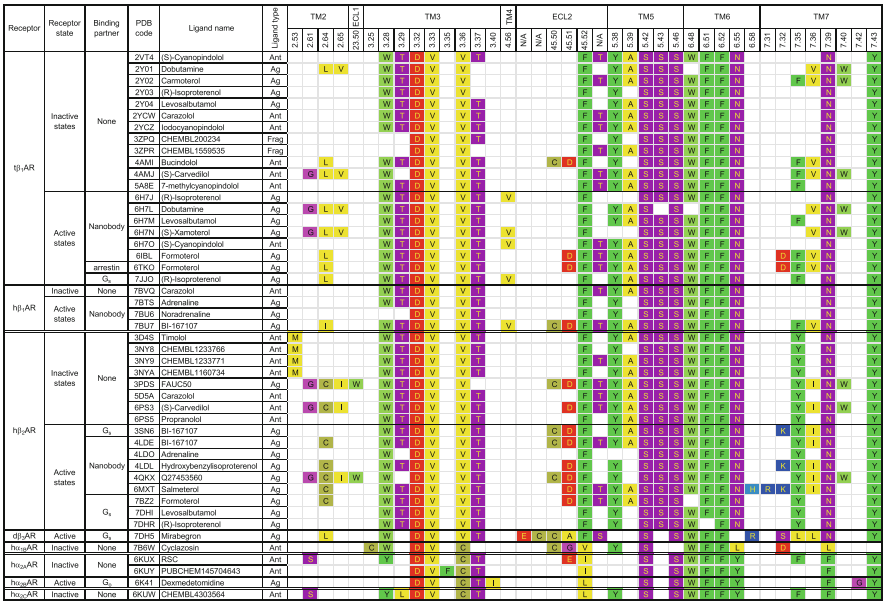


Fig. 1 Structures of the $t\beta_1AR$. (**a**) the inactive state bound to the antagonist cyanopindolol (PDB ID 4BVN; C atoms, grey spheres; water molecules, small red spheres; purple spheres, Na^+ ions), (**b**) the active state bound to the agonist isoproterenol (PDB ID 7JJO; C atoms, yellow spheres) coupled to the G protein G_s, (**c**) the active state bound to formoterol (PDB ID 6TKO; C atoms, yellow spheres) coupled to β-arrestin. In all panels the receptor is in rainbow colouration (N-terminus blue, C-terminus red)

receptor (Serrano-Vega and Tate 2009). The development of a number of generic methodologies was an essential prerequisite to these structures being determined (Tate and Schertler 2009), and these have now enabled the X-ray structure determination of all subsequent GPCRs. These new strategies were necessary because, unlike rhodopsin, β_1AR and β_2AR are unstable in short chain detergents, have higher basal activity and undergo a series of structural transitions between inactive and active states (Nguyen et al. 2017). The three strategies developed were the binding of an antibody (in this case, a F_{ab} fragment: Rasmussen et al. 2007), engineering a fusion protein with T4 lysozyme to facilitate crystal contact formation during crystallogenesis in lipid cubic phase (Rosenbaum et al. 2007), and conformational thermostabilisation through systematic mutagenesis (Serrano-Vega et al. 2008). All three strategies have been used extensively, often in combination, to generate over 600 crystal structures of GPCRs published to date. These include 26 structures of $t\beta_1AR$, 4 structures of $h\beta_1AR$, 37 structures of $h\beta_2AR$, one structure of dog β_3AR and 10 structures of human αARs ($\alpha_{2A}AR$, $\alpha_{2B}AR$, $\alpha_{2C}AR$ and $\alpha_{1B}AR$; see Table 1 for examples). Of note, the first structure determination of a GPCR coupled to a heterotrimeric G protein was in 2011, the β_2AR-G_s complex (Rasmussen et al. 2011), that resulted in the Nobel Prize being awarded to Brian Kobilka in 2012 (Kobilka 2014).

In the last 5 years, there has been a marked change in strategy for structure determination of GPCRs. A series of developments in the field of single-particle cryo-EM over the previous 15 years culminated in better microscopes, faster and more sensitive direct electron detectors, and new algorithms for processing data and

Table 1 Selected structures of adrenoceptors and conserved amino acid residues in the OBP; Ant Antagonist, Ag Agonist, Frag Fragment. Highlighted residues are within 5 Å of the ligand as determined by GPCRdb



determining structures (reviewed in Bai et al. (2015); Fernandez-Leiro and Scheres (2016); McMullan et al. (2016); Vinothkumar and Henderson (2016)). These three factors combined allow structure determination of proteins the size of a GPCR-G protein complex (~120 kDa). This transformed the GPCR field, because it was no longer necessary to spend years engineering a GPCR to improve its stability and/or form well-ordered crystals. Instead, near wild-type receptors can be used and the only protein engineering required may be to improve its production in heterologous expression systems. The first cryo-EM structures of GPCRs coupled to G proteins were published in 2017 (calcitonin receptor, Liang et al. (2017); GLP-1 receptor, Zhang et al. (2017)) and since then over 280 other structures have been determined. These include structures of the $t\beta_1AR$-G_s complex (Su et al. 2020) and $t\beta_1AR$-arrestin complex (Lee et al. 2020), four structures of the $h\beta_2AR$-G_s complex (Zhang et al. 2020; Yang et al. 2021), and the structures of dog β_3AR-G_s (Nagiri et al. 2021) and $h\alpha_{2B}AR$-G_s (Yuan et al. 2020) (see Table 1 for examples). It is interesting to note that the first structures of β_3AR and $\alpha_{2B}AR$ were determined by cryo-EM, whereas previously it would have been easier to determine their inactive state structures by X-ray crystallography. It is also worth noting that structures of GPCRs in lipid nanodiscs have only been determined by cryo-EM and provide a near-native environment for the receptor; this is particularly beneficial in

determining structures in complex with β-arrestin (Lee et al. 2020) that requires lipids for effective association with GPCRs.

To bring the structural biology picture up to the present (July 2022), it is now possible to determine cryo-EM structures of GPCRs in the inactive state using a fusion protein approach and/or when bound to antibodies (Robertson et al. 2021; Bloch et al. 2022; Xu et al. 2022). β_2AR was used as a model Class A receptor for one of the studies and its structure was determined by single-particle cryo-EM in the ligand-free state, and either bound to agonist or antagonist (Xu et al. 2022). It is inevitable that these technologies will generate another surge in GPCR structures over the coming years.

2 Comparison Between Adrenoceptor Structures and How Ligands Bind

Adrenoceptors are divided phylogenetically into two main families, the α-adrenoceptors and β-adrenoceptors, with the α-receptors divided further into the α_1 and α_2 adrenoceptors. Pairwise amino acid sequence analyses show that there is a high conservation in the transmembrane domains (57–87% similarity) suggesting that the overall architecture of the receptors is very similar. This is indeed the case with the active state β-adrenoceptor structures (β_1AR, β_2AR and β_3AR) and the α_{2B}-adrenoceptor (α_{2B}AR) that vary between 0.8 and 1.5 Å RMSD (root mean squared deviation) in all possible pairwise comparisons. The inactive state structures (β_1AR, β_2AR, α_{2A}AR, α_{2C}AR) also show high similarities in structure with RMSDs of pairwise comparisons varying between 0.6 and 1.4 Å. The antagonist-bound α_{1B}AR is a distinct outlier with pairwise comparisons varying between 1.2 and 3.1 Å when compared with the other inactive state adrenoceptor structures. The greatest sequence variation is observed in the loop regions, and the N-terminus and C-terminus, with similarity being as low as 4%. The mechanism of receptor activation is likely to have similarities across the adrenoceptor family. However, both active and inactive state structures are known only for β_1AR and β_2AR, and structures of both conformations are required for elucidating detailed molecular mechanisms of receptor activation; these will be discussed in Sect. 3.

All the adrenoceptors are activated by adrenaline and noradrenaline, and therefore it is perhaps unsurprising that there are some highly conserved residues in the OBP (Table 1). Residues that are within 5 Å of ligands in every adrenoceptor structure determined to date are located in transmembrane helix 3 (H3) ($Asp^{3.32}$, $Val^{3.33}$), H5 ($Ser^{5.42}$) and H6 ($Phe^{6.52}$). Superscripts refer to the Ballesteros-Weinstein numbering system for amino acid residues in GPCRs (Ballesteros et al. 2001). More residues may be included in the list if 44 out of the 47 listed structures (>94%) have a given residue proximal to the ligand; these include residues in H5 ($Ser^{5.46}$), H6 ($Trp^{6.48}$, $Phe^{6.51}$), and H7 ($Tyr^{7.43}$). Another group of residues are those that are also always proximal to the ligand, but they differ in the αARs compared to βARs. For example, residue 3.36 is Cys in αARs and Val in βARs. Similar pairings of residues are 45.52 (Val/Ile/Leu in αARs, Phe in βARs), 6.55 (Leu/Tyr in αARs, Asn in βARs), and 7.39

(Leu/Phe in αARs, Asn in βARs). If all the structures are considered, then the regions that can potentially be proximal to a ligand includes every transmembrane region except H1, and also extracellular loops ECL1 and ECL2. There are very few systematic cases where residues are proximal to a ligand only in one specific conformational state (active or inactive). This is despite the observation that the OBP decreases in volume by up to 41% (Warne et al. 2019) when a G protein mimetic is coupled to the receptor compared to an inactive state (measured in structures bound to the same ligand). Thus, G protein coupling causes an increase in the number of ligand–receptor interactions and/or strength of hydrogen bonds, which increases ligand affinity.

The conservation of adrenoceptor architecture and residues in the OBP suggests that there will be a similarity in how ligands bind. Unfortunately, there is not one ligand that has been used in the structure determination of every adrenoceptor, so a direct comparison between all adrenoceptors cannot be made. In addition, there are currently very few structures of αARs and only very high affinity ligands have been used, because they have been necessary for receptor stabilisation. Comparing the mode of ligand binding between $t\beta_1$AR and $h\beta_2$AR unsurprisingly shows very high similarity, reflecting the high conservation in structure (Fig. 2). Comparisons between other receptors shows more variation, particularly when antagonist-bound structures are compared, although the region where they bind is similar.

The β_1AR (turkey and human) is currently the only GPCR where high-resolution structures have been determined coupled to either a G protein (or G protein mimetic) or β-arrestin (Fig. 3). This allows a direct comparison between the conformation of the receptor and potential changes in the OBP that may illuminate potential mechanisms for ligand bias. Biased ligands signal preferentially through either the G protein or β-arrestin, activating different pathways in the cell and thus have different cellular consequences (Smith et al. 2018; Wootten et al. 2018). It has been suggested that the therapeutic effects of the beta blocker carvedilol are mediated by blocking G protein coupling and allowing arrestin signalling to occur (DeWire and Violin 2011), although this is controversial and has been recently disputed (Benkel et al. 2022). The beneficial effects of salbutamol are thought to be through only the G protein pathway (Nguyen et al. 2017). Comparing the structure of formoterol-bound arrestin-coupled $t\beta_1$AR with the structure of formoterol-bound $t\beta_1$AR coupled to a G protein mimetic, shows a 3 Å shift in the position of ECL3 and also a 1 Å shift of H5 away from the ligand, resulting in decreased ligand-receptor contacts and decreased affinity for the agonist compared to when a G protein is coupled (Lee et al. 2020). Different surfaces at the juxtaposition between the transducer and receptor on the intracellular surface also offers opportunities for the development of novel regulators of signalling. Other structures have been determined with a biased ligand bound, for example carvedilol bound to the inactive state of $t\beta_1$AR (Warne et al. 2012). However, there were no significant differences in conformation when compared to structures bound to other ligands that are not thought to be biased. This really highlights the need of having multiple structures in different conformational states to allow meaningful molecular mechanisms to be established that underpin the structure-activity relationships of ligands.

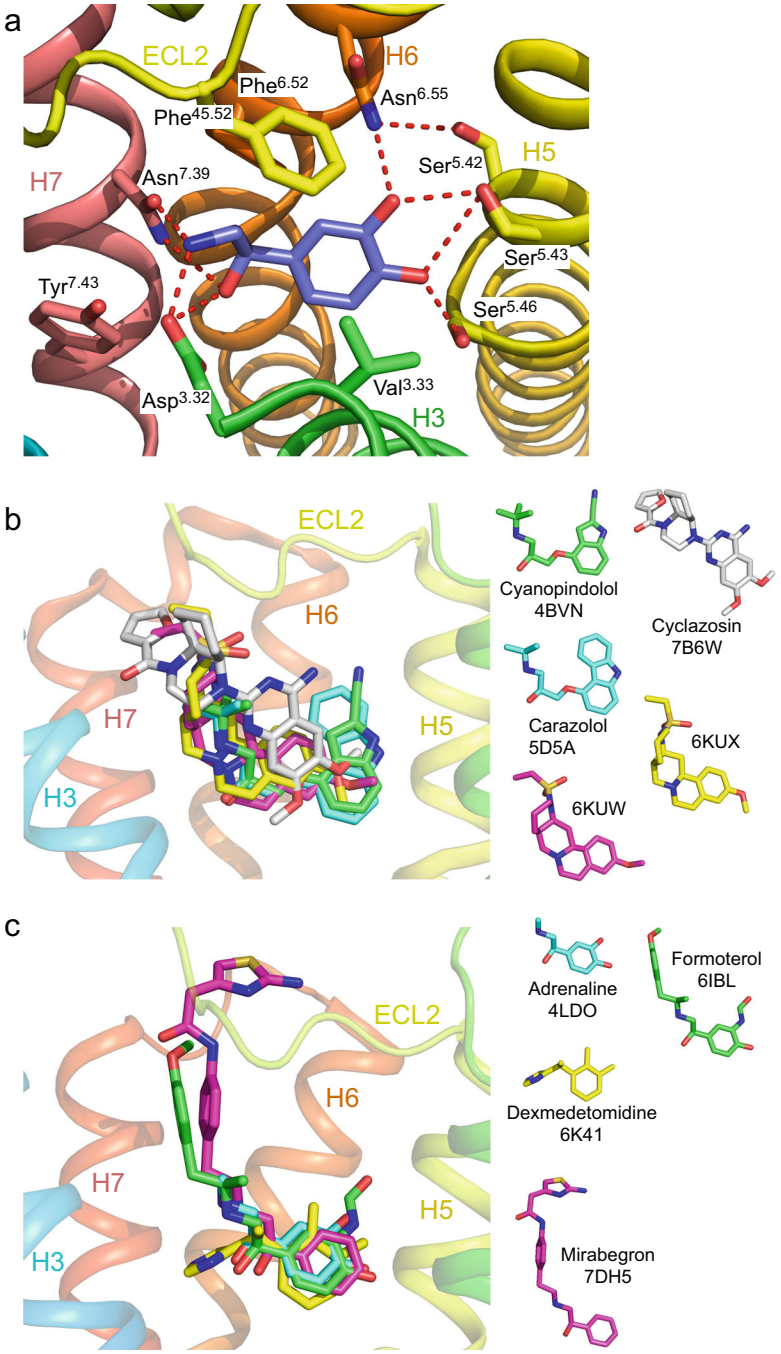


Fig. 2 (**a**) Structure of the orthosteric binding pocket of hβ_1AR bound to noradrenaline, with red dashed lines representing putative hydrogen bonds (PDB code 7BU6), (**b**) binding pose of antagonists in the OBP, (**c**) binding pose of agonists in the OBP. PDB IDs for the structures are given adjacent to the ligand. See Table 1 for species of receptors depicted in (**b**) and (**c**)

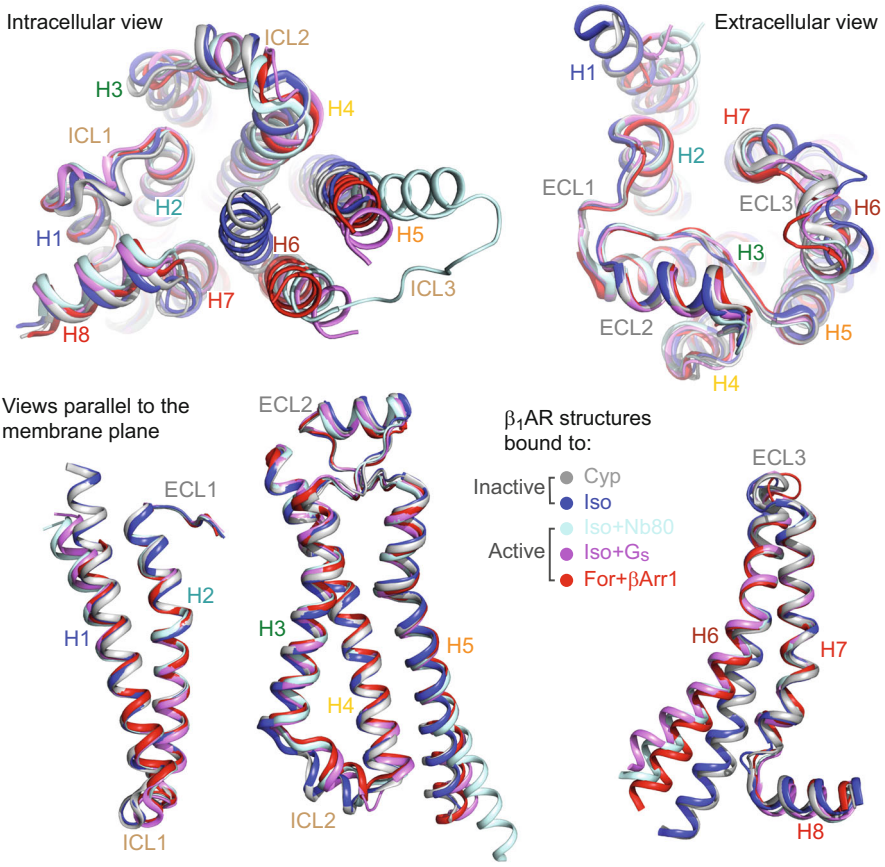


Fig. 3 Different conformational states of $t\beta_1AR$. Overlay of structures in the inactive state bound to cyanopindolol (Cyp) and isoproterenol (Iso), and the active state coupled to the G protein mimetic Nb80, G_s and β-arrestin (For, formoterol)

3 Structure and Activation of β_1AR and β_2AR

The $h\beta_2AR$ and $t\beta_1AR$ were the first two hormone receptor structures determined (Cherezov et al. 2007; Warne et al. 2008). The $h\beta_1AR$ is considerably less thermostable than $t\beta_1AR$ (Serrano-Vega and Tate 2009), so the latter was used extensively for early biochemical work and heterologous expression (Warne et al. 2003). However, even $t\beta_1AR$ was insufficiently stable for crystallisation and therefore it was thermostabilised by the addition of six point mutations (Serrano-Vega et al. 2008). The amino acid sequences of human and turkey β_1AR are 76% identical in the transmembrane regions and there are no significant differences between carazolol-bound structures of $t\beta_1AR$ (PDB code 2YCW; Moukhametzianov et al. (2011)) and the recently determined $h\beta_1AR$ (PDB code 7BVQ; Xu et al. (2021)) structure

(RMSD 0.6 Å, over 1,524 atoms). Thus, conclusions derived from $t\beta_1AR$ structures are applicable to the human receptor, despite there being some differences in pharmacology (Baker 2010), which could arise from kinetic differences in transitions between different conformations or different ligand binding pathways (Xu et al. 2021). There are also no significant structural differences (RMSD 0.6 Å over 1,632 atoms) between carazolol-bound $t\beta_1AR$ (PDB code 2YCW; Moukhametzianov et al. (2011) and $h\beta_2AR$ (PDB code 2RH1; Cherezov et al. (2007)), although intracellular loop 2 (ICL2) contains a short α-helix in $t\beta_1AR$ whereas it is unstructured in $h\beta_2AR$. Structures of $t\beta_1AR$ typically contain a Na^+ ion that appears to stabilise the turn at the end of a short α-helix in extracellular loop 2 (ECL2; (Warne et al. 2008). MD simulations (Dror et al. 2009) of $h\beta_2AR$ resulted in the appearance of an extracellular Na^+ ion and ordering of ICL2 as observed in $t\beta_1AR$. The high-resolution structure of $t\beta_1AR$ at 2.1 Å resolution identified an intramembrane Na^+ ion (Miller-Gallacher et al. 2014) in a similar position to that observed in other receptors (Katritch et al. 2014). However, Na^+ ion concentration does not affect agonist affinity at $t\beta_1AR$ (Miller-Gallacher et al. 2014), unlike in the adenosine A_{2A} receptor ($A_{2A}R$) where Na^+ is an allosteric antagonist (Liu et al. 2012). This is because agonist binding to $A_{2A}R$ results in a transition to an intermediate state very similar to the fully active state (Lebon et al. 2011) where the intramembrane Na^+ ion pocket has collapsed and Na^+ is extruded (presumably down its concentration gradient into the cytoplasm). In contrast, agonist binding to $t\beta_1AR$ does not alter significantly the overall conformation of the receptor (Warne et al. 2011) and so the intramembrane Na^+ binding pocket remains unchanged.

The activation of β_1AR and β_2AR are thought to be essentially identical given the similarities in their overall structures in different conformational states and key amino acid residues are highly conserved. Therefore, in the discussion below residues will be numbered according to the Ballesteros-Weinstein numbering system (Ballesteros et al. 2001), which can be converted conveniently to specific residue numbers using GPCRdb if required (www.gpcrdb.org; Kooistra et al. (2021)). It should be appreciated that structures represent a series of snapshots of selected stable states within the overall conformational landscape of the receptors. However, multiple techniques demonstrate that β_2AR is highly dynamic even in the absence of ligands and appears to access a plethora of different conformations (Manglik et al. 2015). This is consistent with the concept of basal activity, where a receptor in the absence of a ligand can couple functionally to a G protein, implying a structure similar, if not identical, to the structures of agonist-bound receptor-G protein complexes. For clarity, the activation mechanism is given in a linear fashion following a distinct timeline; this might not be the case in reality.

Binding of the full agonist isoproterenol or FAUC50 to $t\beta_1AR$ or $h\beta_2AR$, respectively, resulted in structures showing a 1–2 Å contraction of the OBP and the rotamer change of $Ser^{5.46}$ in comparison with antagonist-bound structures (Rosenbaum et al. 2011; Warne et al. 2011). There were no other significant changes throughout the receptor. When a partial agonist bound, structures showed that there was still the contraction of the OBP, but there was no change in orientation of $Ser^{5.46}$ (Warne et al. 2011). These subtle changes are thought to be sufficient to make the

receptor more likely to transition into an active state capable of coupling to a G protein. The importance of $Ser^{5.46}$ during activation was highlighted through a comparison of the activity and structures of $t\beta_1AR$ bound to cyanopindolol and 7-methylcyanopindolol (7-MeCyp). Cyanopindolol was originally described as an antagonist of β_1AR, but in more sensitive assays it is seen to act as a weak partial agonist (Sato et al. 2015). The cyanopindolol-bound $t\beta_1AR$ structure showed no contraction of the OBP and no rotamer change of $Ser^{5.46}$. However, during activation of the receptor, it would be expected that $Ser^{5.46}$ would have to rotate and therefore modification of cyanopindolol to prevent this, by the addition of a methyl group in the 7 position, would be expected to decrease significantly ligand efficacy. This was indeed the case, and 7-MeCyp acted as a neutral antagonist at $t\beta_1AR$ and a partial inverse agonist at $h\beta_2AR$ (Sato et al. 2015). The rotamer change of $Ser^{5.46}$ reduces the number of van der Waals and polar interactions between transmembrane helices H4, H5 and H6, thus making it more likely that the helices can move into the positions they adopt in active conformations.

No structures of β_1AR or β_2AR intermediates between an agonist-bound inactive state and the G protein-coupled state have been crystallised, although such intermediates are known for other receptors, e.g., $A_{2A}R$ (Lebon et al. 2012). Comparisons between the inactive state and G protein-coupled state shows a number of distinct changes throughout the whole receptor (Rasmussen et al. 2011). There is a closure of the entrance to the orthosteric binding site which reduces the on and off rate of ligands (DeVree et al. 2016). Where structures have been determined in the inactive state and G protein-coupled state bound to the same ligand, it is apparent that there can be up to a 41% decrease in the volume of the OBP due primarily to the inward movement of the extracellular ends of H6 and H7 (Warne et al. 2019). This results in an increase in the number and/or strength of ligand-receptor contacts that are consistent with an increase in ligand affinity when a G protein is coupled. There is a re-arrangement of three residues ($Pro^{5.50}$, $Ile^{3.40}$, $Phe^{6.44}$) at the core of the receptor that is regarded as a key switch in receptor activation (Huang et al. 2015). The contraction of the aqueous cavity off the intramembrane Na^+ binding pocket results in the loss of the Na^+ ion. There are also rearrangements of conserved tyrosine residues $Tyr^{5.58}$ and $Tyr^{7.53}$ that make interactions in the core of the receptor that stabilise the active state (Huang et al. 2015).

The major structural difference between the inactive and active states is on the intracellular surface where the 14 Å outward movement of the intracellular end of H6 (measured at Cα Lys 267 of $h\beta_2AR$) forms a cleft that accommodates the C-terminal α5 helix of the G protein (Rasmussen et al. 2011). There are also additional small changes in the positions of H7 and H5 on G protein coupling. Virtually all the interactions between the heterotrimeric G protein and β_2AR occur via the α-subunit, with only a few minor contacts to the β-subunit (Rasmussen et al. 2011). Of the α-subunit contacts to the receptor, 70% are made by the α5 helix. As is observed in other GPCR-G protein complexes, the majority of contacts are via van der Waals interactions with only a few polar interactions and salt bridges (Garcia-Nafria and Tate 2019).

The structure of tβ_1AR when coupled to β-arrestin is very similar to when it is coupled to either G_s or the G protein mimetic nanobody Nb80 (Lee et al. 2020). However, there are three significant differences. Firstly, the cytoplasmic end of H6 is shifted by only 7 Å when coupled to arrestin compared to 12 Å coupled to G_s (measured at Cα Arg284). Secondly, ECL3 is shifted towards the core of the receptor by 3 Å (measured at Cα Asp318) when arrestin is coupled compared to when G_s is coupled. Thirdly, there is a 1 Å outward movement of H5 away from the ligand in the OBP when arrestin is coupled compared to when nanobody Nb80 is coupled (both structures were determined with formoterol bound). This results in the breakage of hydrogen bonds between the ligand and $Ser^{5.46}$ and $Ser^{5.42}$, which is consistent with a decrease in agonist affinity when arrestin is coupled compared to when G protein is coupled. As mentioned in Sect. 2, these differences are sufficient for the development of novel biased therapeutics to the βARs, but how transferable these findings are to other GPCRs awaits further high-resolution structure determination of cognate pairs of receptors bound to the same ligand and to either a G protein or β-arrestin.

4 Conclusions

The structures of β_1AR and β_2AR have been at the forefront of the GPCR field in understanding receptor conformational changes, efficacy, specificity and transducer coupling. This has been through the determination of multiple structures, bound to different ligands and in multiple conformational states. In comparison, there are only one or two structures of each of the other adrenoceptors and two have yet to have their structures determined. There thus remains considerable work to be done to bring the other receptors up to the level of understanding we have for β_1AR and β_2AR. The recent developments in single-particle cryo-EM will undoubtedly accelerate structure determination of both inactive receptors bound to antagonists and active receptors coupled to G proteins. There are significant technical challenges in determining structures of receptors coupled to β-arrestin, but these are not insurmountable, and many more structures of arrestin-coupled receptors are needed before a detailed understanding of ligand bias can be developed.

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Genetic Variants of Adrenoceptors

Andrea Ahles and Stefan Engelhardt

Contents

Abstract

Adrenoceptors are class A G-protein-coupled receptors grouped into three families (α_1-, α_2-, and β-adrenoceptors), each one including three members. All nine corresponding adrenoceptor genes display genetic variation in their coding and adjacent non-coding genomic region. Coding variants, i.e., nucleotide exchanges within the transcribed and translated receptor sequence, may result

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in a difference in amino acid sequence thus altering receptor function and signaling. Such variants have been intensely studied in vitro in overexpression systems and addressed in candidate-gene studies for distinct clinical parameters. In recent years, large cohorts were analyzed in genome-wide association studies (GWAS), where variants are detected as significant in context with specific traits. These studies identified two of the in-depth characterized 18 coding variants in adrenoceptors as repeatedly statistically significant genetic risk factors – p. Arg389Gly in the β_1- and p.Thr164Ile in the β_2-adrenoceptor, along with 56 variants in the non-coding regions adjacent to the adrenoceptor gene loci, the functional role of which is largely unknown at present. This chapter summarizes current knowledge on the two coding variants in adrenoceptors that have been consistently validated in GWAS and provides a prospective overview on the numerous non-coding variants more recently attributed to adrenoceptor gene loci.

Keywords

Adrenoceptor · Genetic variation · GWAS · Non-coding · Polymorphism

1 Introduction

Genetic variation is defined as the difference in the DNA sequence among individuals. More than 300 million variants have been identified in the human genome, distributed throughout protein-coding and non-coding regions (Lettre 2022). These variants either consist of single nucleotide exchanges (also known as single nucleotide polymorphisms, SNPs), insertion/deletion mutations or tandem repeat polymorphisms. Current classifications define variants occurring at a minor allelic frequency of >5% as common, and a frequency of 0.5–5% and <0.5% determines a low-frequency and rare variant, respectively (Abecasis et al. 2012). Together, several thousand variants associated with adrenoceptor genes have been identified, the largest fraction residing in non-coding genomic regions. Annotated to one reference genome (Morales et al. 2022), these are listed in common databases (Ensembl, NCBI) with univocal genomic location and dbSNP ("rs") number.

All nine adrenoceptor genes contain single nucleotide variations in their coding regions. Except for the α_{1B}- and α_{1D}-adrenoceptor (ADRA1B and ADRA1D), such non-synonymous adrenoceptor variants have been intensely studied both in vitro upon overexpression in cell systems and in vivo in candidate-gene studies for certain disease traits or drug treatment. A detailed overview of all these variants and the corresponding studies published is given in (Ahles and Engelhardt 2014). However, many of these early studies are – due to the nature of their candidate gene-driven approach – necessarily biased toward distinct genomic loci and did not replicate in later genome-wide association studies (GWAS). For GWAS, genotyping is performed using microarrays, whole exome and whole genome sequencing. They are summarized in the GWAS Catalog (Buniello et al. 2019) which to date (Nov

2022) comprises >6,000 studies published within the last decades. GWAS represent an unbiased method to determine the genetic background of complex human diseases and to uncover potentially causative (poly)genetic variants (Duncan and Brown 2018; Tam et al. 2019). They often comprise cohorts of >100,000 individuals, allowing for high statistical power and providing reliable numbers on variant frequency in different populations and disease conditions. With this technological progress, GWAS have become the standard for evaluating the association of a certain genetic variant with physiological and pathological phenotypes. Even rare variants and common variants with relatively small effect sizes (i.e., a rather slight difference between the two allelic variants on the respective phenotypic parameter) can be detected in an appropriately sized cohort. Yet, a GWAS identifies hundreds of associated variants for one trait each alone typically conferring rather little risk. These studies are further limited to phenotypes whose characteristics can be analyzed in a systematic way, while rare or hard to study phenotypes remain underrepresented in GWAS. Statistical analysis of GWAS data and stratification is complex and rather incomprehensive, a problem that is aggravated in meta-analysis of different GWAS, which vastly lack statistical traceability as differently analyzed cohorts are combined (Tam et al. 2019). To circumvent the report of false positive GWAS hits, we do not mention adrenoceptor-associated variants that were found statistically significant in a single cohort for an unrelated trait throughout this chapter. We relate to the GWAS Catalog and further require a GWAS cohort of >1,000 individuals.

Accordingly, two of the 18 previously characterized non-synonymous adrenoceptor variants in the coding region were repeatedly reported as associated with a specific (disease) trait: the β_1-adrenoceptor (ADRB1) variation p.Arg389Gly (rs1801253) and p.Thr164Ile (rs1800888) within the β_2-adrenoceptor (ADRB2) ("protein" nomenclature: prefix p. for protein – major amino acid – position of variant amino acid – minor amino acid). Genetic analysis for GWAS also includes non-coding regions, and non-coding genomic variants are receiving increased attention as they comprise about 90% of all reported associations (Maurano et al. 2012). These non-coding variants are typically mapped to the two protein-coding genes they are located in between and are defined as "regulatory region variant" or "intergenic variant." Regulatory variants induce sequence changes in regulatory DNA elements, such as enhancers, transcription factor binding sites, or methylated DNA regions (Rojano et al. 2019). These alterations may, in turn, affect the binding affinity of transcription factors and subsequently the expression of neighboring genes. In addition, epigenetic patterns intersect with genetic information, potentially fine-tuning the functional properties of a non-coding variant in a cell- and tissue-specific manner (Oh and Petronis 2021; Vohra et al. 2021). A proof of concept for non-coding adrenoceptor variants acting on receptor expression is still lacking. Besides, with the recent release of the complete sequence of a human genome (Nurk et al. 2022), several long-noncoding RNAs (lncRNAs) were newly identified, some of which are even located within an adrenoceptor gene locus. lncRNA sequences are typically transcribed into RNA of >200 bases, and these RNA molecules are reported to modulate the expression of protein-coding genes, yet

their detailed function remains to be determined (Statello et al. 2021). With these novel annotations, some of the non-coding variants are now located within the exon or the intron of an lncRNA. The specific trait associations reported in GWAS for such a variant might refer to the lncRNA's function. To date, the interpretation of GWAS results on non-coding variants necessarily remains somewhat speculative. The detailed and unbiased knowledge of non-coding variants demands new studies for in-depth characterization of these variants and their effect on gene expression.

Structured by adrenoceptor subtypes, this chapter aims to provide a comprehensive summary of non-coding variants attributed to adrenoceptor genes and reports current knowledge on the two coding variants ADRB1-p.Arg389Gly and ADRB2-p.Thr164Ile, which reveal whether these coding variants alter the expression and/or function of the receptor protein or whether the variants just represent markers for the reported traits in GWAS.

2 Variants Associated with α_1-Adrenoceptors

α_1-adrenoceptors are robustly expressed in cardiac and smooth muscle. Their activation by catecholamines contributes to contraction and subsequently controls blood pressure, pupil width, bladder, and prostate tone (O'Connell et al. 2014; Akinaga et al. 2019) (for details on expression, see Chapter "Expression Pattern and Species Differences"). As postsynaptic receptors in the central nervous system, α_1-adrenoceptors stimulate transmitter release (Perez 2020). Three α_1-adrenoceptor subtypes are encoded in the human genome: α_{1A}, α_{1B}, and α_{1D} (*ADRA1A, ADRA1B, ADRA1D*). The *ADRA1A* and the *ADRA1B* locus are subject to alternative splicing resulting in different protein-coding isoforms, while the *ADRA1D* is expressed as one single isoform. For none of these three receptors, a coding variant showed significant association with a specific parameter in a GWAS. Non-coding variants were detected for all three α_1-adrenoceptor subtypes (Table 1).

The *ADRA1A* gene is located on the reverse strand of chromosome 8. The adjacent protein-coding genes for dihydropyrimidinase like 2 (*DPYSL2*) and stathmin-4 (*STMN4*) are about 0.1 and 0.7 Mb distant, respectively. In addition, lncRNAs are located in the *ADRA1A* intronic region (both on the reverse, i.e., *ADRA1A* strand, and the forward strand) and upstream of the *ADRA1A* gene locus. The four non-coding variants associated with *ADRA1A* detected in GWAS are depicted in Fig. 1a, along with adjacent coding genes and annotated lncRNAs. These genomic variants are related to traits of the central nervous system. Two of these variants were found to be associated with total PHF-tau, which is implicated in the pathogenesis of Alzheimer's disease (Wang et al. 2020): rs6998591 located within the intronic region of the *ADRA1A* gene and the intergenic variant rs13273959 attributed to both the *ADRA1A* and the *DPYSL2* gene. The latter encodes a brain enriched protein involved in microtubule assembly and synaptic signaling that might play a role in the development of Alzheimer's disease (Williamson et al. 2011).

Table 1 α_1-adrenoceptor genetic variation. Each variant is defined with its unique dbSNP number and listed with its minor allelic frequency (MAF, minor allele in brackets) and the reported GWAS association

Location	dbSNP	MAF	GWAS association	References
ADRA1A				
Regulatory	rs7845740	0.40 (T)	Bitter alcoholic beverage consumption	Zhong et al. (2019)
Intergenic Intronic	rs13273959 rs6998591	0.08 (G) 0.32 (T)	PHF-tau measurement	Wang et al. (2020)
Intergenic	rs145140079	0.001 (T)	Periventricular white matter hyperintensities	Armstrong et al. (2020)
ADRA1B				
Intergenic	rs148871069	0.0004 (G)	SBP, DBP, pulse pressure Hematocrit, red blood cell count, hemoglobin	Hoffmann et al. (2017) Chen et al. (2020); Sakaue et al. (2021)
ADRA1D				
Regulatory	rs297690	0.29 (T)	DBP	Plotnikov et al. (2022)
Intergenic	rs190933	0.30 (T)		Sakaue et al. (2021)
Intergenic	rs6037811	0.50 (A)	Schizophrenia	Trubetskoy et al. (2022)
Intergenic Intronic	rs3859671 rs835879	0.50 (G) 0.48 (C)	Mononucleosis	Tian et al. (2017)
Intronic	rs835875	0.46 (C)	Heel bone mineral intensity	Kim (2018); Kichaev et al. (2019)

Abbreviations: *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *PHF-tau* paired helical filaments (main constituent: tau protein)

The regulatory region variant rs7845740 was found to be associated with bitter alcoholic beverage consumption in two independent cohorts (Zhong et al. 2019), a complex trait that presumes a central nervous contribution. This variant is located within a binding site of the transcription factor CCTC-binding factor (CTCF), which promotes or represses gene expression and can affect enhancer–promoter interaction (Abecasis et al. 2012). It is thus imaginable that rs7845740 interferes with the expression of the two adjacent protein-coding genes (*ADRA1A* and *DPYSL2*) and of the lncRNAs which were identified within the *ADRA1A* intronic sequence (Fig. 1a). Finally, the rare variant rs145140079 (minor allelic frequency: 0.1%), located upstream of the *ADRA1A* start codon, was associated with white matter hyperintensities in two cohorts (Armstrong et al. 2020). These are supposed to be caused by cerebral small vessel disease and to increase stroke mortality and cognitive and functional impairment, including Alzheimer's disease (Tubi et al. 2020).

The *ADRA1B* gene is located on the forward strand of chromosome 5. Figure 1b depicts the main *ADRA1B* isoform and transcript variant X6. While the distance to the adjacent protein-coding gene 5′ of the gene locus (*IL12B*) is 0.7 MB, the *TTC1*

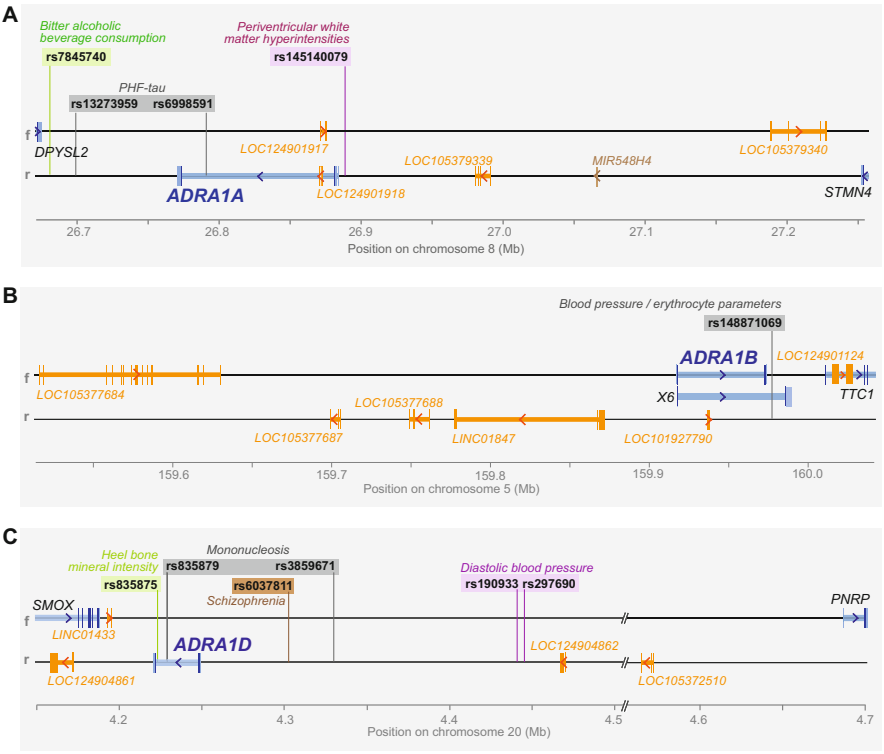

Fig. 1 α_1-adrenoceptor genetic variation. Genomic loci of (**a**) *ADRA1A* (transcript variant 1), (**b**) *ADRA1B* and (**c**) *ADRA1D*. 3′- and 5′-untranslated regions of protein-coding genes are marked in light blue, exons in dark blue. lncRNAs are shown in orange. Genetic variants are depicted according to their genomic location and grouped by reported traits

gene encoding for tetratricopeptide repeat domain 1 is only 36 Kb 3′ of *ADRA1B*. The genomic region further contains several not yet characterized lncRNAs.

For the *ADRA1B*, only one non-coding variant was reported repeatedly in GWAS. rs148871069 displays a rare variant with a minor allelic frequency of 0.04% and is located about 4 kb 3′ of the *ADRA1B* gene (regarding the main *ADRA1B* isoform) in an intergenic region and within the intron of the *ADRA1B* transcript variant X6, respectively (Fig. 1b). The variant was repeatedly detected in GWAS, statistically significant for different traits (Table 1): First, the minor G allele was associated with decreased erythrocyte density which went along with decreased hematocrit and less hemoglobin content (Chen et al. 2020; Sakaue et al. 2021). Second, the same SNP was associated with blood pressure, the minor G allele showing a decrease in both systolic and diastolic blood pressure as well as in pulse pressure (Hoffmann et al. 2017), a physiologic parameter that might be related to α_1-adrenoceptor-mediated contraction of arteries (Akinaga et al. 2019). Whether the non-coding variant rs148871069 affects receptor expression and function, and

whether there is a functional impact of ADRA1B on the modulation of red blood cell composition and pulse pressure, is unclear to date.

The *ADRA1D* gene is located on the reverse strand of chromosome 20, flanked by the genes coding for spermine oxidase (SMOX) and prion protein (PNRP), as well as four lncRNA genes. Six non-coding variants are associated with the *ADRA1D* (Fig. 1c): The intergenic variant rs190933 and the regulatory region variant rs297690 were found to be associated with diastolic blood pressure. The latter variant is located within an enhancer element and thus can potentially affect the expression of *ADRA1D* or the lncRNA *LOC124904862*, which is situated 24 kb upstream of the variant. In addition, an association of the intronic variant rs835875 with heel bone mineral density is reported (Kim 2018; Kichaev et al. 2019). The intergenic variant rs6037811 has been associated with schizophrenia (Trubetskoy et al. 2022), a trait that is linked to alterations in the sympathetic nervous system (Perez 2020). Finally, one single GWAS reported an association with "susceptibility to mononucleosis measurement" with two hits associated with the *ADRA1D*: the intergenic variant rs3859671 located 5′ of the *ADRA1D* gene, and rs835879 in the intronic region of the *ADRA1D* (Tian et al. 2017). The potential link of the latter traits with the *ADRA1D* gene remains to be determined.

In summary, eleven SNPs located in the non-coding regions adjacent to or within α_1-adrenoceptor genes have been validated in GWAS. Although some traits reported directly relate to the function of the respective adrenoceptor, a direct link of one of the nucleotide alterations to altered receptor expression and signaling remains undetermined to date.

3 Variants Associated with α_2-Adrenoceptors

α_2-adrenoceptors are expressed in multiple organs, including adipose tissue, smooth muscles, and the brain (for details on expression, see Chapter "Expression Pattern and Species Differences"). They are involved in regulating blood pressure, pain, and neurotransmitter release. α_2-adrenoceptor signaling further stimulates platelet aggregation and inhibits lipolysis and insulin release from the pancreas (Giovannitti et al. 2015). All three α_2-adrenoceptor subtypes (*ADRA2A, ADRA2B*, and *ADRA2C*) contain variants in their coding and non-coding regions. The insertion/deletion mutation c.901_909del (p.Glu301_Glu303del, resp. rs28365031) located in the third intracellular loop of the ADRA2B protein was found to be associated with diastolic blood pressure in one GWAS (Sakaue et al. 2021). Due to the lack of confirmation by a second cohort, this coding variant is not discussed in detail in this chapter. GWAS hits in non-coding regions attributed to α_2-adrenoceptor loci are limited to the *ADRA2A* (Table 2).

The *ADRA2A* gene is located on the forward strand of chromosome 10. The adjacent protein-coding gene 5′ of the *ADRA2A* is located at a distance of about 63 kb (SHOC2, leucine-rich repeats scaffold protein), while 3′ the distance to the following protein-coding gene comprises nearly 1 Mb.

Table 2 Genetic variation of the α_2-adrenoceptor *ADRA2A*. Each variant is identified by its unique dbSNP number and listed with its minor allelic frequency (MAF, minor allele in brackets) and the reported GWAS association

Location	dbSNP	MAF	GWAS association	References
Regulatory Intergenic	rs869244 rs4545476	0.38 (A) 0.48 (T)	Platelet aggregation	Johnson et al. (2010); Chen et al. (2019)
Intergenic Intergenic	rs12775580 rs12244654	0.08 (T) 0.17 (T)	Glucose levels	Richardson et al. (2022) Sakaue et al. (2021)
Intergenic	rs11195508	0.11 (G)	Hemoglobin A1c levels Glycated hemoglobin levels	Sakaue et al. (2021) Sinnott-Armstrong et al. (2021)

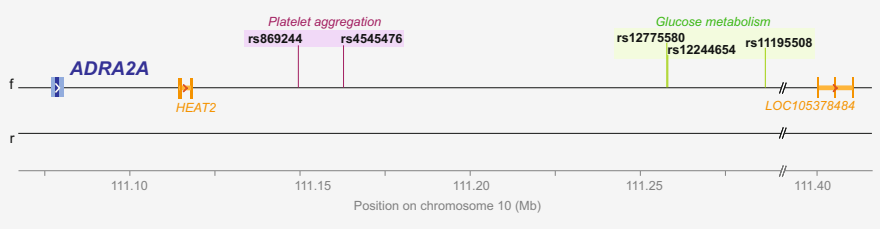

Fig. 2 Variants attributed to the human α_{2A}-adrenoceptor. Genomic locus of *ADRA2A*. 3′- and 5′-untranslated regions of protein-coding genes are marked in light blue, exons in dark blue. lncRNAs are shown in orange. Genetic variants are depicted according to their genomic location and grouped by reported traits

All five detected variants are located 3′ of the *ADRA2A* gene and of the gene encoding lncRNA HEart disease-Associated Transcript 2 (*HEAT2*) (Fig. 2). The traits they are associated with coincide with α_2-adrenoceptor function. Both rs869244 and rs4545476 are associated with differences in platelet aggregation (Johnson et al. 2010; Chen et al. 2019). While the latter is defined as an intergenic variant, rs869244 is localized in an enhancer sequence, allowing for variant-specific regulation of ADRA2A expression and the expression of the immune cell-enriched lncRNA *HEAT2* (Boeckel et al. 2019) (Fig. 2). In addition, an association with glucose regulation has been reported for three intergenic non-coding variants (rs12775580, rs12244654, and rs11195508) that are attributed to the *ADRA2A* gene (Table 2). Although an impact of intergenic variants on the expression of neighboring genes is not described to date, these associations match the reported function of the ADRA2A, namely the inhibition of insulin secretion from the pancreas by ADRA2A (Fagerholm et al. 2011).

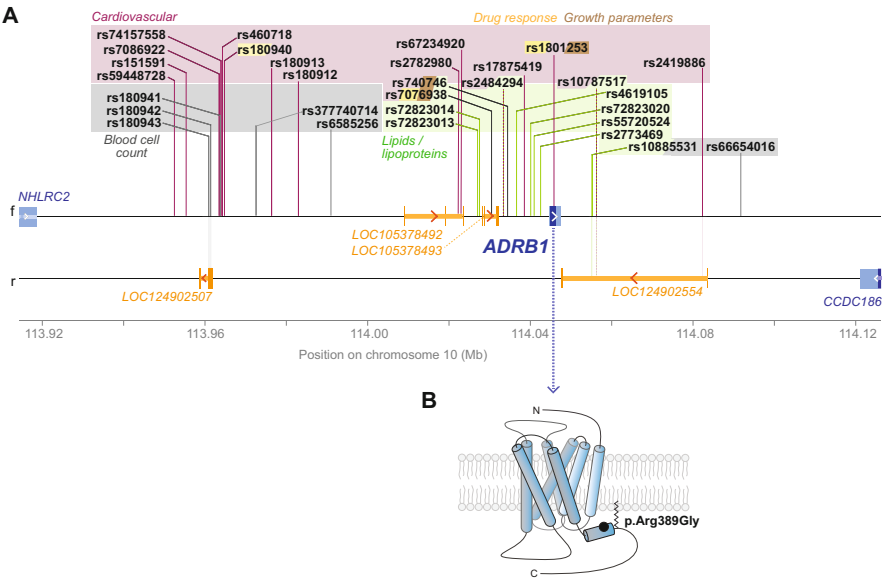

Fig. 3 β_1-adrenoceptor genetic variation. (**a**) *ADRB1* gene locus and adjacent protein-coding genes (blue), lncRNAs (orange) and variants repeatedly associated with a certain trait in GWAS (grouped by reported traits). (**b**) ADRB1 protein and location of the coding variant p.Arg389Gly (rs1801253)

4 Variants Associated with the β_1-Adrenoceptor

The β_1-adrenoceptor (ADRB1) stimulates cardiac output and renin release and thereby maintains blood pressure (Dorn 2010) (for details on ADRB1 signaling, see Chapter "Signal Transduction, Canonical and Alternative Pathways"; for its implication in cardiovascular disease, see Chapters "Cardiovascular: Heart Failure, Ischemic Heart Disease, Arrhythmia" and "Cardiovascular: Hyper- and Hypotension, Shock"). Apart from its robust expression in cardiac myocytes, *ADRB1* expression is high in adipose tissue, where it has been reported to stimulate lipolysis in adipocytes (Riis-Vestergaard et al. 2020) (See also Chapter "Expression Pattern and Species Differences"). With the coding variant p.Arg389Gly and 29 associated non-coding variants, the *ADRB1* locus contains by far the most GWAS hits of the nine adrenoceptor subtypes (Fig. 3a), underscoring its essential role in various physiological systems and diseases.

4.1 The ADRB1 Coding Variant p.Arg389Gly

The intron-less *ADRB1* gene encodes a receptor protein of 477 amino acids. Position 389 is located in helix 8, which is formed between the distal end of transmembrane domain 7 and the C-terminal palmitoylation site (Fig. 3b). Here, arginine is

substituted by glycine at a mean minor allelic frequency of 30%. Evidence toward a functional role of this common variation has been detected in vitro, and there is strong indication for its relevance from multiple clinical studies, including GWAS.

In early in vitro studies conducted in cell lines, the Arg389-variant displayed higher basal and agonist-induced adenylyl cyclase-mediated cAMP formation compared to the Gly389-variant (Mason et al. 1999; Joseph et al. 2004; Ahles et al. 2015). The beating frequency of isolated rat cardiac myocytes was likewise higher when expressing the human Arg389-variant. Using fluorescence resonance energy transfer to monitor conformational changes within the receptor proteins upon application of different ligands, the β-blocker carvedilol induced larger changes in the conformation of the Arg389-variant compared to the Gly389-variant (Rochais et al. 2007).

Alternative signaling, i.e., the interaction of the ADRB1 with arrestins and subsequent receptor desensitization, has been investigated in a variant-specific manner. Here, norepinephrine-induced receptor phosphorylation at intracellular serine and threonine residues by G protein-coupled receptor kinases (GRKs) was stronger for the Arg389-variant, which subsequently led to enhanced recruitment of β-arrestin (Ahles et al. 2015; McCrink et al. 2016). In agreement with its more potent interaction with arrestins, the Arg389-ADRB1 exhibited greater agonist-promoted desensitization than the Gly389-variant (Liggett et al. 2006). Upon cardiomyocyte-specific transgenic overexpression of the human ADRB1 variants in mice, increased basal and dobutamine-induced contractility levels have been reported for the Arg389- compared to the Gly389-variant (Mialet Perez et al. 2003). In addition, desensitization was enhanced for the Arg389-variant in older animals pointing toward a "hyperfunctionality" of the Arg389-variant, and vice versa a "hypofunctionality" for the Gly389-variant.

The elucidation of the crystal structure of the turkey ADRB1 then provided a structural basis for the increased functionality of the Arg389-variant. In these structures, helix 8 is well resolved and contains an arginine at the respective conserved site, whose side chain is oriented toward helix 1 (Warne et al. 2012) (for details on ADRB1 structure, see Chapter "Structures of Adrenoceptors"). In the corresponding model of the human ADRB1, the polar side chains of residues Lys85 and Thr86 in helix 1 are in juxtaposition with Arg389. While Thr86 could interact with Arg389 via hydrogen bonding, repulsion forces are prominent between the helix1-Lys85 and the helix8-Arg389. These electrostatic interactions are different in the Gly389-ADRB1 and might increase the dynamics of the receptor protein, thereby providing a structural basis for the variant-dependent functionality of the ADRB1 (Ahles et al. 2015). Experimentally, the speed of receptor activation has been assessed in dependence on the helix1/helix8 interface by fluorescence resonance energy transfer. Here, the Arg389-ADRB1, expressed in HEK293 cells, showed a faster activation than the Gly389-variant when repeatedly stimulated. This difference was not detected anymore when the helix1/helix8 interface was disrupted by mutating Lys85 and Thr86 to unpolar residues (Ahles et al. 2015).

The functional impact of p.Arg389Gly has finally been validated in GWAS in recent years. These studies typically comprise >100,000 individuals of different

ethnic backgrounds. The first GWAS in which p.Arg389Gly appeared as a hit was on association with birth weight in an analysis of nearly 70,000 Europeans (Horikoshi et al. 2013), with Gly389 being linked to a lower birth weight compared to Arg389. This association has been confirmed in two further GWAS (Warrington et al. 2019; Plotnikov et al. 2020). The authors link a lower birth weight to higher blood pressure in adulthood. The underlying mechanism of this proposed relation remains elusive. Yet, the p.Arg389Gly variation has subsequently been detected as associated with the trait systolic and/or diastolic blood pressure in five GWAS (Surendran et al. 2016; Hoffmann et al. 2017; Feitosa et al. 2018; Sung et al. 2018; Giri et al. 2019). This association with blood pressure is not limited to the coding variation, as it has also been detected for non-coding variants attributed to the *ADRB1* gene (Table 3), the latter presumably acting through modulation of ADRB1 expression. p.Arg389Gly was further found to be associated with cardiovascular disease; the pathologic details were not further specified by the authors (Kichaev et al. 2019). In contrast, p.Arg389Gly was not associated with heart failure risk in any of the >30 GWAS investigating this trait.

These unbiased results from GWAS are reflected by the general outcome of clinical studies that have been conducted in the pre-GWAS era to assess the relevance of the ADRB1 variation p.Arg389Gly in hypertension and heart failure: In the three candidate-gene association studies on hypertension comprising the largest cohorts (n > 1,000), the Arg389-variant was associated with a higher risk for hypertension (Gjesing et al. 2007; Tikhonoff et al. 2008; Johnson et al. 2011), while studies on variant-specific prevalence in heart failure did not result in a univocal association (Ahles and Engelhardt 2014). Importantly, candidate-gene studies show evidence that p.Arg389Gly affects the response to β-blockers: On the one hand, the effect of β-blockers has been studied in healthy individuals under conditions of increased heart rate and blood pressure (by exercise or dobutamine infusion). While basal and maximal hemodynamics did not differ between Arg389 and Gly389 homozygotes, the relative decrease evoked by β-blockers was greater for Arg389 in four out of five study groups (Ahles and Engelhardt 2014). On the other hand, candidate-gene studies on patients treated with β-blockers comprising cohort sizes >1,000, demonstrated improved survival for Arg389 with heart failure (Liggett et al. 2006; O'Connor et al. 2012; Aleong et al. 2013) or coronary artery disease (Pacanowski et al. 2008) compared to Gly389 carriers. Also, a meta-analysis of three smaller studies (in total 504 heart failure patients) revealed a significantly greater improvement of left ventricular ejection fraction for Arg389 homozygotic individuals when treated with β-blockers compared to Gly389 carriers (Muthumala et al. 2008). The five large published GWAS that tested for genetic variation associated with β-blocker response did not report any association – in contrast to the candidate-gene studies mentioned above. A definite statement on the impact of p.Arg389Gly on drug response awaits further GWAS and ideally a subgrouping for different β-blockers. Comedication with additional antihypertensive agents might be considered for analysis, as p.Arg389Gly was associated with treatment with diuretics and drugs acting at the renin-angiotensin-aldosterone-system in two independent GWAS (Wu et al. 2019; Sakaue et al. 2021).

Table 3 β_1-adrenoceptor genetic variants associated with cardiovascular traits. Each variant is defined with its unique dbSNP number and listed with its minor allelic frequency (MAF, minor allele in brackets) and the reported GWAS association

Location	dbSNP	MAF	Reported trait	References
5' of ADRB1				
Intergenic	rs59448728	0.25 (A)	SBP	Sakaue et al. (2021)
Regulatory	rs151591	0.28 (A)	SBP, DPB	Plotnikov et al. (2022)
Intergenic	rs7086922	0.07 (T)	DPB	Plotnikov et al. (2022)
Intergenic	rs74157558	0.07 (A)	SBP	Plotnikov et al. (2022)
Intergenic	rs460718	0.39 (A)	SBP	Sakaue et al. (2021)
Intergenic	rs180940	0.39 (A)	SBP, DBP (+ smoking)	Sung et al. (2018)
Intergenic	rs180913	0.41 (T)	SBP, DBP, MAP	Takeuchi et al. (2018)
Intergenic	rs180912	0.46 (T)	Hypertension	Takeuchi et al. (2018)
lncRNA LOC105378492, intron Regulatory	rs2782980	0.28 (T)	SBP, DBP (± smoking) MAP	Wain et al. (2011, 2017a); Sung et al. (2018); Kichaev et al. (2019); Sakaue et al. (2021)
lncRNA LOC105378492, intron	rs67234920	0.17 (A)	PR interval	Ntalla et al. (2020)
lncRNA LOC105378493, intron	rs7076938	0.30 (C)	MAP	Liu et al. (2016)
Intergenic	rs2484294	0.29 (G)	SBP, DBP, MAP (± alcohol)	Feitosa et al. (2018); Plotnikov et al. (2022)
Regulatory	rs740746	0.29 (G)	SBP, DBP, MAP (± alcohol) Electrocardiography	Ehret et al. (2016); Feitosa et al. (2018); Plotnikov et al. (2022) Verweij et al. (2020)
Intergenic	rs17875419	nd	DBP	Warren et al. (2017)

(continued)

Table 3 (continued)

Location	dbSNP	MAF	Reported trait	References
ADRB1 exon				
	rs1801253 p.Arg389Gly	0.30 (G)	SBP (± smoking) DPB (± alcohol/ smoking) Cardiovascular disease	Surendran et al. (2016); Hoffmann et al. (2017); Giri et al. (2019) Feitosa et al. (2018); Sung et al. (2018) Kichaev et al. (2019)
3' of ADRB1				
lncRNA LOC124902554, intron	rs10787517	nd	SBP	Wain et al. (2017b)
lncRNA LOC124902554, intron	rs2419886	0.24 (T)	Serum calcium measurement	Sakaue et al. (2021); Young et al. (2021)

Abbreviations: *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *MAP* mean arterial pressure, *nd* not determined

4.2 Non-coding Variants Attributed to the ADRB1

The *ADRB1* gene is located on the forward strand of chromosome 10, flanked by lncRNAs, with the adjacent 3′ and 5′ protein-coding genes NHL repeat-containing protein 2 (*NHLRC2*), a not yet fully characterized protein, and coiled-coil domain containing 186 (*CCDC186*, also known as CTCL tumor-associated antigen) about 120 and 70 kb distant, respectively. Both 3′ and 5′ of the *ADRB1* locus, lncRNAs are annotated (Fig. 3a), whose role in physiology and disease is vastly unknown to date. The traits reported for *ADRB1*-associated variants can be subdivided into four major groups: cardiovascular, blood cell count, lipids/lipoproteins, and growth parameters, with some variants associated with multiple of these groups.

In line with ADRB1 function and the coding variant p.Arg389Gly, an association with a cardiovascular trait was reported for 16 non-coding *ADRB1* variants. The majority of these are linked to the traits systolic and/or diastolic blood pressure (Table 3). In particular, the regulatory region variants are suggestive of influencing ADRB1 expression and function, thereby resulting in variant-specific differences in blood pressure: rs151591 is part of a CTCF binding site (Plotnikov et al. 2022), rs2782980 (Wain et al. 2011, 2017a; Sung et al. 2018; Kichaev et al. 2019; Sakaue et al. 2021) and rs740746 (Ehret et al. 2016; Feitosa et al. 2018; Plotnikov et al. 2022) localize in enhancer regions. Moreover, five SNPs are located within the intron of lncRNAs (rs2782980 and rs67234920 in *LOC105378492*; rs7076938, rs10787517, and rs2419886 in *LOC124902554*), hypothesizing a relation of the reported cardiovascular trait and the (patho)physiological function of the respective lncRNA.

Table 4 β_1-adrenoceptor genetic variants associated with drug treatment. Each variant is defined with its unique dbSNP number and listed with its minor allelic frequency (MAF, minor allele in brackets) and the reported GWAS association

Location	dbSNP	MAF	Reported trait	References
5' of ADRB1				
Intergenic	rs180940	0.39 (A)	Beta-blocking agent use	Sakaue et al. (2021)
lncRNA LOC105378493, intron	rs7076938	0.30 (C)	Diuretics use	Wu et al. (2019)
ADRB1 exon				
	rs1801253 p.Arg389Gly	0.30 (G)	Medication use (diuretics, RAAS agents)	Wu et al. (2019); Sakaue et al. (2021)

Of note, two variations 5′ of the *ADRB1* have been additionally found associated with drug treatment (Table 4). First, the intergenic variant rs1800940 was associated with β-blocker use (Sakaue et al. 2021), presuming an influence of the variant on receptor function. Second, rs7076938, located in the intronic region of lncRNA *LOC105378493,* was detected as one determinant of diuretics treatment (Wu et al. 2019), a first-line medication for hypertension.

Besides the multiple associations with cardiovascular traits, seven non-coding variants were reported in GWAS for blood cell count. Five of these were linked to alterations in the number of white blood cells (in general or specifically neutrophils, leukocytes, lymphocytes), in line with the immunomodulatory action of ADRB1, if indeed a genomic link can once be attested between non-coding variants and the receptor gene (Table 5). Among these, rs6585256 displays a regulatory region variant as it is annotated in an enhancer element, modulating both lymphocyte count and platelet crit (i.e., the proportion of blood volume occupied by platelets) (Vuckovic et al. 2020). The variants rs180943 and rs180942 are located within an exon of the lncRNA LOC124902507, which is annotated 5′ of the *ADRB1* gene. These represent nucleotide substitutions that are transcribed and alter the sequence of the lncRNA, which might, in turn, alter lncRNA expression or folding and subsequent interaction with specific proteins or RNA molecules. The link to white blood cell composition regarding the GWAS results (Kichaev et al. 2019; Chen et al. 2020; Vuckovic et al. 2020; Sakaue et al. 2021) remains to be determined. Also, rs10885531, located in the intron of lncRNA *LOC124902554* 3′ of the *ADRB1* gene and associated with reticulocyte count (Dastani et al. 2012), might determine the (unknown) function of this lncRNA.

Furthermore, 11 non-coding variants are associated with traits on lipids (triglycerides, cholesterol), (apo)lipoproteins (very low density, low density, and high density lipoprotein), and the respective ratios (Table 5). As β_1-adrenoceptor activation induces lipolysis, such GWAS hits appear logical. Here, rs740746, located in an enhancer element and already a hit for cardiovascular traits

Table 5 β_1-adrenoceptor genetic variants associated with blood cell count, lipid metabolism, and growth parameters. Each variant is defined with its unique dbSNP number and listed with its minor allelic frequency (MAF, minor allele in brackets) and the reported GWAS association

Location	dbSNP	MAF	Reported trait	References
			Blood cell count	
5' of ADRB1				
lncRNA LOC124902507, exon	rs180943	0.38 (G)	Neutrophil count Leukocyte count	Kichaev et al. (2019); Vuckovic et al. (2020); Sakaue et al. (2021)
lncRNA LOC124902507, exon	rs180942	0.46 (T)	White blood cell count	Chen et al. (2020)
Intergenic	rs180941	0.44 (A)	Neutrophil count White blood cell count	Chen et al. (2020)
Intergenic	rs377740714	nd	Lymphocyte count Leukocyte count	Sakaue et al. (2021)
Regulatory	rs6585256	0.41 (A)	Lymphocyte count Platelet crit	Vuckovic et al. (2020)
3' of ADRB1				
lncRNA LOC124902554, intron	rs10885531	0.45 (T)	Reticulocyte count	Vuckovic et al. (2020)
Intergenic	rs66654016	0.12 (C)	Lymphocyte count	Chen et al. (2020)
			Lipids and lipoproteins	
5' of ADRB1				
Intergenic	rs72823013	0.05 (A)	Lipoproteins / cholesterol, aspartate aminotransferase	Klarin et al. (2018); Klimentidis et al. (2020); Sakaue et al. (2021); Richardson et al. (2022)
Intergenic	rs72823014	0.06 (A)	(Apo) lipoproteins/ cholesterol, aspartate aminotransferase	Richardson et al. (2020, 2022); Chen et al. (2021)
lncRNA LOC105378493, intron	rs7076938	0.30 (C)	Lipoproteins/ cholesterol	Liu et al. (2017)
Intergenic	rs2484294	0.29 (G)	Lipoproteins/ triglycerides	Richardson et al. (2022)

(continued)

Table 5 (continued)

Location	dbSNP	MAF	Reported trait	References
Regulatory	rs740746	0.29 (G)	Lipoproteins/ triglycerides	Qi and Chatterjee (2018); Huang et al. (2021)
Intergenic	rs4619105	0.05 (A)	Lipoproteins	Hoffmann et al. (2018)
Intergenic	rs72823020	0.07 (A)	(Apo) lipoproteins	Richardson et al. (2020, 2022)
Intergenic	rs55720524	0.05 (T)	Lipoproteins / cholesterol	Richardson et al. (2022)
Regulatory	rs2773469	0.29 (A)	Triglycerides	Richardson et al. (2020)
3' of ADRB1				
lncRNA LOC124902554, intron	rs10885531	0.45 (T)	Adiponectin	Dastani et al. (2012)
lncRNA LOC124902554, intron	rs10787517	nd	Lipoproteins/ cholesterol	Ripatti et al. (2020); Sakaue et al. (2021); Richardson et al. (2022)
			Birth/growth parameters	
5' of ADRB1				
lncRNA LOC105378493, intron	rs7076938	0.30 (C)	Birth weight Body height	Horikoshi et al. (2016); Warrington et al. (2019); Sakaue et al. (2021)
Regulatory	rs740746	0.29 (G)	Birth weight/ body height, infant head circumference	Yang et al. (2019)
ADRB1 exon				
	rs1801253 p.Arg389Gly	0.30 (G)	(offspring) Birth weight Body height	Horikoshi et al. (2013); Kichaev et al. (2019); Warrington et al. (2019); Plotnikov et al. (2020)

Abbreviation: *nd* not determined

(Table 3), is associated with lipid traits (levels of cholesterol, triglycerides, LDL, and HDL) (Qi and Chatterjee 2018; Huang et al. 2021). A second regulatory region variant is solely associated with triglyceride levels (Richardson et al. 2020): rs2773469 locates to an open chromatin region, i.e., a region that can be assessed by DNA regulatory elements and thus is important for transcriptional regulation of neighboring genes. Next to six intergenic variants, the intron of lncRNA *LOC105378493* (located 5′ of *ADRB1*) and of lncRNA *LOC124902554* (3′ of *ADRB1*) both contain variants associated with lipid metabolism traits (Dastani et al. 2012; Chen et al. 2020; Ripatti et al. 2020; Sakaue et al. 2021; Richardson et al. 2022).

Finally, the complex traits of body height and birth weight, the coding *ADRB1* variant p.Arg389Gly is associated with, were repeatedly reported for two non-coding variants (Table 5): again, the regulatory region variant rs740746 (Yang et al. 2019) and rs7076938 (Horikoshi et al. 2016; Warrington et al. 2019; Sakaue et al. 2021) located in the intron of lncRNA *LOC105378493*, which already was detected in studies on lipid traits, mean arterial pressure, and diuretics use (see Tables 3, 4, and 5).

In summary, the *ADRB1* genetic region is subject to numerous GWAS reports, including the common coding polymorphism p.Arg389Gly. These unbiased results acknowledge the established critical role of the β_1-adrenoceptor in blood pressure regulation, and in addition suggest major implications of the *ADRB1* genetic region on blood cell composition, lipid metabolism, and body growth. These associations should especially be considered when elucidating the function of the lncRNAs located adjacent to the *ADRB1* gene.

5 Variants Associated with the β_2-Adrenoceptor

β_2-adrenoceptors (ADRB2) are expressed throughout various cell types including smooth muscle and immune cells (for details on expression, see Chapter "Expression Pattern and Species Differences"). Their activation induces vasodilation and relaxation of bronchial and uterine smooth muscle. β_2-Agonists are applied to treat broncho-constrictive diseases and preterm labor. Next to the rare *ADRB2* coding variant p.Thr164Ile (rs1800888), 11 non-coding variants attributed to the *ADRB2* were repeatedly reported to be significantly associated with specific traits in GWAS (Fig. 4a).

5.1 The ADRB2 Coding Variant p.Thr164Ile

The intron-less *ADRB2* gene contains three non-synonymous variations in its open reading frame. The two common coding variants p.Gly16Arg and p.Gln27Glu – both located in the extracellular N-terminus of the ADRB2 – were reported to affect the functional properties of the ADRB2 in some but not all in vitro studies (Ahles and Engelhardt 2014). Subsequently, these variants have to date not been found

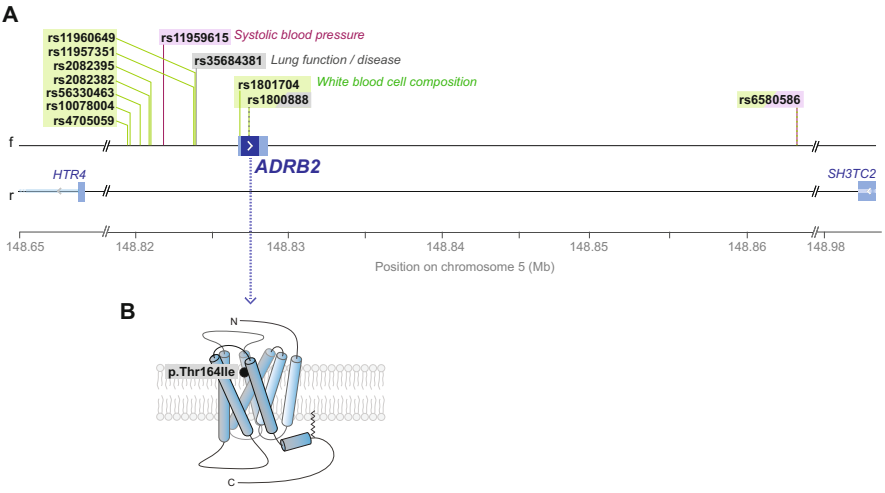

Fig. 4 β_2-adrenoceptor genetic variation. (**a**) *ADRB2* gene locus and adjacent protein-coding genes. Light blue – 3′-and 5′-untranslated regions, dark blue- exons. Variants are depicted according to their genomic location and grouped by reported traits. (**b**) ADRB2 protein and location of the coding variant p.Thr164Ile (rs1800888)

associated with any trait in a GWAS. In contrast, the p.Thr164Ile variant is rare, with isoleucine occurring at an allelic frequency of 2% and in a heterozygous state. This variation locates within the lipid bilayer in transmembrane helix 4 (Fig. 4b), a well-conserved region that has been resolved in the numerous crystal structures published (see Chapter "Structures of Adrenoceptors"). As these structures typically contain threonine at position 164 and an effort to crystallize an Ile164-ADRB2 protein has not been undertaken to date, conclusions arising from structures of the ADRB2 protein are limited to modeling studies and considerations on chemical charges, i.e., the exchange of the polar threonine residue to hydrophobic isoleucine. Position 164 is located near the ligand binding pocket of the ADRB2 and a determinant of the helix4/helix5 interface with Thr164 putatively forming hydrogen bonds with two conserved serines (Ser203 and Ser207) in transmembrane domain 5 (Archala et al. 2022). Hence, p.Thr164Ile is suggested to modulate ligand binding affinities and to influence ADRB2 activation dynamics (Warne et al. 2008, 2011).

Indeed, signaling defects of the Ile164-ADRB2 have already been observed in vitro. When overexpressed in CHW-1102 cells, the Ile164-variant displayed a three- to fourfold lower binding affinity for catecholamines and the β-agonist isoproterenol compared to the Thr164-variant. Consequently, the interaction of the agonist-stimulated ADRB2 with the stimulatory G protein Gs was also decreased for Ile164 (Green et al. 1993), in line with a decrease in basal and agonist-stimulated adenylyl cyclase activity as determined in CHW-1102 cells and transgenic mice overexpressing the different ADRB2 variants (Turki et al. 1996). The loss of function of p.Thr164Ile was further confirmed for the endogenous receptor by analyzing isoproterenol-stimulated cAMP formation in lymphocytes, comparing

Ile164 carriers and Thr164 homozygotes (Büscher et al. 2002). The potency of β-agonist-induced lipolysis (Hoffstedt et al. 2001) and inhibition of IgE-mediated histamine release (Kay et al. 2003, 2007) were reduced in Ile164 carriers.

The effects of the p.Thr164Ile variation observed in vitro were confirmed in large cohorts (Table 6): two GWAS on lung function found the Ile164 variant to be associated with decreased forced expiratory volume (FEV1) (Wain et al. 2017a; Shrine et al. 2019). Consequently, the FEV/FEC ratio was reduced, and peak expiratory flow was significantly decreased (Kichaev et al. 2019; Shrine et al. 2019). Moreover, in a study comprising different ethnic groups, the variant was associated with chronic obstructive pulmonary disease (Moll et al. 2021). The eosinophil percentage of neutrophils was increased for Ile164 (Vuckovic et al. 2020), a finding that might be associated with the severity of asthma and COPD exacerbations (Barnes 2019). Of note, p.Thr164Ile was not detected as a relevant contributor in GWAS for asthma or any cardiovascular disease.

GWAS on treatment efficacy of β-agonists to prevent bronchoconstriction in asthmatic or COPD patients or on their effect on cardiovascular parameters are lacking to date, yet small studies on the response to β_2-agonists predict a significantly reduced response in Ile164 carriers: Both in healthy volunteers (Dishy et al. 2004; Bruck et al. 2005) and patients with congestive heart failure (Barbato et al. 2007) the dilatative effect and the cardiac response, respectively, to β-agonists were less pronounced in Ile164 carriers compared to non-carriers.

5.2 Non-coding Variants Attributed to the ADRB2

The *ADRB2* gene is located on the forward strand of chromosome 5. The genes 5′ and 3′ adjacent to the *ADRB2* locus are >150 kb distant and encode HTR4 (5-hydroxytryptamine receptor) and SH3TC2 (SH3 domain and tetratricopeptide repeat-containing protein 2). Not a single lncRNA is annotated in the intergenic regions 5′ and 3′ of the receptor sequence. Eleven non-coding variants are associated with a trait related to either blood pressure, lung function/disease, or white blood cell composition with only one of these variants located 3′ of the *ADRB1* gene locus (Fig. 4a). The two intergenic variants rs11959615 and rs6580586 are associated with systolic blood pressure (Kulminski et al. 2018; Kichaev et al. 2019), a trait that coincides β_2-adrenoceptor expression in smooth muscles of blood vessels and the vasodilatory effect of ADRB2 activation. In line with ADRB2 function in the lung, the intergenic variant rs35684381 was found associated with chronic obstructive pulmonary disease (Sakornsakolpat et al. 2019), a trait to which the coding variant p.Thr164Ile has also been linked. The majority of GWAS hits associated with the *ADRB2*, however, are related to the composition of white blood cells, consistent with the receptor's expression in immune cells and the established concept of sympathetic control of immune responses by norepinephrine secreted from sympathetic nerves activating immune cell ADRB2 (Udit et al. 2022). This obvious relation suggests that the nine non-coding variants associated with immune cell count alter ADRB2 expression and thereby modulate immune cell composition. This regulation is an

Table 6 β_2-adrenoceptor genetic variants. Each variant is defined with its unique dbSNP number and listed with its minor allelic frequency (MAF, minor allele in brackets) and the reported GWAS association

Location	dbSNP	MAF	Reported trait	References
			Blood pressure	
5' of ADRB2				
Intergenic	rs11959615	0.35 (T)	SBP	Kichaev et al. (2019)
3' of ADRB2				
Intergenic	rs6580586	0.28 (C)	SBP	Kulminski et al. (2018)
			Lung function/disease	
5' of ADRB2				
Intergenic	rs35684381	0.25 (C)	COPD	Sakornsakolpat et al. (2019)
ADRB2 exon	rs1800888 p.Thr164Ile	0.004 (T)	Lung function COPD	Wain et al. (2017a); Kichaev et al. (2019); Shrine et al. (2019); Moll et al. (2021)
			White blood cells	
5' of ADRB2				
Intergenic	rs4705059	0.35 (C)	Leucocyte/ eosinophil count	Astle et al. (2016); Sakaue et al. (2021)
Intergenic	rs10078004	0.35 (G)	Neutrophil % of leucocytes	Vuckovic et al. (2020)
Regulatory	rs56330463	0.35 (T)	White blood cell count and composition (e.g., eosinophils, neutrophils)	Astle et al. (2016); Kichaev et al. (2019); Chen et al. (2020); Vuckovic et al. (2020); Kachuri et al. (2021)
Regulatory	rs2082382	0.15 (G)	White blood cell count (e.g., leucocytes, neutrophils, granulocytes)	Astle et al. (2016); Kichaev et al. (2019); Vuckovic et al. (2020); Kachuri et al. (2021); Sakaue et al. (2021)
Regulatory	rs2082395	0.35 (A)	Monocyte % of leucocytes	Vuckovic et al. (2020)
Intergenic	rs11957351	0.35 (C)	Eosinophil count	Höglund et al. (2022)
Intergenic	rs11960649	0.35 (A)	Neutrophil/ white blood cell count	Chen et al. (2020)
ADRB2 - 5'-UTR	rs1801704	0.21 (C)	Neutrophil-to-lymphocyte ratio	Kachuri et al. (2021)
ADRB2 - exon	rs1800888 p.Thr164Ile	0.004 (T)	Eosinophil % of leukocytes	Vuckovic et al. (2020)
3' of ADRB2				
Intergenic	rs6580586	0.28 (C)	Eosinophil count Eosinophil % of leucocytes	Vuckovic et al. (2020)

Abbreviations: *SBP* systolic blood pressure, *COPD* chronic obstructive pulmonary disease

established mechanism for 5' UTR variants as they induce mutations in transcription factor binding sites. Here, rs1801704 is located within the *ADRB2* 5' UTR and leads to a nucleotide exchange in the binding motives for the transcription factors Myb-like protein 1 (MYBL1, variant position: 2 of 17) and glial cells missing transcription factor 1 (GCM1, variant position: 8 of 28). Altered transcription factor binding and activation might alter ADRB2 expression in a cell-type specific manner and be an explanation for a variant-dependent neutrophil-to-lymphocyte ratio as determined by GWAS (Kachuri et al. 2021).

Furthermore, variants in regulatory regions adjacent to the *ADRB2* gene locus might also modify ADRB2 expression by altering the sequence of a CTCF binding site (rs2082382, rs56330463, and rs2082395) (Table 6). Particularly for rs2082382 and rs56330463 an association with blood cell composition has been independently reported in several cohorts: rs2082382 determines neutrophil, leukocyte, and myeloid white blood cell count and is significantly associated with the traits "sum of neutrophil and eosinophil counts" as well as "sum of basophil and neutrophil counts" (Astle et al. 2016; Kichaev et al. 2019; Vuckovic et al. 2020; Kachuri et al. 2021; Sakaue et al. 2021). Likewise, rs56330463 has been repeatedly associated with eosinophil count and the percentage distribution of different immune cell types (Astle et al. 2016; Kichaev et al. 2019; Chen et al. 2020; Vuckovic et al. 2020; Kachuri et al. 2021). Additionally, four intergenic variants 5′ of the *ADRB2* gene locus and one variant 3′ have reports on association with different white blood cells (Table 6), underlining the importance of the *ADRB2* gene and genomic region concerning interindividual immune responses.

In summary, GWAS hits within the *ADRB2* gene and adjacent to the gene locus are coherent with receptor function on vaso-/bronchodilation and immune responses. Results from GWAS have confirmed the importance of the rare coding polymorphism p.Thr164Ile, while the functional impact of the intensely studied N-terminal coding variants p.Arg16Gly and p.Gln27Glu remains uncertain as they were not associated with any trait in any GWAS published to date.

6 Variants Associated with the β_3-Adrenoceptor

The β_3-adrenoceptor (ADRB3) is expressed in human bladder, parts of the gastrointestinal tract and female genital (for details on expression, see Chapter "Expression Pattern and Species Differences"). The relaxation effect of β_3-agonists is therapeutically exploited for the treatment of overactive bladder syndrome (Michel and Korstanje 2016; Schena and Caplan 2019).

Within the *ADRB3* gene, four non-synonymous variations have been characterized in vitro. In particular the common variation p.Trp64Arg, located at the intracellular end of transmembrane domain 1, was investigated in various candidate-gene studies on cardiac disease, obesity, diabetes, and hyperuricemia. Overall, these studies presume the Arg64-allele as a risk factor specifically for overactive bladder syndrome/hyperuricemia and type 2 diabetes (Ahles and Engelhardt 2014; Michel 2023). However, this conclusion was not validated by

GWAS, which also have been published for the trait "hyperuricemia" and to a great extent for "diabetes": The ADRB3 was not associated with any trait in a single GWAS, i.e., none of the *ADRB3* coding variants or attributed non-coding variants has been reported as a hit in a large cohort.

7 Conclusions

GWAS test thousands of genetic variants across many individual human genomes (and ethnic groups) to identify those statistically associated with a specific trait or disease (Duncan and Brown 2018). Due to the effective suppression of bias and the inherent statistical power, this approach has largely replaced earlier candidate-gene studies. As a result of this paradigmatic change and the rapidly increasing number and scope of GWAS, also much of the earlier literature on adrenoceptor variants has to be scrutinized (Ahles and Engelhardt 2014) leaving us with a sobering number of two trustworthy adrenoceptor coding variants. These two variants are p.Arg389Gly in the β_1-adrenoceptor and the rare p.Thr164Ile variation in the β_2-adrenoceptor.

The genome-wide approach has however added another layer of complexity through the identification of numerous non-coding genetic variants, many of them within regulatory regions of adrenoceptor genes. While the mechanistic basis of the effects assigned to the coding variants appears largely resolved, we are only at the very beginning to understand how non-coding genetic variation determines traits and disease risk. Regarding the adrenoceptor-associated non-coding variants, a first layer of investigation will need to define whether their effect is indeed related to the regulation of expression of the adjacent receptor gene. If so, multiple candidate mechanisms come into play, ranging from altered binding of the transcriptional machinery to the evolving world of non-coding RNAs, many of which are also transcribed from adrenoceptor loci and which we have reported in this chapter.

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Adrenoceptors: Receptors, Ligands and Their Clinical Uses, Molecular Pharmacology and Assays

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Abstract

The nine G protein-coupled adrenoceptor subtypes are where the endogenous catecholamines adrenaline and noradrenaline interact with cells. Since they are important therapeutic targets, over a century of effort has been put into developing drugs that modify their activity. This chapter provides an outline of how we have arrived at current knowledge of the receptors, their physiological roles and the methods used to develop ligands. Initial studies in vivo and in vitro with isolated organs and tissues progressed to cell-based techniques and the use of cloned adrenoceptor subtypes together with high-throughput assays that allow close examination of receptors and their signalling pathways. The crystal structures of many of the adrenoceptor subtypes have now been determined opening up new possibilities for drug development.

Keywords

α-Adrenoceptor · β-adrenoceptor · Adrenoceptor ligands · Affinity · Agonist · Antagonist · Efficacy · Pharmacology · Pharmacological assays

Abbreviations

ADHD	Attention deficit/hyperactivity disorder
AF	Atrial Fibrillation
AR	Adrenoceptor
Bmax	Maximum number of binding sites
BPH	Benign prostatic hyperplasia
cAMP	Cyclic adenosine monophosphate
COPD	Chronic obstructive pulmonary disease
CRE	cAMP response element
CREB	cAMP response element binding protein
Cryo-EM	Cryo-electron microscopy
DAG	Diacylglycerol
DARPin	Designed ankyrin repeat protein
EC_{50}	Concentration required to stimulate a half maximum response in that system
ECAR	Extracellular acidification rate
EGFR	Epidermal growth factor receptor
EL	Extracellular loop
Epac	Exchange protein directly regulated by cAMP
FA	Full agonist
GDP	Guanosine diphosphate
GPCR	G protein-coupled receptor
GRK	G protein receptor kinase
GTP	Guanosine trisphosphate
IA	Inverse agonist
IC	Intracellular loop
IP_3	Inositol trisphosphate
ISA	Intrinsic sympathomimetic activity
ISH	In situ hybridisation
JGA	Juxta glomerular apparatus
Jnk	Jun N-terminal kinase
K_b	Dissociation constant for an antagonist as calculated from parallel shift of an agonist ligand concentration response curve in the absence and presence of a known concentration of the antagonist (e.g. Gaddum equation)
K_d	Dissociation constant = concentration required to bind half of the receptors
K_i	Dissociation constant as calculated from inhibition of another ligand e.g. in a radioligand binding assay (e.g. Cheng-Prusoff equation)

Mab	Monoclonal antibody
MAPK	Mitogen-activated protein kinase
mTORC2	Mammalian target of rapamycin complex 2
NA	Neutral antagonist
NAM	Negative allosteric modulator
nLuc	Nano luciferase
PA	Partial agonist
PAM	Positive allosteric modulator
PIP2	Phosphatidylinositol 4,5-bisphosphate
PK	Pharmacokinetic
PKA	Protein kinase A
PTSD	Post-traumatic stress disorder
SPA	Scintillation proximity assay
TM	Transmembrane domain

1 General Introduction

Adrenoceptors (AR) comprise a group of nine G protein-coupled receptors that are the targets of the endogenous catecholamines adrenaline and noradrenaline. They are divided into 3 subgroups, α_1, α_2 and β that have 3 subtypes in each, namely α_{1A}, α_{1B}, α_{1D}; α_{2A}, α_{2B}, α_{2C}; and β_1, β_2 and β_3 (Altosaar et al. 2019). The canonical signalling pathway utilised by α_1-AR is $G_{q/11}$ coupling to phospholipase C to cause hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) to inositol trisphosphate (IP_3) and diacylglycerol (DAG) with a consequent increase in intracellular Ca^{2+} and activation of protein kinase C (PKC). The α_2-AR subgroup are G_i-coupled to inhibit adenylyl cyclase and reduce the production of cAMP, whereas β-AR are G_s-coupled to stimulate adenylyl cyclase and increase cAMP production (Altosaar et al. 2019). However, many of the AR subtypes can couple to multiple G proteins and may have G protein independent actions and display complex signalling profiles although the physiological and clinical roles of some of these are currently uncertain (Littmann et al. 2015; da Silva Junior et al. 2017; Woo et al. 2019; Proudman and Baker 2021; De Pascali et al. 2022; Proudman et al. 2022a). The receptors are expressed in a very wide variety of cell types, and the pattern of expression determines the type of response observed following stimulation of the sympathetic nervous system. The targeting of AR has produced antagonist and agonist drugs useful for the treatment of many diseases which are currently used for coronary artery disease, hypertension, cardiac arrythmias, heart failure, portal hypertension, hyperthyroidism, migraine, glaucoma, anxiety, benign prostatic hyperplasia (BPH), overactive bladder, post-traumatic stress disorder (PTSD), asthma and chronic obstructive pulmonary disease (COPD), hypotension and shock, anaphylaxis, sedation, drug (e.g. opiate, alcohol, benzodiazepine) withdrawal, attention deficit hyperactivity disorder (ADHD), delirium, nasal decongestion, rosacea and muscle spasm and may have roles in

depression. The drugs currently in use are generally agonists, antagonists or inverse agonists, but there is great interest in the development of biased agonists and allosteric modulators that potentially promise even more selective actions associated with minimalisation of side effects. Most of the data presented in this chapter relates to human receptors (unless otherwise stated) and whilst there are examples highlighted, this is not an exhaustive review of all the published literature.

2 History of Drugs Acting at Adrenoceptors

By the turn of the twentieth century, the pharmacological properties of catecholamines were being described. Oliver, experimenting with organ extracts, discovered that ingestion of sheep adrenal gland caused constriction of the radial artery (Oliver and Schäfer 1894) and subsequently in animal experiments showed that adrenal medullary extract caused vasoconstriction and increased blood pressure and heart rate (Oliver and Schafer 1895). There then began a series of attempts to isolate the active compound culminating in the isolation of crystalline adrenaline by Takamine in 1901 (Yamashima 2003). The history of the development of drugs acting selectively at ARs really began with the work of Sir Henry Dale (Dale 1906). He recognised that stimulation of the sympathetic nervous system could produce a variety of effects including vasoconstriction in some regions and vasodilation in others, contraction or relaxation of smooth muscle, positive inotropic and chronotropic effects in the heart and metabolic changes. Dale showed that the actions of adrenaline were altered by preexposure to ergotoxine, and he interpreted that the change from a vasoconstrictor to a vasodilator response indicated a mixed response under normal conditions and that ergotoxine caused a selective paralysis of myoneural junctions responsible for the vasoconstrictor response. Later Cannon and Rosenblueth (1937) suggested an alternative explanation, namely that nerve terminals released two transmitters sympathin E (excitatory) and sympathin I (inhibitory) an idea that gained support in the 1930s/40s.

However, a landmark publication from Raymond Ahlquist in 1948 (Ahlquist 1948) adopted an approach based on the rank order of potency of a series of natural and synthetic agonists leading to the suggestion that the actions of the sympathetic neurotransmitter adrenaline (later corrected to noradrenaline) were mediated by two groups of receptors – α and β. He postulated that these receptor groups could not be classified purely on the basis of excitatory or inhibitory actions and used the rank order of potency of a series of 6 sympathomimetic amines in a number of assay systems – vasoconstriction, contraction of the uterus and ureters, contraction of the nictitating membrane of the eye, dilatation of the pupil, inhibition of the gut and stimulation of the heart – to define his receptors. He concluded that *most* of the excitatory functions – vasoconstriction, uterine contraction, nictitating membrane contraction, and dilator pupillae were mediated by α receptors – whereas *most* of the inhibitory functions – vasodilation, relaxation of uterine and bronchial smooth muscle – and *one* excitatory function – cardiac stimulation – were mediated by β receptors. This classification also provided an explanation for the actions of