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REVIEW

The rush to adrenaline: drugs in sport acting on the β-adrenergic system

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Athletes attempt to improve performance with drugs that act on the β-adrenergic system directly or indirectly. Of three β-adrenoceptor (AR) subtypes, the β_2-AR is the main target in sport; they have bronchodilator and anabolic actions and enhance anti-inflammatory actions of corticosteroids. Although demonstrable in animal experiments and humans, there is little evidence that these properties can significantly improve performance in trained athletes. Their actions may also be compromised by receptor desensitization and by common, naturally occurring receptor mutations (polymorphisms) that can influence receptor signalling and desensitization properties in individuals. Indirectly acting agents affect release and reuptake of noradrenaline and adrenaline, thereby influencing all AR subtypes including the three β-ARs. These agents can have potent psychostimulant effects that provide an illusion of better performance that does not usually translate into improvement in practice. Amphetamines and cocaine also have considerable potential for cardiac damage. β-AR antagonists (β-blockers) are used in sports that require steadiness and accuracy, such as archery and shooting, where their ability to reduce heart rate and muscle tremor may improve performance. They have a deleterious effect in endurance sports because they reduce physical performance and maximum exercise load. Recent studies have identified that many β-AR antagonists not only block the actions of agonists but also activate other (mitogen-activated PK) signalling pathways influencing cell growth and fate. The concept that many compounds previously regarded as 'blockers' may express their own spectrum of pharmacological properties has potentially far-reaching consequences for the use of drugs both therapeutically and illicitly.
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Abbreviations: AR, adrenoceptor; Gs, stimulatory G-protein; Erk1/2, extracellular-signal regulated kinase; TNFα, tumour necrosis factor α

There has been considerable interest expressed in the use of drugs by athletes to improve performance. As the physiological secretion of adrenaline into the bloodstream in response to stress, fright or physical exercise undeniably affects performance, it is not surprising that a number of categories of drugs that are used by some athletes to alter performance have their effects on the adrenergic system. The main categories of drugs acting on the adrenergic system that have been used acutely include stimulants such as cocaine, amphetamines and ephedrine (see Docherty, 2008), and blockers such as the β-AR antagonists (The World Anti-Doping Code—The 2008 Prohibited List International Standard). Most of the desired effects are produced by these drugs acting directly or indirectly on β-ARs both in the brain and in peripheral tissues. More recently, β-AR agonists have been used as anabolic agents to increase body weight and build muscle strength. This review will examine not only the characteristics of β-ARs, their distribution in the body, their functions and how they are influenced by drugs but also how the variations in receptors between individuals affect drug responses.

The endogenous ligands acting in the adrenergic system are noradrenaline, which is released as a neurotransmitter from the majority of sympathetic nerves, and adrenaline, which is released not only from the adrenal medulla as a circulating hormone but also can act as a neurotransmitter at a few neurons in the central nervous system. Both catecholamines are agonists with broadly similar pharmacology except that noradrenaline displays some selectivity for β_1-ARs compared with β_2-ARs (Alexander *et al.*, 2007). This may be one reason why most of the attention in terms of enhancing performance in sport has fallen on adrenaline. Injection of adrenaline intravenously produces a relatively short-lasting increase in blood pressure and fallen rate and force. The action of adrenaline on blood vessels is complex and broadly reflects the distribution and relative expression of AR subtypes. In vessels where α-ARs predominate (such as

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skin), the dominant effect is vasoconstriction, whereas in those where β-ARs are more numerous (such as heart and skeletal muscle), the dominant effect is vasodilatation. Thus, adrenaline produces an increased cardiac output while at the same time dilating coronary arteries, and it also has a powerful vasodilator effect on blood vessels in skeletal muscle, thus preparing the body for physical activity (Rang *et al*., 2003).

Adrenoceptors

Noradrenaline and adrenaline produce their effects in the body by acting on a group of nine G-protein-coupled receptors termed adrenoceptors (Bylund *et al*., 1994). Of these, the β-ARs are one of the three main types that are further subdivided into three subtypes, which are encoded by distinct genes. The genes are 40–50% similar and encode single polypeptide chains of between 400 and 500 amino acids. The genes encoding β_1-ARs and β_2-ARs are intronless, whereas those encoding the β_3-AR have introns with part of the C terminus encoded by the second exon (Bylund *et al*., 1994). All three receptors possess the characteristic seven hydrophobic transmembrane segments of the prototypical G-protein-coupled receptor, and all of these segments contribute to the binding pocket occupied by the ligand. In addition, they all have N-linked N-terminal glycosylation sites but differ in the C terminus in that the β_3-AR is shorter than the β_1-AR and β_2-AR and does not contain phosphorylation sites for either PKA or G-protein receptor kinases (GRKs). All three subtypes when activated can cause increases in intracellular levels of cAMP, although many recent studies demonstrate that this is not the sole signalling pathway (Lefkowitz *et al*., 2002; Galandrin and Bouvier, 2006). There are selective agonists and antagonists for all three receptor subtypes (Alexander *et al*., 2007).

β_1-Adrenoceptors

β_1-Adrenoceptors are found in many areas of the body including the heart, kidney, white adipose tissue and the brain, where, in particular, high concentrations are found in the pineal gland (Frielle *et al*., 1987) (Table 1). β_1-ARs in the

Table 1 Distribution and function of β-AR subtypes

Tissue or organ with parameter measured	β_1	β_2	β_3
Cardiac—rate, force (Kaumann, 1989)	Increase	Increase	
Cardiac—lusitropic (Kaumann *et al*., 1999)	Increase	Increase	
Lung—bronchi tone (Barnes *et al*., 1983)		Dilate	
Lung—parenchyma tone (Bertram *et al*., 1983)	Relax	Relax	
Lung—mediator release (Undem and Buckner, 1984)		Decrease	
Lung—surfactant production/mucus secretion (Warburton *et al*., 1987)		Increase	
Small coronary arteries—tone (Vatner *et al*., 1986)		Dilate	
Large coronary arteries—tone (Purdy *et al*., 1988)	Dilate		
Skeletal muscle blood vessels—tone (Bowman and Anden, 1981)		Dilate	
Saphenous vein—tone (Ikezono *et al*., 1987)		Dilate	
Cerebral arteries—tone (Edvinsson and Owman, 1974)	Dilate	Dilate	
Sympathetic neurotransmission—activity (Majewski, 1983)		Facilitate	
Parasympathetic neurotransmission—activity (Vermeire and Vanhoutte, 1979)	Inhibit		
CNS—blood pressure, heart rate (Day and Roach, 1974)		Increase	
CNS—inhibitory synaptic transmission (Waterhouse *et al*., 1982)	Facilitate		
CNS—clonidine withdrawal (Jonkman *et al*., 1984, 1998)		Attenuate	
CNS—appetite (Tsujii and Bray, 1992)			Inhibit
CNS—memory (Gibbs and Summers, 2002)		Facilitate	Facilitate
GI tract—gastric, fundus—tone (McLaughlin and MacDonald, 1991)			Relax
GI tract—ileum, colon—tone (Roberts *et al*., 1995; De Ponti *et al*., 1996)			Relax
GI tract—gastric acid (Stevens *et al*., 1986; Canfield and Paraskeva, 1992b)		Suppress	Secrete
GI tract—caecum bicarbonate (Canfield and Abdul-Ghaffar, 1992a)			Secrete
GI tract—pancreas exocrine (Chariot *et al*., 1988)		Secrete	
Genitourinary tract—uterus—tone (Krstew *et al*., 1982)		Relax	Relax
Genitourinary tract—vas deferens—tone (Kenakin, 1982)		Relax	
Genitourinary tract—costo-uterine muscle—tone (Hartley and Pennefather, 1985)		Relax	
Genitourinary tract—bladder detrusor—tone (Oshita *et al*., 1997)		Relax	Relax
Genitourinary tract—corpus cavernosum—tone (Hedlund and Andersson, 1985)	Relax	Relax	
Kidney—renin release (Buhler *et al*., 1975)	Increase		
Eye—aqueous humor formation (Nathanson, 1980)		Increase	
Eye—aqueous humor outflow (Potter, 1981)	Decrease		
Eye—ciliary muscle (Rang *et al*., 2003)		Relax	
Skeletal muscle tremor (Bowman and Anden, 1981)		Increase	
Skeletal muscle—growth, glycogenolysis, speed of contraction (Bowman and Anden, 1981; Rothwell and Stock, 1987)		Increase	
White adipose tissue—lipolysis (Lafontan *et al*., 1983)	Increase	Increase	Increase
Brown adipose tissue—thermogenesis (Nedergaard *et al*., 2007)			Increase
Pancreas—glucagon, insulin secretion (Mariani *et al*., 1971; Schuit and Pipeleers, 1985)		Increase	
Liver—glycogenolysis (Kennedy and Ellis, 1969)		Increase	

Abbreviations: β-AR, β-adrenoceptor; CNS, central nervous system; GI, gastrointestinal.

heart mediate positive chronotropic and inotropic responses; those in the kidney control renin release from the juxtaglomerular apparatus, whereas those in adipose tissue control lipolysis (Brodde, 2008). In the brain, β_1-ARs control the secretion of melatonin from the pineal gland and also appear to have a role in mood alterations (Leonard, 1997). In blood vessels, β_2-ARs have classically been considered to be dominant but in a number of arteries, including the coronary, mesenteric and saphenous, β_1-ARs also mediate vasodilatation (Molenaar *et al*., 1988a, b; Briones *et al*., 2005; Neidhold *et al*., 2007). Studies in β_1-AR knockout mice also suggest a more important role for β_1-ARs in vascular relaxation than was previously supposed (Chruscinski *et al*., 2001).

Selective agonists and antagonists exist or have been developed for the β_1-AR. These include the physiological neurotransmitter noradrenaline, which has modest selectivity for the β_1-AR, and the synthetic agonists xamoterol, RO363 and denopamine (Alexander *et al*., 2007). It is clear that prolonged activation of β_1-ARs has deleterious effects on the heart, as the use of xamoterol as an inotropic agent is associated with increased mortality (Persson *et al*., 1996), and transgenic mice with relatively mild cardiac overexpression of β_1-ARs rapidly develop cardiac failure (Engelhardt *et al*., 1999). Selective β_1-AR agonists are still used as inotropic agents for the acute treatment of cardiac failure but only for short periods under carefully controlled conditions. Chronic activation of cardiac β_1-ARs is associated with apoptosis of cardiomyocytes. This may be an important factor in the greatly increased risk of heart failure in people taking cocaine or amphetamines for sporting or other reasons and in the great success associated with the use of β-AR antagonists for the treatment of cardiac failure. Selective β_1-AR antagonists include CGP20712A, betaxolol and atenolol, and some of these drugs are important therapeutic agents (Alexander *et al*., 2007).

β_2-Adrenoceptors

β_2-Adrenoceptors have an even wider distribution than β_1-ARs and control a wide variety of functions in the body (Brodde, 2008). They also mediate positive inotropic and chronotropic effects in the heart. The human heart, unlike many other animal species, has a significant (up to 40% of total β-ARs) population of β_2-ARs (Buxton *et al*., 1987) and these are well coupled to cAMP production. In the lung, the activation of β_2-ARs causes not only the well-recognized bronchodilator effect but also reduces the release of bronchoconstrictor mediators and increases the release of surfactants and mucus (Rang *et al*., 2003). β_2-ARs mediate a powerful vasodilator effect in small coronary blood vessels (Vatner *et al*., 1986) and skeletal muscle blood vessels (Guimaraes *et al*., 2001). Other effects seen in skeletal muscle include increased growth and speed of contraction, glycogenolysis and tremor (Bowman and Anden, 1981). Skeletal muscle tremor is a well-recognized side effect of adrenaline and β_2-AR agonists, and it probably results from increased muscle spindle discharge and effects on muscle contraction leading to less well-coordinated contraction of muscle fibres (Bowman and Anden, 1981). In the pancreas, there is an increase in both insulin and glucagon secretion, and glycogenolysis in the liver is increased (Rang *et al*., 2003).

Owing to their important role as anti-asthmatic drugs, there has been a huge range of selective β_2-AR agonists developed. Although adrenaline has strong actions at β_2-ARs, its action at all other AR subtypes and short half-life limit its therapeutic usefulness that is confined largely to the treatment of anaphylactic shock. Salbutamol and terbutaline are selective β_2-AR agonists given by aerosol to produce bronchodilatation in asthma. They are highly effective and have a medium duration of action ($t_{1/2} \sim 4$ h). Salmeterol and formoterol are more recently developed long-acting β_2-AR selective agonists. Clenbuterol appears to have similar pharmacology to the other long-acting β_2-AR agonists and is used as an anti-asthmatic drug in many countries, but it has been widely used illicitly in sport as an anabolic agent (The World Anti-Doping Code—The 2008 Prohibited List International Standard). Unlike β_1-ARs, chronic activation of β_2-ARs does not appear to have major deleterious effects apart from possible downregulation and desensitization. The experience in humans is supported by studies in transgenic mice where animals expressing extraordinarily high levels of β_2-ARs do not develop heart failure, even though they have high heart rates (Milano *et al*., 1994). Thus, even though β_1-ARs and β_2-ARs share cAMP accumulation as a common signalling mechanism, there must be major differences to explain the contrasting effects on cardiac myocytes. All antagonists acting at β_2-ARs that are approved for human therapeutic use, such as propranolol, are non-selective and block both β_1-ARs and β_2-ARs. Selective compounds such as ICI118551 are available for experimental use but have never been developed for human use. β-AR antagonists are widely used therapeutically to treat heart failure, high blood pressure, cardiac arrhythmias, angina and glaucoma, but they have also been used illicitly in sport to reduce tremor, particularly in pistol shooting and motor racing (The World Anti-Doping Code—The 2008 Prohibited List International Standard).

β_3-Adrenoceptors

The third β-AR subtype has been relatively less studied and exploited therapeutically than β_1-ARs or β_2-ARs. Although pharmacological studies in the gut (Furchgott, 1972) and adipose tissues (Zaagsma and Nahorski, 1990) suggested the presence of an additional β-AR subtype, based on the inability of conventional β-AR antagonists to block responses to β-AR agonists, this was not widely accepted until the development of novel atypical β-AR agonists, such as BRL37344 (Arch *et al*., 1984; Wilson *et al*., 1984), and the cloning of the human β_3-AR (Emorine *et al*., 1989). The β_3-AR is widely distributed in the gut, brain, genitourinary tract, uterus and white and brown adipose tissue (McLaughlin and MacDonald, 1991; Summers *et al*., 1995; Evans *et al*., 1996). The presence of the receptor in fat stimulated activity in the pharmaceutical industry to develop anti-obesity and anti-diabetic β_3-AR agonists that has so far been a success in rodents but not in humans (Arch *et al*., 1984; Arch, 2000).

Selective β_3-AR agonists may also have utility for the treatment of irritable bowel syndrome and as tocolytic agents in threatened abortion where their resistance to desensitization would be an advantage (Rouget *et al*., 2005; Bardou *et al*., 2007).

There are a number of selective β_3-AR agonists available, although these demonstrate wide species variations in their efficacy. Many of the earlier compounds, such as BRL37344 and CL316243, are highly effective agonists in rodents but have low, if any, efficacy at the human β_3-AR (Arch, 2000), whereas compounds such as L755507 potently activate both human and rodent receptors (Fisher *et al*., 1998; Sato *et al*., 2007). Selective antagonists are harder to find, and although SR59230A has been described as such (Manara *et al*., 1996), many studies show that whereas it has reasonable potency at the β_3-AR, it blocks β_1-ARs and β_2-ARs at similar concentrations (Arch, 2000) and also blocks α_1-ARs (Briones *et al*., 2005). Nevertheless, it has proved a useful tool experimentally in studies where β_3-ARs are the dominant receptor expressed (Sato *et al*., 2007). Another selective β_3-AR antagonist, L748337, competitively blocks responses to agonists in Chinese hamster ovary cells expressing human β_3-ARs and also inhibits the lipolytic response to the β_3-AR agonist L742791 in isolated non-human primate adipocytes (Candelore *et al*., 1999). Currently, there are no accepted therapeutic or sporting uses for β_3-AR agonists or antagonists.

Functional domains of β-ARs

All three β-AR subtypes are classical biogenic amine-liganded G-protein-coupled receptors (Bylund *et al*., 1994). The binding pocket lies in the core of the receptor formed by the transmembrane domains with Asp^{113} (for the β_2-AR) forming a salt bridge with the amino group found in all β-AR ligands, and Ser^{204} and Ser^{207} in TM5 forming hydrogen bonds with the *meta*- and *para*-hydroxyls on the catechol ring of the catecholamines. The Phe^{290} in TM6 is believed to stabilize the structure, and conformational changes in this region are believed to be important for receptor activation (Strosberg and Gerhardt, 2000). Although these features are common to all subtypes, there are clear differences that explain the subtype specificity of agonists and antagonists. The binding pocket of the β_3-AR has been suggested to be less encumbered than either the β_1-AR or β_2-AR allowing β_3-AR agonists (many of which are antagonists at β_1-ARs and β_2-ARs) to interact with additional side chains in TM7, TM1 and TM2. A major difference between the three receptor subtypes lies in the C-terminal tail in that the β_1-AR and particularly the β_2-AR contain phosphorylation sites for PKA and GRK that are important for receptor desensitization. In contrast, the β_3-AR C terminus contains no PKA and few GRK sites, and it is resistant to desensitization (Strosberg and Gerhardt, 2000).

Signal transduction pathways

Classically, β-ARs couple through Gs to activate adenylate cyclase and increase intracellular levels of cAMP (Rang *et al*., 2003; Alexander *et al*., 2007). However, in recent years, the field of ARs has developed many new layers of complexity, and the receptors and their associated G-proteins have been shown to influence an almost bewildering array of signalling mechanisms (Lefkowitz *et al*., 2002). The third intracellular loop of ARs is recognized as important for G-protein coupling, but two additional factors that are emerging as important determinants of the signalling pathways utilized are protein-binding motifs in the C-terminal region of ARs and the agonist used to activate the receptor. Comparison of β_1-ARs and β_2-ARs shows that the β_1-AR couples to Gs and does not internalize well, whereas the β_2-AR shows robust internalization and can couple to both Gs and Gi. The β_1-AR has a PDZ domain (ESKV) at the C terminus that determines binding to scaffolding proteins in the cell membrane (Xiang *et al*., 2002). A mutant β_1-AR with the PDZ motif mutated (EAAA) internalizes and couples to Gi like the β_2-AR. The β_2-AR contains another PDZ motif (DSPL) at the C terminus that controls receptor recycling and coupling to Gi (Xiang and Kobilka, 2003). Another factor that is an important influence on coupling to signalling pathways is the phosphorylation state of the receptor. For example, phosphorylation of β_2-AR switches coupling from Gs to Gi (Lefkowitz *et al*., 2002).

Although activation of the human β_3-AR increases cAMP accumulation, the receptor also couples to Gi, modulates adenylyl cyclase activation and causes stimulation of the Erk1/2 mitogen-activated PK pathway (Gerhardt *et al*., 1999; Soeder *et al*., 1999). Unlike the β_1-ARs and β_2-ARs, the coupling to Gi does not involve receptor phosphorylation or internalization, as the β_3-AR does not display putative phosphorylation sites in the C terminus and the receptor does not internalize.

The concept of ligand-directed signalling is a topic of immense interest and it has been explained in terms of the ability of ligands to form distinct conformational complexes with the receptor (Kenakin, 2003; Urban *et al*., 2007), resulting in qualitatively different responses (Swaminath *et al*., 2005). Several recent studies describe the activation of Erk1/2-phosphorylation by drugs classified as β-AR antagonists in cells expressing β_1-AR or β_2-AR (Azzi *et al*., 2003; Baker *et al*., 2003). It has been demonstrated that a wide range of β-AR ligands have complex efficacy profiles for cAMP generation and Erk1/2 activation at both β_1-ARs and β_2-ARs (Galandrin and Bouvier, 2006). In addition, recent studies in β_3-ARs showed that SR59230A acts as a classical competitive antagonist for cAMP accumulation, but it is a powerful agonist for Erk1/2 and p38 mitogen-activated PK activation (Sato *et al*., 2007). These studies suggest that many compounds previously thought to interact with receptors to block the actions of agonists (as antagonists or inverse agonists) may, in fact, have the ability to selectively activate discrete pathways by inducing or interacting with particular conformations of the receptor. The concept that many compounds previously regarded as 'blockers' may express their own spectrum of pharmacological properties has potentially far-reaching consequences for the use of drugs both therapeutically and illicitly.

Drugs used in sport acting directly on β-ARs

β_2-AR agonists: ergogenic effects

β_2-Adrenoceptor agonists are used therapeutically for the treatment of asthma and exercise-induced asthma owing to their potent bronchodilator activity. However, the use of β_2-AR agonists by athletes is prohibited under the World Anti-Doping Agency (WADA) World Anti-Doping Code, with the exception of formoterol, salbutamol, salmeterol and terbutaline, when administered by inhalation if the athlete has been granted a therapeutic use exemption (The World Anti-Doping Code—The 2008 Prohibited List International Standard). Actions and potential actions of β-agonists that could lead to the enhancement of performance in sport include bronchodilation, anabolic and anti-inflammatory actions.

β_2-AR agonists: bronchodilator actions

Numerous studies have investigated the effect of inhaled β_2-AR agonists in non-asthmatic athletes. However, although all demonstrated a bronchodilator action (Morton and Fitch, 1992; Norris *et al*., 1996; Larsson *et al*., 1997; Goubault *et al*., 2001), there were varying effects on physical performance. After inhalation of therapeutic doses of salbutamol, a limited number of studies have reported an increase in exercise performance (Bedi *et al*., 1988; Signorile *et al*., 1992; van Baak *et al*., 2004), whereas the majority of studies failed to demonstrate an ergogenic effect (Morton and Fitch, 1992; Norris *et al*., 1996; Goubault *et al*., 2001). Furthermore, although a small increase in endurance cycling performance was reported after inhalation of a supratherapeutic dose (800 μg) of salbutamol (van Baak *et al*., 2004), a similar dose had no effect on endurance performance in non-asthmatic triathletes (Goubault *et al*., 2001). The few studies that investigated the effects of other inhaled β_2-AR agonists have also failed to demonstrate an effect on performance despite an improvement in lung function (Larsson *et al*., 1997; Carlsen *et al*., 2001; Riiser *et al*., 2006). Overall, the acute use of the current inhaled β_2-AR agonist anti-asthma medications by non-asthmatic or asthmatic athletes seems unlikely to provide any improvement in performance in addition to that provided by the control of their condition. Of more concern is the adequacy of the tests required to obtain a therapeutic use exemption, as they may be inappropriate for diagnosing exercise-induced asthma under the conditions experienced in competition (Naranjo Orellana *et al*., 2006).

One situation in which improved lung function afforded by inhaled β_2-AR agonists has anecdotally been of benefit is the administration of these drugs to swimmers immediately prior to a race, when the increase in oxygen intake could increase the time before surfacing after a dive, thus saving fractions of seconds off race times (Verroken, 2005). However, this has been countered by the governing body that requires athletes to move away from the competition area during treatment and allow time for recovery before competing.

β_2-AR agonists: anti-inflammatory actions

There has been a great deal of interest in possible anti-inflammatory actions of β_2-AR agonists driven mainly by their widespread use for the treatment of asthma, now overwhelmingly regarded as an inflammatory disease of the airways. Asthma is associated with the release of a wide variety of inflammatory mediators, including TNFα, eotaxin and a wide variety of interleukins and other cytokines (for details, see Barnes and Drazen, 2002; Broadley, 2006). Earlier *in vitro* studies suggested that β_2-AR agonists, including salbutamol, salmeterol and formoterol, were capable of inhibiting inflammatory mediator release from mast cells (Broadley, 2006). Studies *in vivo*, however, are less convincing and many of the effects could be explained by the bronchodilator effects of β_2-AR agonists. Although salmeterol, in particular, affects cell adherence and chemotaxis, the release of inflammatory mediators does not appear to be influenced. The current consensus is that β_2-AR agonists do not have direct anti-inflammatory properties. However, there is convincing evidence from both basic and clinical studies that the use of β_2-AR agonists in combination with inhaled steroids provides a control of asthma symptoms that is far better than with the use of either agent alone, suggesting synergism (Nelson, 2005). In a recent study, in a human bronchial epithelial cell line, β_2-AR agonists caused an enhancement of the level of glucocorticoid response element-dependent transcription by corticosteroids to levels 2–3 times greater than that achieved by corticosteroids alone (Kaur *et al*., 2008). This was a class action of β_2-AR agonists with similar effects being produced by salbutamol, salmeterol or formoterol in combination with dexamethasone, budesonide or fluticasone. In the clinical treatment of asthma, the most commonly used glucocorticoids are budesonide, fluticasone, mometasone, triamcinolone and flunisolide, and they have been selected for their high levels of first pass metabolism to minimize systemic effects. However, systemic effects such as growth suppression, reduction of bone density, changes in metabolism and suppression of the pituitary–adrenal axis are not uncommon, particularly in children (Topliss *et al*., 2003; Nieto *et al*., 2007). These are normally minimized by adjustment of the dose of corticosteroids. However, as combinations of long-acting β_2-AR agonists and inhaled corticosteroids are now being widely used for the treatment of asthma, a variety of patients including athletes may experience systemic effects, particularly if doses higher than the normal therapeutic dose are administered.

β_2-AR agonists: anabolic effects

Although there is little evidence that the inhalation of therapeutic doses of β_2-AR agonists enhances performance, the anabolic effects on muscle could potentially be observed if very high doses were inhaled, or if another route of administration was used. Anabolic effects have been studied particularly for clenbuterol, a long-acting β_2-AR agonist that is licensed for the treatment of asthma in a limited number of countries (but not including the United Kingdom, the United States or Australia). It is of interest that clenbuterol was originally developed as a non-steroidal anabolic agent to improve the conversion rate (or efficiency of converting food into body mass) in animals.

The anabolic effects of oral β_2-AR agonists have been well documented in animals (Zeman *et al*., 1988; Moore *et al*., 1994; Ryall *et al*., 2006) and are associated with an increase in skeletal muscle protein, largely due to the inhibition of protein degradation (Reeds *et al*., 1986; Yang and McElligott, 1989; Maltin *et al*., 1993). In addition to the increase in muscle mass, β_2-AR agonists also decrease body fat (Yang and McElligott, 1989), hence their classification as 'repartitioning agents'. The increase in muscle mass is associated with an increase in muscle force production (Zeman *et al*., 1988; Dodd *et al*., 1996). The β_2-AR agonist-induced skeletal muscle hypertrophy is evident in fast- (Type II) and slow- (Type I) twitch muscles (Zeman *et al*., 1988; Dodd *et al*., 1996), although in some muscles a change in the proportion of fast- to slow-twitch fibres may occur (Zeman *et al*., 1988; Baker *et al*., 2006; Burniston *et al*., 2007). However, whether this is accompanied by an increase in exercise performance is less clear. It has been reported that there was no effect of clenbuterol on exercise performance in rats (Torgan *et al*., 1995; Murphy *et al*., 1996). In contrast, it has been reported that there is a decrease in exercise performance (Ingalls *et al*., 1996) and a decrease in sprint and swimming performance after the administration of clenbuterol to mice (Duncan *et al*., 2000). In the latter study, three mice died of sudden cardiac failure and clenbuterol had a deleterious effect on cardiac muscle (Duncan *et al*., 2000). Other studies have shown an increase in fatigue associated with these anabolic effects (Burniston *et al*., 2007).

Another β-AR agonist on the WADA list of banned substances is zilpaterol. Like clenbuterol, this was introduced as a growth promoter in cattle and is used for this purpose in South Africa and Mexico. From the limited information available, it has a similar pharmacological profile to clenbuterol but is less potent and has little chemical resemblance to isoprenaline, salbutamol, formoterol and clenbuterol, which are all phenylethanolamines (Verhoeckx *et al*., 2005). Although little scientific work has been performed on the anabolic properties of zilpaterol in humans, it is widely touted as an anabolic agent by bodybuilding forums.

The effects of β_2-AR agonists on muscle strength and mass, and physical performance have been shown to be dependent on the route of administration. In rats, intraperitoneal injections of salmeterol and formoterol cause muscle hypertrophy (Moore *et al*., 1994; Ryall *et al*., 2006), but much higher doses of oral salmeterol are required to produce a similar effect (Moore *et al*., 1994). It was suggested that the lower potency of orally administered salmeterol reflects its low oral bioavailability (Moore *et al*., 1994), which is also a common feature with other agonists including salbutamol and may decrease the abuse potential of these agents (Moore *et al*., 1994). In humans, there is little evidence that an increase in physical performance occurs after inhalation of salbutamol (Meeuwisse *et al*., 1992; Morton and Fitch, 1992), salmeterol (Morton *et al*., 1996), formoterol (Carlsen *et al*., 2001) or terbutaline (Larsson *et al*., 1997). In contrast, however, oral administration of salbutamol has been shown to have a positive effect on performance (Moore *et al*., 1994; Caruso *et al*., 1995; van Baak *et al*., 2000). A single oral dose of salbutamol increased isokinetic muscle strength and endurance performance in healthy male volunteers (van Baak *et al*., 2000), although these ergogenic effects were not evident in 4 (of 16) participants who experienced adverse side effects, including nausea, dizziness and nervousness. The anabolic activity of β_2-AR agonists is dependent on the receptor density of particular muscles (Beermann, 2002), and as chronic administration of β_2-AR agonists is associated with a receptor downregulation (Kim *et al*., 1992; Johnson, 2006), this may also limit the effectiveness of these agents.

Adverse actions of β_2-AR agonists

The β_2-AR agonists were originally developed to specifically target the bronchial β_2-ARs and to have minimal effects on the heart. However, it has subsequently been established that the human heart has a significant population of β_2-ARs (Buxton *et al*., 1987). Thus, tachycardia that is a common feature of the inhalation of β_2-AR agonists probably results from a combination of a direct effect on the heart and vasodilation of blood vessels, particularly those in skeletal muscle. This, together with the hypokalaemia resulting from the activation of Na^+/K^+ ATPase in skeletal muscle, may provoke cardiac arrhythmias.

The metabolic effects of activation of β_2-ARs include increases in plasma glucose levels resulting from glycogenolysis predominantly in the liver (Broadley, 2006). This may be of advantage to athletes but not to diabetic patients. Recent studies have also established that β_2-ARs mediate glucose uptake into skeletal muscle (Nevzorova *et al*., 2006) by an insulin-independent mechanism, although it is not clear whether this mechanism operates in humans.

Although bronchodilatation is the key action of β_2-AR agonists for the treatment of asthma and also potentially in sport, it has also been suggested that the dilated airway allows greater penetration of allergens into the airways and may therefore increase the inflammatory response (Broadley, 2006). In a sporting context, this may be particularly important in venues where there are high levels of atmospheric particulate matter.

β-AR antagonists

As noted earlier, β-AR antagonists (β-blockers) are used therapeutically for the treatment of cardiovascular diseases, such as cardiac failure, angina and hypertension, where the beneficial effects result mainly from actions on the heart. In sport, the main ergogenic effect of β-AR antagonists relates to their ability to decrease heart rate and hand tremor (Bowman and Anden, 1981) that is likely to be of benefit in sports that require steadiness and accuracy (for example, archery, shooting). In addition, their actions to relieve the symptoms of anxiety, manifested as tachycardia and skeletal muscle tremor (Reilly, 2005), may enhance performance in some sports. These potential ergogenic effects of β-AR antagonists have led to their prohibition in competition in a number of sports, including archery, billiards, boules, gymnastics, shooting and modern pentathlon disciplines

involving shooting (The World Anti-Doping Code—The 2008 Prohibited List International Standard). They are also prohibited out of competition for archery and shooting.

However, β-AR antagonists are unlikely to be of benefit in endurance sports, as they reduce physical performance and maximum exercise load (Kaiser *et al*., 1985). Antagonism of β-ARs is associated with decreased anaerobic capacity (Rusko *et al*., 1980) owing to the effects on metabolism such as decreased glycogenolysis in skeletal muscle and decreased lipolysis (Head, 1999). Furthermore, the effect of endurance training on cardiovascular parameters (decreased heart rate and increased stroke volume for work of a given intensity together with the restriction of muscle blood flow due to blockade of β_2-AR-mediated vasodilatation) most likely interferes with an athlete's ability to compensate for a β-blocker-induced decrease in heart rate (Head, 1999). The effects of non-selective β-AR antagonists on exercise performance are more pronounced than those of cardioselective antagonists (Lundborg *et al*., 1981; Laustiola *et al*., 1983).

Drugs used in sport acting indirectly on β-ARs

An additional group of agents that have the potential to add to performance in sport practice and competition and are prohibited by the WADA World Anti-Doping Code (The World Anti-Doping Code—The 2008 Prohibited List International Standard) include indirectly acting sympathomimetic agents and noradrenaline reuptake blockers (see Smith and Perry, 1992 and Docherty, 2008). WADA classifies these agents as stimulants, and specific agents that are prohibited include amphetamine, cocaine, dimethylamphetamine, ephedrine, mephenteramine, methamphetamine, methylenedioxymethamphetamine, methylephedrine and methylphenidate (see also Docherty, 2008). Interestingly, pseudoephedrine was removed from the banned list in 2004 with the proviso that WADA would monitor the presence of pseudoephedrine in athletes. Problematically, however, the use of high concentrations of pseudoephedrine can lead to high concentrations of cathine (pseudonorephedrine), a metabolite of pseudoephedrine; cathine is active as an indirectly acting sympathomimetic (see Table 2) and is prohibited when its concentration in urine is greater than $5\,mg\,mL^{-1}$. Other agents that are monitored but not prohibited include phenylephrine, phenylpropanolamine and synephrine (The World Anti-Doping Code—The 2008 Prohibited List International Standard). Ephedrine appears to be tolerated by WADA at about therapeutic levels but prohibited when its concentration in urine is greater than $5\,mg\,mL^{-1}$ (Tseng *et al*., 2006).

The potential central psychostimulant benefits of these agents include the reduction of fatigue, increased concentration and alertness, whereas the potential peripheral physiological advantage of these agents includes the indirect activation of β-ARs and the stimulation of cardiovascular function and metabolic activity. The relative potencies of several of these compounds on synaptosomal noradrenaline uptake and release are listed in Table 2 (Rothman *et al*., 2001, 2003; see also Docherty, 2008).

Table 2 Relative potency on noradrenaline release of indirectly acting sympathomimetics illegally used in sport

Compound	*EC_{50} (nM)*
S(+)methamphetamine	12.3
S(+)amphetamine	7.1
S(−)methcathionine	13.1
S(−)cathinone	12.4
(−) ephedrine[a]	43.1
(+) ephedrine	218
(−) pseudoephedrine[b]	4092
(+) pseudoephedrine[b]	224
(−) pseudonorephedrine[c]	30.1
(+) pseudonorephedrine[d]	15
(−) norephedrine	42.1
(+) norephedrine	137

The phenylpropanolamines (ephedrine, pseudoephedrine and pseudonorephedrine) are less active on dopamine uptake and release, and largely inactive on 5-HT uptake and release, whereas the phenylisopropylamines (amphetamines) are much less selective (Rothman *et al*., 2001, 2003).
[a,b](−) ephedrine and (−) pseudoephedrine are the pharmacopoeial preparations.
[c]Also known as (−) cathine.
[d]Also known as (+) cathine.

Interestingly, there is some propensity for some of these agents, notably ephedrine, to act directly at β-ARs (Vansal and Feller, 1999; Cheng *et al*., 2001).

Ergogenic effects of ephedrine, pseudoephedrine, amphetamine and related compounds

There is a perception that these compounds have direct ergogenic effects on sport performance and that they will improve aerobic performance and endurance, reduce muscle fatigue and increase strength. There have been a number of studies that have examined these agents in sport with variable outcomes, and the majority of studies fail to provide substantive evidence for such direct effects. A recent randomized controlled clinical trial showed that pseudoephedrine failed to affect ergometer cycling time trial performance, isometric contraction and time to fatigue in athletes (Gillies *et al*., 1996), with similar negative findings using a variety of measures for strength and fatigability being reported in athletes for pseudoephedrine and phenylpropanolamine (Chester *et al*., 2003); pseudoephedrine (Hodges *et al*., 2003) and ephedrine (Sidney and Lefcoe, 1977); and in healthy individuals for pseudoephedrine (Chu *et al*., 2002). In contrast, in a similarly controlled trial (Gill *et al*., 2000), pseudoephedrine significantly improved specific performance variables in exercise, including maximum torque in knee extension and peak power during cycling performance. In another positive study, improvements in muscle strength were noted for amphetamine (Chandler and Blair, 1980). The mechanism(s) underlying these effects are, however, unclear; in *in vitro* studies, high concentrations ($\sim 10^{-4}$ M) of ephedrine and pseudoephedrine have direct inhibitory effects at the motor endplate rather than any facilitatory effect (Sieb and Engel, 1993; Milone and Engel, 1996). In a further study (Hodges *et al*., 2006), pseudoephedrine ($2.5\,mg\,kg^{-1}$)

significantly improved 1500 m time trial performance in athletes by a modest 2.1%. Interestingly, these authors suggested that this effect was likely to be related to the psychostimulant effects of pseudoephedrine rather than any peripheral ergogenic effect (see below).

Ephedrine has additionally been purported to have 'fat burning' activity; however, a meta-analysis examining the efficacy of ephedrine (in doses ranging from 20–150 mg day^{-1}) on weight loss suggests that this effect is small, with a short-term loss of approximately 0.9 kg $month^{-1}$ more than placebo (Boozer *et al*., 2002; Shekelle *et al*., 2003).

Although the direct ergogenic benefit of these agents may be doubtful, they do have the potential for mediating indirect ergogenic effects by the release of endogenous amines (see Docherty, 2008) that affect both cardiovascular and respiratory functions (Drew *et al*., 1978); specifically, the question is: do these agents enhance performance in sport practice and competition by stimulating cardiovascular performance to provide sufficient O_2 to working muscles? Some studies do indeed show significant increases produced by pseudoephedrine (a single dose of 180 mg) on FEV_1 (forced expiratory volume per s) and FVC (forced expiratory vital capacity) as well as significant increases in heart rate (Gill *et al*., 2000). However, other studies failed to show any effect of pseudoephedrine on respiratory or cardiovascular function (Bright *et al*., 1981; Bell *et al*., 1998). In a recent randomized controlled trial, non-professional cyclists were given placebo, 1 or 2 mg kg^{-1} pseudoephedrine, 0.33 or 0.66 mg kg^{-1} phenylpropanolamine and exercise performance was tested on a cycle ergometer. In both cases, both pseudoephedrine and phenylpropanolamine not only failed to affect exercise performance but, more importantly, had little or no effect on peak systolic, diastolic or peak pulse pressures (1 mg kg^{-1} but not 2 mg kg^{-1} increases systolic pressure by 10 mm Hg) or on maximum oxygen uptake (VO_{2max}; Swain *et al*., 1997). In a study on non-athletes, a single dose of 40 mg of ephedrine did not significantly improve oxygen consumption, respiratory efficiency or ventilation, or significantly alter heart rate or systolic blood pressure on cycle ergometer testing (DeMeersman *et al*., 1987). This was supported by a subsequent study (Clemons and Crosby, 1993) where 60 mg pseudoephedrine also failed to alter these key parameters during exercise tests. These data point to the likelihood that these agents, at these doses, have an inconsistent effect at best, on improving cardiorespiratory function potentially mediated via β-ARs activated by endogenously released amines. An important corollary was that the doses used, although said to approximate to the standard therapeutic dose, were in fact quite variable.

The indirectly acting sympathomimetic amines have ergogenic potential mediated via their psychostimulant effects. The most potent psychomotor agents include amphetamine, related congeners and cocaine (see also Docherty, 2008). The effects of these agents on mood, alertness, concentration and perception of fatigue are mediated by the elevation of brain levels of dopamine and noradrenaline. There are few controlled studies with amphetamines and cocaine in athletes; anecdotally, the suggestion is that the perception of improvement may not be matched by actual performance (Eichner, 1993; Avois *et al*., 2006). Discussions have largely centred on the possibility that any benefit may be short-term 'power' performance rather than 'endurance' performance (Bohn *et al*., 2003). Interestingly, in a study examining the effects of cocaine (12.5 and 20 mg kg^{-1}) in rats, the contrary was suggested, with cocaine additionally accelerating glycogen degradation and lactate accumulation during exercise (Braiden *et al*., 1994). There have been several studies examining the potential psychostimulant effects of pseudoephedrine and ephedrine. DeMeersman *et al*. (1987) asked subjects to rate their level of exertion in an attempt to quantify the potential psychostimulant effect of ephedrine on perceived exercise performance. Subjects given ephedrine did not report any difference from those given placebo in terms of their perceived exertion level. In a similar study, both pseudoephedrine (60 mg) and phenylpropanolamine (25 mg) failed to significantly change the perceived exertion rating (Chester *et al*., 2003). This suggests that the sense of euphoria may provide the illusion of better performance when, in actuality, performance was not improved or was impaired.

Factors affecting actions of drugs at β-ARs

Desensitization

Many G-protein-coupled receptors display desensitization in response to continuous exposure to agonists (Krupnick and Benovic, 1998; Ferguson, 2001). The three β-AR subtypes are no exception to this rule but differ with regard to the mechanisms involved. The desensitization of responses involves three distinct stages: receptor phosphorylation, internalisation and downregulation. Receptor phosphorylation can occur to PKA (cAMP-dependent protein kinase), PKC or GRKs and begins within seconds of agonist exposure (Luttrell, 2005). Because phosphorylated receptors uncouple from G-proteins, this causes impairment of signalling and desensitization (Krupnick and Benovic, 1998). PKA and PKC target consensus sites in the C terminus or intracellular loops of β_1-ARs or β_2-ARs and cause phosphorylation of specific serine or threonine residues. As PKA and PKC can be activated after stimulation of a variety of G-protein-coupled receptors, this type of desensitization is termed heterologous and can be triggered by low receptor occupancy (Luttrell, 2005). GRKs target serine and threonine residues in the C terminus of agonist-occupied receptors, and this type of desensitization is therefore homologous and is associated with high receptor occupancy (Luttrell, 2005). The susceptibility of the three subtypes to this form of desensitization is directly related to the number of domains available for phosphorylation in each receptor. Thus, the β_2-AR, β_1-AR and β_3-AR have, respectively, 2, 1 and 0 PKA consensus phosphorylation sites and 13, 10 and 3 potential GRK phosphorylation sites. Not surprisingly, given the paucity of phosphorylation sites, the β_3-AR does not display desensitization associated with phosphorylation (Strosberg and Gerhardt, 2000).

Internalization of receptors occurs within minutes of agonist exposure and involves the interaction between GRK-phosphorylated receptors and the cytosolic protein β-arrestin. The receptor/β-arrestin complexes accumulate in clathrin-coated pits, which are pinched off by another protein dynamin to form endosomal vesicles. The inter-

nalized receptors do not signal and may be recycled to the cell membrane or undergo degradation (Luttrell, 2005). β_2-ARs are the most susceptible to this process, whereas β_1-ARs are more resistant and β_3-ARs do not internalize (Strosberg and Gerhardt, 2000).

Continuous long-term exposure of β-ARs to agonists results in the downregulation of mRNA and receptor protein. The β_2-AR is more susceptible than the β_1-AR, whereas the β_3-AR initially shows marked downregulation, but even in the continued presence of agonist recovers to basal levels within 24 h (Bengtsson *et al*., 1996).

Thus, all three β-AR subtypes can show desensitization, mediated by a variety of mechanisms, in response to agonist exposure. Clearly, this can have important implications for the chronic use of β_2-AR agonists to improve performance, given that the β_2-AR, the subtype that theoretically has the greatest ergogenic potential, is also the subtype most susceptible to desensitization. Whether desensitization is a problem in practice is inconclusive. Historically, β_2-AR agonists have largely been used episodically to treat acute episodes of asthma; they are relatively short acting ($t_{1/2} = 3$–6 h), and therefore desensitization is not usually a problem. Long-term use of fenoterol in New Zealand was associated with increased asthma mortality (Pearce *et al*., 1989), and more recent studies suggest that treatment with the long-acting β_2-AR agonist formoterol causes a desensitization of bronchodilator responses to salbutamol (Haney and Hancox, 2005). However, desensitization as a potential problem is recognized on many of the websites describing the use of β_2-AR agonists (particularly clenbuterol) as 'repartitioning agents'.

Genetic factors affecting responses to drugs acting at β-ARs

Genetic polymorphisms are subtle changes in the genetic sequence that can result in minor differences in the structure of the resulting proteins. Polymorphisms in critical areas of receptors for drugs and hormones can either alter the ability of these compounds to bind to receptors or change the ability of receptors to elicit their responses to stimulation. Single nucleotide polymorphisms can result in amino-acid substitutions, ablation or creation of restriction enzyme recognition sites resulting in restriction fragment length polymorphisms, or they may be 'silent' owing to redundancy in the genetic code where multiple codons encode the same amino acid. Polymorphisms in genes coding for receptors can have significant effects on the function of the resulting receptor protein, influencing receptor signalling, receptor desensitization and sequestration, and binding of agonists and antagonists. Polymorphisms in the upstream non-coding region or in adjacent genes may result in altered levels of receptor expression.

β_1-AR polymorphisms

A number of polymorphisms of the β_1-AR have been identified with seven of these single nucleotide polymorphisms resulting in amino-acid substitutions that result in 11 different genotypes (Kirstein and Insel, 2004; Brodde, 2008). Two of the more common and interesting polymorphisms are the Gly389Arg and Ser49Gly variants (Table 3). Up to 46% of the population have the Gly389Arg mutation and up to 28% the Ser49Gly variant, but note the marked inter-ethnic differences (Table 3). Functional studies have been performed on cells expressing the Gly389 and Arg389 receptor variants and have found that Arg389 form couples more efficiently to Gs and adenylate cyclase (Kirstein and Insel, 2004; Brodde, 2008). In the Gly49 variant, there is a higher basal and agonist-stimulated activity and greater downregulation than the Ser49 variant. Given these differences, a number of studies have carried out population-based analyses to search for a possible association between

Table 3 Polymorphisms of β-AR subtypes that influence responsiveness or receptor regulation in response to activation by directly or indirectly acting agonists

Mutation	*Substitution*	*Functional changes*	*Allele frequency by race/ethnicity*			
			Caucasian	*Afro-American*	*Asian*	*Hispanic*
β_1-AR polymorphisms						
145 A>G	Ser49Gly	Gly49 variant shows constitutive activity and enhanced downregulation	0.12–0.16	0.13–0.15 0.23–0.28	0.15	0.20–0.21
1165 C>G	Gly389Arg	Gly389 variant is 'hypofunctional'	0.24–0.34	0.39–0.46	0.2–0.3	0.31–0.33
β_2-AR polymorphisms						
46 A>G	Arg16Gly	Gly16 variant more susceptible to downregulation	0.38–0.46	0.49–0.51	0.54–0.59	NA
79 C>G	Gln27Glu	Glu27 variant resistant to downregulation	0.35–0.46	0.20–0.27	0.07–0.20	NA
491 C>T	Thr164Ile	Ile164 variant shows reduced binding, signalling and internalization	0.02–0.04	0.02–0.04	0–0.01	0.03
β_3-AR polymorphisms						
190 T>C	Trp64Arg[a]	Arg64 variant shows reduced cAMP in response to stimulation	0.08	0.10	0.18[b]	0.16

Abbreviations: β-AR, β-adrenoceptor; NA, not applicable.
Only the more commonly found polymorphisms or those displaying a clear phenotype are shown. For detailed information, see Kirstein and Insel (2004) and Brodde (2008).
[a]American Pima Indians have an incidence of 0.31 and also have a high incidence of obesity and type II diabetes.
[b]Japanese Americans.

polymorphic β_1-ARs and cardiac function. These studies have given inconsistent results. For the Ser49Gly β_1-AR, there is a significant association for blood pressure and heart rate but not for hypertension or the stimulant effects of noradrenaline. With regard to cardiac failure, both positive and negative associations have been reported (Kirstein and Insel, 2004; Brodde, 2008). The Gly389Arg mutation does not appear to be associated with the changes in the haemodynamic response to noradrenaline, blood pressure or heart rate. However, patients with the Arg389 variant show larger changes in blood pressure when treated with β-AR antagonists. Overall, although there are clear differences between polymorphisms of the β_1-AR in their properties studied *in vitro*, further studies are required to establish what role these polymorphisms may have in altered responses to drugs.

β_2-AR polymorphisms

Nine single-nucleotide polymorphisms of the β_2-AR have been identified in both heterozygous and homozygous forms, and four of these mutations cause amino-acid substitutions (Brodde, 2008). Interestingly, some of these mutations are quite common with up to 46% of the caucasian population with the Gly 16 variant. Arg16Gly and Gln27Glu are the most common substitutions with allelic frequencies of 38-59% for Gly16 and 7-46% for Glu27 (note, however, that there are marked inter-ethnic differences in allelic frequencies) (Table 3). Approximately 25% of subjects are homozygous for both polymorphisms (Brodde, 2008). The pharmacological characterization of these variants shows significant alterations in β_2-AR function. The Gly16 variant is more susceptible to agonist-promoted downregulation than the wild type (the first form of the receptor described), whereas the Glu27 variant is resistant to such downregulation (Brodde, 2008). Ligand binding, G-protein coupling and desensitization are unchanged. The Ile164 mutation occurs adjacent to a serine at codon 165 that plays a key role in agonist binding. This relatively rare polymorphism (<4% of the population) is associated with reduced binding to agonists containing β-hydroxyl groups (adrenaline, noradrenaline and isoprenaline, but not dobutamine or dopamine), agonist-promoted receptor sequestration, coupling to Gs and adenylate cyclase, and elimination of high-affinity binding. Heart rate, adenylyl cyclase and physiological responses to agonist are also reduced in transgenic mice expressing the polymorphism.

The relatively common occurrence of some of these polymorphisms is of particular relevance to people taking β_2-AR agonists, given their clearly different properties. Individuals with the Gly16 variants would be expected to show rapid downregulation of β_2-ARs and a loss of responsiveness to β_2-AR agonists, whereas those with the Glu27 variant would be resistant to these effects. There is some evidence to support this idea from the literature but again there are some inconsistencies. Further studies of some of the more common variants may provide clear-cut results that may have particular relevance to individuals taking long-acting β_2-AR agonists as anabolic agents.

β_3-AR polymorphisms

Only one variant of the β_3-AR has been identified, the Trp64Arg variant, which occurs with varying frequencies in populations with different ethnic backgrounds (Kirstein and Insel, 2004) (Table 3). Caucasians have an allelic frequency of 8–10%, African Americans 12%, Mexican Americans 13%, Japanese 20% and Pima Indians, a population with a high incidence of obesity and type 2 diabetes 31%. The pharmacological analysis of this polymorphism shows that there is no effect on ligand binding, desensitization or adenylyl cyclase activation but that the maximal amount of cAMP produced in response to stimulation is markedly reduced. The mutation may be a genetic determinant for obesity and type 2 diabetes as the β_3-AR is involved in lipolysis and thermogenesis, but this is still a matter of controversy (for review see Kirstein and Insel, 2004).

Conclusions

None of the drugs acting directly on β_1-ARs are used to enhance performance in sport. Agonists acting at this subtype have the potential to cause cardiac damage, whereas β_1-AR selective antagonists, although useful therapeutically as antihypertensives and in the treatment of cardiac failure, do not possess the ergogenic effects required. The major target for many illicitly used drugs in sport is the β_2-AR that is found in the heart, lungs and skeletal muscle where it controls rate and force, relaxes tone and stimulates growth, respectively. β_2-AR agonists are demonstrably powerful bronchodilators, anabolic agents and, in combination with corticosteroids, powerfully enhance their anti-inflammatory actions. However, there is little evidence from animal or human studies that these effects translate into an improvement in performance. Indeed, many of the drugs developed to treat asthma are partial agonists that may limit the effectiveness of adrenaline in producing cardiovascular and metabolic responses. There is the potential to cause cardiac arrhythmias and to exacerbate allergic reactions by improving the access of allergens to the lung. The anti-anxiety effects of β-AR antagonists may be potentially advantageous in some sports, but their negative effects on physical performance will be a handicap in endurance sports. Indirectly acting sympathomimetics will not only have similar actions to adrenaline and noradrenaline on the cardiovascular and metabolic systems but may also have psychostimulant effects that reduce fatigue and give an illusion of better performance. Again, the results of controlled trials reveal a disappointing lack of convincing improvement of trained athletes. There is also a real risk of cardiac damage associated with the prolonged use of these agents. Although β_2-ARs are the most attractive drug target, they are also the most susceptible to downregulation. The effects of drugs would be expected to be transient in nature, and this is borne out in practice with many reports of loss of efficacy after a few weeks. There are also genetic variations in the receptors that influence their effectiveness and regulation on exposure to β_2-AR agonists.

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Conflict of interest

The authors state no conflict of interest.

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