British Journal of Pharmacology (2006) 147, 583–586
www.nature.com/bjp

COMMENTARY

Cardiac implications for the use of β_2-adrenoceptor agonists for the management of muscle wasting

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There are proposals for the implementation of β_2-adrenoceptor agonists for the management of muscle wasting diseases. The idea has been initiated by studies in animal models which show that β_2-adrenoceptor agonists cause hypertrophy of skeletal muscle. Their use in clinical practice will also need an understanding of possible effects of activation of human heart β_2-adrenoceptors. Consequences could include an increased probability of arrhythmias in susceptible patients.
British Journal of Pharmacology (2006) **147,** 583–586. doi:10.1038/sj.bjp.0706670;
published online 23 January 2006

Keywords: Skeletal muscle; muscle wasting; human heart; arrhythmias; β_2-adrenoceptor; formoterol; salmeterol

Abbreviations: CGP 20712A, 2-hydroxy-5(2-((2-hydroxy-3-(4-((1-methyl-4-trifluoromethyl) 1*H*-imidazole-2-yl)-phenoxy) propyl) amino) ethoxy)-benzamide monomethane sulphonate; ICI 118, 551 (erythro-DL-1(7-methylindan-4-yloxy)-3-isopropylamino-butano-2-ol)

Muscle wasting is a collection of complex clinical syndromes associated with many conditions including ageing (sarcopenia, Argilés *et al*., 2005a), cancer (cancer cachexia, Argilés *et al*., 2005b), chronic obstructive lung disease (Hansen *et al*., 2005), muscle dystrophies (Kissel *et al*., 1998; 2001) and chronic heart failure (Anker & Coats, 1999). The degree of muscle wasting is a predictor of mortality for cancer, chronic obstructive lung disease and chronic heart failure (Anker *et al*., 1997; Argilés *et al*., 2005a; Hansen *et al*., 2005). Mechanisms of muscle wasting possibly include proinflammatroy cytokines, tumour necrosis factor-α, interleukin-1, interleukin-6 and interferon-γ (Argilés *et al*., 2005a; Hansen *et al*., 2005) and these have been considered as therapeutic targets (Argilés *et al*., 2005b).

The potential use of β-adrenoceptor agonists for the management of muscle wasting has been raised (Kissel *et al*., 1998; 2001; Busquets *et al*., 2004; Ryall *et al*., 2006). Chronic administration of β-adrenoceptor agonists causes skeletal muscle hypertrophy in many animal species (Kim *et al*., 1992; Hinkle *et al*., 2002; Busquets *et al*., 2004; Ryall *et al*., 2006) and humans (Kissel *et al*., 2001). The successful implementation of β-adrenoceptor agonists for this purpose would include an ability to reverse muscle loss, promote muscle gain, strength and mobility without inducing adverse effects. With respect to the latter, some considerations are given for possible effects in the heart.

High doses of formoterol ($2\,mg\,kg^{-1}$ body weight, i.p.) administered to rats or mice with induced cachectic cancer tumours resulted in a reversal of skeletal and heart muscle wasting (Busquets *et al*., 2004). Formoterol caused an inhibition of skeletal muscle proteolysis and increased protein synthesis (Busquets *et al*., 2004). Ryall *et al*. (2006) report the potential use of *low-dose* formoterol and salmeterol for pathologies of muscle wasting and weakness. Currently, both drugs are used for the management of asthma and are characterized by relatively high lipophilicity, high affinity for the β_2-adrenoceptor and consequently long duration of action (Teschemacher & Lemoine, 1999). In rats, subcutaneous administration of both salmeterol and formoterol caused skeletal and heart muscle hypertrophy. To avoid cardiac complications, it would be desirable to target therapeutic strategies to skeletal muscle. In the study of Ryal *et al*. (2006), formoterol exhibited $\sim$8–16-fold hypertrophic selectivity on the basis of pEC_{50} values for skeletal *versus* heart muscle, while salmeterol was essentially nonselective for both muscle groups ($\leqslant$2-fold *heart* selective). The relative potencies of formoterol and salmeterol for causing skeletal and heart muscle hypertrophy by Ryall *et al.* are interesting in the context of previously determined affinities at β_1- and β_2-adrenoceptors. Salmeterol is 65–2818-fold selective for guinea-pig and human β_2- *versus* β_1-adrenoceptors on the basis of affinity (Roux *et al*., 1996; Hoffmann *et al*., 2004; Baker, 2005), and formoterol 60–346-fold β_2-adrenoceptor selective (guinea-pig β-adrenoceptors, Lemoine *et al*., 1991; 1992; Roux *et al*., 1996). Both agonists have the ability to form guanine nucleotide-sensitive high-affinity binding sites (H) at guinea-pig β_1- and β_2-adrenoceptors (Roux *et al*., 1996). The high-affinity guanine nucleotide-sensitive binding site (H) is presumably responsible for agonist effects. Salmeterol (117-fold) and formoterol (10-fold) are selective for guanine nucleotide-sensitive, high-affinity guinea-pig β_2- *versus* β_1-adrenoceptor binding sites (Roux *et al*., 1996). In functional studies, formoterol was $\sim$680–1700-fold selective for causing relaxation of 0.1 μM carbachol contracted guinea-pig tracheal strips ($\beta_2 > \beta_1$-adrenoceptors) than for causing positive inotropic effects in guinea-pig left ventricular papillary muscle (β_1-adrenoceptors, Trofast *et al*., 1991).

In several experimental conditions, rodent hearts or cardiomyocytes are more susceptible to hypertrophy through β_1- than β_2-adrenoceptors. Isoprenaline caused hypertrophy of neonatal rat cardiomyocytes *in vitro* and adult rat heart *in vivo* which was blocked by the β_1-blocker betaxolol but not the

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β_2-blocker ICI 118551 (Morisco *et al*., 2001). Cardiac overexpression (5–15-fold) of *human* β_1-adrenoceptors in mice caused progressive myocyte hypertrophy, measurable within weeks of birth (Engelhardt *et al*., 1999). On the other hand, 60-fold cardiac overexpression of wild-type *human* β_2-adrenoceptors did not show any evidence of cardiac hypertrophy up to the age of 1 year but did at higher expression levels (Liggett *et al*., 2000). In mice, 30-fold overexpression of β_2-adrenoceptors prevented Gαq-protein overexpression ($\sim$5-fold)-induced hypertrophy (DORN *et al*., 1999). This prompts the question which receptors (β_1-, β_2-) were responsible for salmeterol- and formoterol-induced hypertrophy in skeletal and heart muscle preparations in the study of Ryall *et al*.? Rat soleus muscle comprises 20 : 80% β_1- : β_2-adrenoceptors (Kim *et al*., 1991) and rat ventricle $\sim$73:27% β_1- : β_2-adrenoceptors (Sarsero & Molenaar, 1995). In rat soleus and EDL muscles *in vitro*, a high concentration of ICI 118 551 (10 μM) reversed 100 μM adrenaline- and 10 μM clenbuterol-mediated inhibition of proteolysis (Navegantes *et al*., 2001). The use of low concentrations of β_2-adrenoceptor selective agonists together with previous studies with selective blockers suggests that β_2-adrenoceptors mediate hypertrophy in skeletal muscle. Whether 'break-through' of β_1-adrenoceptors by salmeterol and formoterol was partly or solely responsible for hypertrophy in the heart would need to be determined with selective β_1- and β_2-adrenoceptor blockers (Morisco *et al*., 2001).

Is the progression of this potential therapeutic strategy to humans feasible? The use of highly selective β_2-adrenoceptor agonists, possibly in conjunction with a selective β_1-blocker where possible, could ensure prevention of unintended β_1-adrenoceptor activation. This is particularly important for cardiovascular β_1-adrenoceptors, where chronic activation of β_1-adrenoceptors is contraindicated for prevalent cardiac and vascular disorders including hypertension, ischaemic heart disease, arrhythmias and heart failure where β-blockers are indicated in some cases. A pathological role of the β_1-adrenoceptor was confirmed in transgenic mice with 15-fold overexpression which exhibited an enhancement of heart function (dP/dt) followed by progressive deterioration involving hypertrophy and heart failure (Engelhardt *et al*., 1999). The importance of blocking β_1-adrenoceptors in heart failure to abrogate cardiotoxic β_1-adrenoceptor-mediated effects is well understood (Molenaar & Parsonage, 2005).

Assuming selective activation of β_2-adrenoceptors is achievable, progression of this potential therapeutic strategy from rats to humans requires consideration of the potential effects of chronic activation of β_2-adrenoceptors in human heart. Inhalational formulations of β_2-adrenoceptor agonists used for chronic management of airways diseases are designed for local application and systemic effects are unintended. Nevertheless, frequent use resulting in higher doses can result in cardiac events (Newhouse *et al*., 1996), while effects on muscle mass and function *via* this route have not been reported. Systemic administration of β_2-adrenoceptor agonists will result in widespread β_2-adrenoceptor activation including the heart. Preliminary studies are often carried out in rodents and therefore differences between rodent and human cardiac β_2-adrenoceptor systems should be noted. Briefly, evidence, mostly from Rui-Ping Xiao's laboratory, indicates dual coupling of β_2-adrenoceptors to stimulatory Gsα- and inhibitory Giα-proteins (reviewed most recently by Xiao, 2001; Xiao *et al*., 2004; Zheng *et al*., 2005). As is the case for β_1-adrenoceptor–Gsα-protein–cyclic AMP–PKA signalling in heart, coupling of the β_2-adrenoceptor to the same signalling pathway causes enhanced cell shortening and hastening of relaxation. Coupling of the β_2-adrenoceptor to pertussis toxin-sensitive Giα-protein and activation of phosphoinositide 3-kinase (PI3K) essentially opposes and bluntens the effects of β_2-adrenoceptor–Gsα-protein coupling. Prolonged stimulation also enables the β_2-adrenoceptor to couple to Giα-protein–PI3K–Akt (protein kinase B) pathways to exert an antiapoptotic effect. In human right atrium, it was demonstrated that the β-adrenoceptor agonist isoprenaline in the presence of CGP 20712A but not ICI 118 551 (all drugs 100 μM) were used to show Giα-protein coupling (Kilts *et al*., 2000). Additionally, pertussis toxin enhanced adenylyl cyclase activity in the presence of isoprenaline and CGP 20712A and isoprenaline in the presence of ICI 118 551 (all drugs 100 μM). However, the concentrations of drugs used in that study were particularly high and several studies investigating functional responses suggest predominant coupling to Gsα-proteins. In human atrium (Kaumann *et al*., 1996; Kaumann & Molenaar, 1997; Krause *et al*., 2004) and ventricle (Kaumann *et al*., 1999; Molenaar *et al*., 2000), activation of β_2-adrenoceptors caused increases in contractile force and hastening of relaxation associated with phosphorylation of phospholamban and troponin I, consistent with β_2-adrenoceptor coupling to Gsα-protein–cyclic AMP–PKA. In fact, the cardiostimulant effects mediated through activation of β_2-adrenoceptors were as great as or nearly as great as those mediated through activation of β_1-adrenoceptors. This observation has implications for the systemic use of β_2-adrenoceptor agonists for the purposes of inducing skeletal muscle growth in humans. Activation of human heart β_2-adrenoceptors causes increased rate, force (Fowler *et al*., 1982; Hall *et al*., 1989; Hall *et al*., 1990; Brodde, 1991; Kaumann *et al*., 1999; Molenaar *et al*., 2000) and induction of arrhythmias (Kaumann & Sanders, 1993; Kaumann *et al*., 1995). Salbutamol, a drug used for the management of asthma, causes an increase in contractile force in human right atrium *in vitro* (Hall *et al*., 1990) and causes tachycardia when administered directly into the right coronary artery (Hall *et al*., 1989). Salbutamol infusion into the right femoral vein causes an increase in dispersion of the QTend interval duration representing variations in ventricular repolarization and increase in repolarization heterogeneity (Lowe *et al*., 2001). This has been correlated with an increase in arrhythmia risk (Lowe *et al*., 2001). Thus, activation of ventricular β_2-adrenoceptors in susceptible individuals may cause an increase in triggered arrhythmias (Lowe *et al*., 2001). The clinical cardiovascular safety of β_2-adrenoceptor agonists has remained contentious. A recent meta-analysis of randomized controlled studies in patients with obstructive airways disease suggests that there may be increased risk of adverse cardiac events in this population (Salpeter, 2004). Orally administered salbutamol to 20 patients with advanced congestive heart failure was associated with ventricular tachycardia in six patients and atrial fibrillation in another (Mettauer *et al*., 1985). Such observations provide a caution that in certain large groups of patients with conditions associated with skeletal muscle wasting, obstructive airway disease and chronic heart failure, there may be particular risks associated with the administration of β_2-adrenoceptor agonists. Current clinical information concerning salmeterol and formoterol is restricted to inhalation administration. Both

drugs are considered relatively safe with little evidence of cardiac adverse events except possibly in patients predisposed to arrhythmias (Cazzola *et al*., 1998). It was concluded from a review of multiple studies that *inhaled* salmeterol 100 μg could lead to changes in the ECG in a small number of patients from some but not all studies, suggesting 'doubtful clinical meaning'.

β_2-Adrenoceptor agonists may have a role in the management of muscle wasting diseases in selected patients; however, their use may be contraindicated in some patients, particularly those predisposed to the occurrence of cardiac arrhythmias.

Research was supported by grants from the National Health and Medical Research Council of Australia and The Prince Charles Hospital Foundation. Lu Chen is supported by an Endeavour International Postgraduate Research Scholarship (IPRS) at the University of Queensland.

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(Received November 17, 2005
Accepted November 22, 2005
Published online 23 January 2006)