Published in final edited form as:

Exp Physiol. 2006 November ; 91(6): 1041–1049. doi:10.1113/expphysiol.2006.035014.

Relative myotoxic and haemodynamic effects of the β -agonists fenoterol and clenbuterol measured in conscious unrestrained rats

Jatin G Burniston¹, Lip-Bun Tan², and David F Goldspink¹

¹Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, Webster Street, Liverpool, L3 2ET, England. ²Academic Unit of Molecular Vascular Medicine, University of Leeds, Leeds, LS2 9JT, England.

Abstract

The β_2 -adrenoceptor (β_2 -AR) agonists clenbuterol and fenoterol have similar beneficial effects in animal models of heart failure. However, large doses of clenbuterol can induce cardiomyocyte death and it is not known which of these agents has the most favourable therapeutic profile. We have investigated the cardiotoxicity of clenbuterol and fenoterol alongside that of isoproterenol, and compared their haemodynamic effects. Wistar rats ($n=6$, per group) were subcutaneously injected with each β -agonist ($0.003 \text{ mmol kg}^{-1}$ to 3 mmol kg^{-1}) or saline and cardiomyocyte apoptosis was detected by caspase 3 immunohistochemistry. In a separate experiment rats ($n=4$) were given equivalent doses to those used in the myotoxicity studies, in a randomised crossover design, and their blood pressure recorded via radio telemetry. Injection of 0.3 mmol kg^{-1} fenoterol or isoproterenol, but not clenbuterol, induced significant cardiomyocyte apoptosis ($0.4 \pm 0.05\%$; $P < 0.05$). At 3 mmol kg^{-1} , all agonists induced apoptosis (fenoterol $1.1 \pm 0.1\%$; isoproterenol $0.9 \pm 0.8\%$; clenbuterol $0.4 \pm 0.07\%$; $P < 0.05$). β_1 -AR antagonism (10 mg kg^{-1} bisoprolol) prevented (92% ; $P < 0.05$) apoptosis induced by all 3 agonists, but clenbuterol-induced apoptosis could also be prevented (96% ; $P < 0.05$) by β_2 -AR antagonism (10 mg kg^{-1} ICI118551). Clenbuterol decreased diastolic (1.3 – 1.6 fold; $P < 0.05$) and systolic (1.3 fold; $P < 0.05$) blood pressure and doses $> 0.3 \text{ mmol kg}^{-1}$ increased heart rate (1.4 fold; $P < 0.05$). Fenoterol increased heart rate (1.2 – 1.4 fold; $P < 0.05$) and doses $> 0.3 \text{ mmol kg}^{-1}$ decreased diastolic blood pressure (1.3 fold; $P < 0.05$). In conclusion, the cardiotoxicity of fenoterol was similar to isoproterenol and greater than clenbuterol, and fenoterol had less desirable haemodynamic effects.

Keywords

apoptosis; cardiomyocyte; isoprenaline; blood pressure; skeletal muscle

Introduction

The cardiotoxicity of endogenous catecholamines and their synthetic analogue, isoproterenol, is widely recognised (Mann et al., 1992; Communal et al., 1998; Ng et al., 2002; Goldspink et al., 2004). Mann et al. in (1992) revealed this cardiotoxicity to be mediated by the β -adrenergic receptor (β -AR), and later work by Communal et al. (1999) showed that it is the β_1 - rather than β_2 -AR that mediates β -agonist-induced cardiomyocyte death. This difference is due to disparate subcellular signalling of β_1 - and β_2 -AR and this

has been extensively studied in vitro (Communal et al., 1999; Zaugg et al., 2000; Xiao et al., 2003; Communal & Colucci, 2005). β_1 -AR couple with the stimulatory G protein (G_{α_s}) and induce cardiomyocyte apoptosis via cAMP and Ca^{2+} entry-dependent mechanisms, whereas, β_2 -AR couple with both G_{α_s} and the pertussis toxin (PTX)-sensitive inhibitory G protein (G_{α_i}) and do not induce cardiomyocyte apoptosis in vitro (Communal & Colucci, 2005). For the most part, the observations in vitro are substantiated by studies using genetically manipulated animals, confirming that elevated β_1 -AR signalling (15-fold over-expression of the β_1 -AR) induces cardiomyopathy (Engelhardt et al., 1999), whereas, 60-fold overexpression of the β_2 -AR is not detrimental (Liggett et al., 2000). Based on this body of evidence there is growing interest in the therapeutic potential of β_2 -AR agonists for the treatment of heart failure. For example, in addition to their more well established anabolic effects (Yacoub, 2001; Soppa et al., 2005), β_2 -agonists have been proposed as a potential anti-apoptotic therapy (Ahmet et al., 2004; Xydas et al., 2006) and a means of providing inotropic support to the failing heart (Xiao et al., 2003).

It is important to recognise, however, that studies in vitro and transgenic models are not analogous to the proposed therapeutic use in which exogenous β_2 -AR agonists would be given in vivo. We have shown that administration of the β_2 -agonist clenbuterol in vivo induces myocyte death in cardiac and skeletal muscle (Burniston et al., 2002; Burniston et al., 2005e). Our findings do not contradict those of studies in vitro. In fact, our work using wild-type animals confirms that apoptosis is induced by stimulation of the cardiomyocyte β_1 -AR. But, when clenbuterol is given in vivo its stimulation of β_2 -AR of the peripheral vasculature and sympathetic nervous system potentiates norepinephrine release from the sympathetic varicosities and this is sufficient to stimulate cardiomyocyte β_1 -AR and induce apoptosis (Burniston et al., 2005e). Therefore, the use of exogenous β_2 -agonists as a therapy for heart failure, or other chronic diseases, needs to be considered carefully.

Recently, clenbuterol (Xydas et al., 2006) and another commonly investigated β_2 -AR agonists, fenoterol, (Ahmet et al., 2004) have each been shown to have beneficial effects on the myocardium of animals subjected to coronary artery ligation. Because fenoterol has been reported to be a more potent anabolic agent than clenbuterol (Ryall et al., 2002) fenoterol may be the preferred therapeutic option. However, fenoterol stimulates the β_1 -AR as well as the β_2 -AR (Hoffmann et al., 2004) and its stimulation of the β_2 -AR is selectively mediated via the G_{α_s} pathway (Xiao et al., 2003), which is similar to the pro-apoptotic β_1 -AR signalling pathway. Therefore, we hypothesised that, when given to whole animals in vivo, fenoterol will be more myotoxic and have less favourable haemodynamic effects than clenbuterol. To test this hypothesis we first measured the incidence of myocyte apoptosis in the heart and soleus muscle of animals administered equimolar doses of fenoterol, clenbuterol or isoproterenol. Then, in a second group of animals, implanted with telemetric blood pressure transducers, we measured the effects on blood pressure and heart rate of equimolar doses of fenoterol and clenbuterol.

Methods

Animal husbandry

All experiment procedures were conducted under the British Home Office Animals (Scientific Procedures) Act 1986 and according to UK Home Office guidelines. Male Wistar rats (289 ± 19 g) were bred in-house in a conventional colony, housed in controlled conditions of 20 °C, 45 % relative humidity and a 12 h light (06:00 – 18:00) and 12 h dark cycle, with water and food available *ad libitum*.

β -Agonist-induced myocyte apoptosis

The dose-dependency of β -agonist-induced apoptosis was investigated from 0.003 mmol kg⁻¹ to 3 mmol kg⁻¹. Independent groups of rats ($n = 6$, in each group) were given single subcutaneous injections of either β -agonist (fenoterol, clenbuterol or isoproterenol) or saline vehicle and were killed 4 h later. To investigate the β -AR subtype mediating myocyte apoptosis, animals ($n = 6-8$, in each group) were given single subcutaneous injections of either 10 mg of bisoprolol kg⁻¹ (β_1 -AR selective antagonist) or ICI 118 551 kg⁻¹ (β_2 -AR selective antagonist) 1 h before injection of the most damaging dose of β -agonist. Previous experiments using this model (Tan et al., 2003; Burniston et al., 2005e) have shown that such administrations provide selective β_1 - or β_2 -AR antagonism, respectively.

At the end of each experiment the rats were concussed and killed by cervical dislocation, and their heart and soleus muscles isolated. The heart was mounted apex uppermost on a cork disc and a segment of the mid-belly of each soleus was mounted in transverse section. Muscles were snap-frozen in super-cooled isopentane and stored at -80°C , prior to cryo-sectioning (5- μm thick). Apoptosis was detected on cryosections in vitro using an anti-caspase 3 antibody (R&D Systems, Minneapolis, USA) as described previously (Goldspink et al., 2004; Burniston et al., 2005c; Burniston et al., 2005d; Burniston et al., 2005e; Burniston et al., 2006). Our previous work (Goldspink et al., 2004) and that of others (De Angelis et al., 2002) has shown that caspase-3 activity co-localises with dUTP nick-end labelling on cryosections in vitro, and with annexin V-biotin detection of phosphatidylserine externalisation in vivo (Burniston et al., 2005e), validating this method of detecting apoptosis.

The incidence of β -agonist-induced myocyte death in the heart and soleus muscles was quantified from cryosections stained with caspase 3. Cardiomyocyte death was measured in the left ventricular subendocardium. For each heart, six to eight fields of view (x100 magnification), encompassing the entire subendocardial region (approximately 10^4 cells), were digitised. Positive staining (apoptosis) was differentiated from the haematoxylin background and the incidence of myocyte death expressed as percent area relative to each field of view. To quantify myocyte death in the soleus, three random fields of view (x100 magnification) across each transverse section were digitised. Both injured and viable fibres were counted (>700 fibres), and the number of damaged fibres expressed as a percentage of the total.

The haemodynamic effects of β -agonist (fenoterol or clenbuterol) administration were investigated in a separate group of animals surgically implanted with telemetric blood pressure transducers.

Surgical procedures

Prophylactic antibiotic (2 mg kg⁻¹ Baytril) was administered 2 h prior to surgery. Anaesthesia was induced in a chamber using 4 % isoflurane in medical oxygen and the animals intubated (18-G catheter) and mechanically ventilated (~60 breaths per minute, 300 ms inspiration time) with 1.5 % isoflurane. The animal's heart rate and arterial O₂ saturation were monitored via pulse oximetry and their body temperature was monitored via a rectal probe and maintained using a heated mat. The abdomen was prepared, a laparotomy performed and the intestine parted and held in place with a retractor and surgical gauze. A section of the aorta (~10 mm length) was isolated immediately distal to the renal bifurcation and two lengths of suture passed underneath to allow proximal and distal occlusion. The catheter probe of the telemetric blood pressure transducer (TA11 PA-C40; Data Sciences International, St Paul, MN) was introduced cranial to the distal occlusion and advanced proximally into the vessel. A small amount of tissue adhesive and a cellulose patch were

used to cover the site of the catheter insertion and the patency of the vessel and performance of the transducer verified using the telemetric receiver. The transducer body was placed in the abdominal cavity and the incision closed in two layers, incorporating the suture rib of the transducer in the muscular wall of the abdomen. Anaesthesia was withdrawn and each animal received fluid therapy (1.5 ml sterile saline i.p.) and was allowed immediate access to food and water. Analgesia (0.05 mg kg⁻¹ Buprenorphine) was given (i.m.) peri-operatively and at 12-h intervals for 48 h post-operatively.

Measurement of blood pressure and heart rate

The output from the animal's blood pressure transducer was monitored via radio telemetry by placing the receiver underneath the animal's cage. Throughout data collection the animals were conscious and able to move freely around their cage. The blood pressure waveform, displayed in real-time, was analysed using AcqKnowledge software (Biopac systems Inc, Goleta, CA) to give systolic and diastolic blood pressures and heart rate. Seven days after implanting the transducer, the animal's heart rate and blood pressure responses to different doses (0.003 mmol kg⁻¹ to 3 mmol kg⁻¹) of β -agonist or saline were measured using a randomised crossover design. Animals ($n = 4$) were given a bolus injection (i.p.) of either clenbuterol or fenoterol and their blood pressure and heart rate response recorded for 24 h; a 2 d wash out period was allowed before the next administration. The muscles of these animals were not harvested.

Statistical analyses

Data were analysed by two-way analysis of variance, employing either an unrelated factorial design to investigate differences in incidence of myocyte apoptosis or a repeated measures design to investigate differences in the magnitude of the haemodynamic response. In all cases, differences were considered statistically significant if $p < 0.05$.

Results

Injection of each β -agonist induced myocyte apoptosis in the heart (Figure 1A) and soleus muscle (Figure 1B). In contrast, no apoptosis was detected in the striated muscles (Figure 1C & D) of control animals. In the heart, a significant incidence of apoptosis was measured after injection of 0.3 mmol kg⁻¹ of either fenoterol or isoproterenol but not clenbuterol (Figure 2A). At 3 mmol kg⁻¹, each agonist induced significant ($P < 0.05$) cardiomyocyte death but clenbuterol ($0.34 \pm 0.07\%$) induced less than fenoterol ($1.1 \pm 0.09\%$) or isoproterenol ($0.9 \pm 0.08\%$). In the soleus, fenoterol and clenbuterol induced significant ($P < 0.05$) myofibre apoptosis at doses of 0.03 mmol kg⁻¹ or greater whereas only 3 mmol kg⁻¹ of isoproterenol induced significant ($P < 0.05$) myofibre death (Figure 2B). Thus, the relative myotoxicity of fenoterol, clenbuterol and isoproterenol was different in the heart (Figure 2C) and skeletal (Figure 2D) muscle.

Prior β_1 -AR selective antagonism (bisoprolol) significantly protected the myocardium from apoptosis induced by fenoterol, clenbuterol and isoproterenol (Figure 3A). However, clenbuterol-induced apoptosis could also be prevented by β_2 -AR selective antagonism (ICI 118 551). In skeletal muscle, the β -AR mediating apoptosis was the same for all three β -agonists, in that only β_2 -AR blockade was effectual (Figure 3B).

Radio telemetric recording of aortic blood pressure in conscious unrestrained animals revealed marked differences in the haemodynamic effects of fenoterol and clenbuterol (Figure 4). In response to injection of 3 mmol of β -agonist kg⁻¹, the greatest change in heart rate was measured after 2 min and the greatest change in blood pressure after 10 min (Figure 5), and these time points were used to collect dose-response data shown in Figure 6. The half-life of the β -agonist-induced haemodynamic response after administration of this dose

was approximately 6 h and 16 h for fenoterol and clenbuterol, respectively. Administration of 0.003 mmol kg⁻¹ fenoterol significantly ($P<0.05$) elevated heart rate whereas heart rate was only significantly ($P<0.05$) increased after 0.03 mmol kg⁻¹ clenbuterol. At doses of 0.003 mmol kg⁻¹ and 0.03 mmol kg⁻¹, the positive chronotropic effect of fenoterol was significantly ($P<0.05$) greater than clenbuterol (Figure 6A). All doses of clenbuterol significantly ($P<0.05$) depressed diastolic blood pressure, and this hypotensive effect was significantly ($P<0.05$) greater than that of fenoterol at doses of 0.003 mmol kg⁻¹ and 0.03 mmol kg⁻¹ (Figure 6B). Systolic blood pressure significantly ($P<0.05$) decreased after administration of 0.003 mmol kg⁻¹ clenbuterol, and administration of larger doses (0.3 mmol kg⁻¹ and 3 mmol kg⁻¹) of either fenoterol or clenbuterol significantly ($P<0.05$) decreased systolic blood pressure (Figure 6C).

Discussion

The current work reveals that the cardiotoxicity of fenoterol is equivalent to that of the widely recognised cardiotoxic agent, isoproterenol, and much greater than that of clenbuterol (Figure 2). Fenoterol is a potent stimulator of the β_2 -AR (January et al., 1997), but our β -blockade studies (Figure 3) suggest that fenoterol's greater cardiotoxicity over clenbuterol is due to its stimulation of β_1 -AR (similar to isoproterenol), rather than its greater potency at the β_2 -AR or its selectivity for the β_2 -AR G α_s signalling pathway (Xiao et al., 2003). These findings agree with previous studies in vitro (Hoffmann et al., 2004) and clinical data (Burgess et al., 1991) that have each suggested that fenoterol stimulates both β_1 - and β_2 -AR. In light of this evidence, we should reappraise our classification of fenoterol as a selective β_2 -AR agonist. Indeed, fenoterol-induced cardiomyocyte apoptosis (Figure 1) may underlie the previously observed myocardial scarring after intravenous infusion of this agent (Pack et al., 1994), and it could be speculated that the similar cardiotoxicity of isoproterenol and fenoterol might also link their respective associations with the asthma mortalities of the 1960s and 1980s (Sears & Taylor, 1994; Beasley et al., 1999).

In agreement with the myotoxicity data, the poorer selectivity of fenoterol for the β_2 -AR was also evident in its effects on the heart rate and blood pressure (Figure 6). Fenoterol had a greater positive chronotropic effect than clenbuterol but our data do not provide evidence for a causal link between raised heart rate and myocyte death. Studies conducted in vitro (Communal & Colucci, 2005) and our previous work in vivo (Burniston et al., 2005b; Burniston et al., 2005e) have each demonstrated that the myotoxic effects of β -agonists are mediated by over stimulation of the β_1 -AR and its downstream signalling cascade. The haemodynamic changes caused by clenbuterol (Figure 6) agree well with previous data from anaesthetised rats (Jones et al., 2004) and, as opposed to fenoterol, conform to the expected paradigm for a β_2 -AR selective agonist. Jones et al. (2004) also investigated the β_2 -agonist pro-drug BRL-47672, reporting that it had a lesser effect on heart rate and mean arterial pressure than clenbuterol. Muscle anabolism induced by BRL-47672 (Jones et al., 2004) may be similar to clenbuterol (Rajab et al., 2000) and, therefore, BRL-47672 may represent a more acceptable means of inducing muscle growth. However, caution should still be advocated because BRL-47672 is metabolised to a β_2 -AR agonist (Sillence et al., 1995) and the potential myotoxicity of this metabolite is unknown.

In support of previous work from our laboratory (Burniston et al., 2002; Ng et al., 2002; Tan et al., 2003; Goldspink et al., 2004; Burniston et al., 2005a; Burniston et al., 2005e) skeletal myofibre death was mediated only via the β_2 -AR, irrespective of the agonist administered (Figure 3B). But, when the hearts from these animals were investigated the effect of β_1 - or β_2 -AR selective antagonism was different depending on which agonist was used (Figure 3A). Clenbuterol-induced cardiomyocyte apoptosis could be prevented by either β_1 -AR or β_2 -AR blockade whereas only β_1 -AR selective blockade prevented fenoterol- or

isoproterenol-induced cardiomyocyte death. Previously, we have shown that clenbuterol induces cardiomyocyte death in vivo by modulating the sympathetic nervous system (Burniston et al., 2002; Burniston et al., 2005e). Because of this, the cardiotoxicity of β_2 -AR selective agonists, such as clenbuterol (Figure 2A) or salbutamol (Pack et al., 1994), is less than that of agonists, such as fenoterol and isoproterenol, that also stimulate the cardiomyocyte β_1 -AR and, thereby, directly induce cardiomyocyte death. The cardiotoxicity of isoproterenol and fenoterol was decreased, rather than increased, after β_2 -AR antagonism (Figure 3A). This effect, which is contrary to expectations from studies in vitro showing an anti-apoptotic effect of β_2 -AR agonism, exemplifies the need to conduct studies, such as the current work, that replicate the intended use of these agents in vivo.

Because of differences in body size and pharmacobiodynamics it is not possible to extrapolate doses used here for rats to likely doses in humans. However, our haemodynamic data can be used to identify doses that induced changes within the animals' physiological range. Administration of either fenoterol or clenbuterol at doses of 0.3 mmol kg^{-1} or greater induced maximal increases in heart rate ($563.5 \pm 8.6 \text{ beats min}^{-1}$; Figure 6A), which represent the upper limit of the animals' physiological range. At the lesser dose of $0.03 \text{ mmol kg}^{-1}$, fenoterol increased the animals' heart rate to 78 % and clenbuterol to 42 % of their heart rate reserve (maximum heart rate – resting heart rate). At this dose, fenoterol and clenbuterol each induced significant myocyte apoptosis in the soleus muscle (Figure 2B) while the incidence of apoptosis in the heart failed to reach statistical significance (Figure 2A). These findings show the potential of these agents to induce myocyte death at 'physiological' doses and are especially worrisome with regard the illicit use of these agents. The pseudo-science associated with the abuse of β_2 -agonists for promoting muscle growth suggests that the user increase the dose until the side effects, which include muscle tremors and elevated heart rate, can no longer be tolerated (Duchanie, 1992). This form of prescription could be responsible for the reported cases of myocardial infarction in body builders abusing clenbuterol (Goldstein et al., 1998; Kierzkowska et al., 2005). Whether endogenous catecholamine release could also cause myocyte death at levels eliciting submaximal changes in heart rate cannot be extrapolated from these findings using synthetic agents but is worthy of investigation.

Our finding that fenoterol and clenbuterol cause cardiomyocyte apoptosis in healthy animals does not contradict recent work by Ahmet et al. (2004) and Xydas et al. (2006) reporting that these agents reduce cardiomyocyte apoptosis in a model of myocardial infarction induced by coronary artery ligation. When administered to normal healthy animals in vivo, β_2 -agonists induce cardiomyocyte death by neuromodulation of the SNS and stimulation of the cardiomyocyte β_1 -AR. The apparent disparity between the findings of the current work and those of Ahmet et al. (2004) and Xydas et al. (2006) is explained by the desensitisation of β_1 -AR signalling pathway that is associated with this model of cardiac damage (Anthonio et al., 2000). In agreement with this, each of these studies (Ahmet et al., 2004; Xydas et al., 2006) reported that the anti-apoptotic effect of β_1 -AR antagonism was less than that of β_2 -agonism and that there was no synergistic effect when the two interventions (β_1 -AR antagonism and β_2 -agonism) were combined. Similarly, the finding that clenbuterol administration is therapeutic and augments recovery of the unloaded myocardium (Yacoub, 2001; Hon & Yacoub, 2003) can be reconciled with the current findings by appreciating that these patients also receive 'combination therapy' that includes β_1 -AR blockade. However, it is important to note that these protective mechanisms (selective desensitisation of β_1 -AR or co-administration of a β_1 -AR antagonist) are unlikely to be present in other chronic diseases, such as cancer cachexia or sarcopenia, for which β_2 -AR agonist therapy is also being considered. In each of these situations the myotoxic effects of β_2 -AR stimulation on the skeletal muscle will not have been ablated. However, we have recently discovered that

clenbuterol's hypertrophic effects can be separated from its myotoxic effects in the heart and skeletal muscle by carefully controlling the dose administered (Burniston et al., 2006).

In conclusion, comparison of the dose-dependent myotoxic and haemodynamic effects of fenoterol and clenbuterol revealed that each of these agents induced significant skeletal myocyte death and considerable cardiomyocyte death at a dose ($0.03 \text{ mmol kg}^{-1}$) eliciting a less than maximal change in heart rate. At this dose fenoterol induced a greater increase in heart rate whereas clenbuterol had a greater hypotensive effect. The cardiotoxic effects of these agents in vivo contrast with the effects predicted from studies in vitro and highlight the dangers of arbitrarily translating findings in vitro to whole animal models.

Acknowledgments

This research was funded by a British Heart Foundation Junior Research Fellowship (FS/04/028) awarded to JGB.

References

- Ahmet I, Krawczyk M, Heller P, Moon C, Lakatta EG, Talan MI. Beneficial effects of chronic pharmacological manipulation of beta-adrenoreceptor subtype signaling in rodent dilated ischemic cardiomyopathy. 2004; 110:1083–1090. [PubMed: 15313944]
- Anthonio RL, Brodde OE, van Veldhuisen DJ, Scholtens E, Crijns HJ, van Gilst WH. Beta-adrenoceptor density in chronic infarcted myocardium: a subtype specific decrease of beta1-adrenoceptor density. Int J Cardiol. 2000; 72:137–141. [PubMed: 10646955]
- Beasley R, Pearce N, Crane J, Burgess C. Beta-agonists: what is the evidence that their use increases the risk of asthma morbidity and mortality? J Allergy Clin Immunol. 1999; 104:S18–30. [PubMed: 10452785]
- Burgess CD, Windom HH, Pearce N, Marshall S, Beasley R, Siebers RW, Crane J. Lack of evidence for beta-2 receptor selectivity: a study of metaproterenol, fenoterol, isoproterenol, and epinephrine in patients with asthma. Am Rev Respir Dis. 1991; 143:444–446. [PubMed: 1671326]
- Burniston JG, Chester N, Clark WA, Tan L-B, Goldspink DF. Dose-dependent apoptotic and necrotic myocyte death induced by the β_2 -adrenergic receptor agonist, clenbuterol. Muscle Nerve. 2005a; 32:767–774. [PubMed: 16007677]
- Burniston JG, Clark WA, Tan L-B, Goldspink DF. Dose-dependent separation of the hypertrophic and myotoxic effects of the β_2 -adrenergic receptor agonist clenbuterol in rat striated muscles. Muscle & Nerve. 2006; 33:655–663. [PubMed: 16411205]
- Burniston JG, Ellison GM, Clark WA, Goldspink DF, Tan L-B. Relative toxicity of cardiotonic agents: some induce more cardiac and skeletal myocyte apoptosis and necrosis in vivo than others. Cardiovasc Toxicol. 2005b; 5:355–364. [PubMed: 16382173]
- Burniston JG, Ng Y, Clark WA, Colyer J, Tan L-B, Goldspink DF. Myotoxic effects of clenbuterol in the rat heart and soleus muscle. J Appl Physiol. 2002; 93:1824–1832. [PubMed: 12381771]
- Burniston JG, Saini A, Tan L-B, Goldspink DF. Aldosterone induces apoptosis in the heart and skeletal muscles of normotensive rats in vivo. J Mol Cell Cardiol. 2005c; 39:395–399. [PubMed: 15907929]
- Burniston JG, Saini A, Tan L-B, Goldspink DF. Angiotensin II induces apoptosis in vivo in skeletal, as well as cardiac, muscle of the rat. Exp Physiol. 2005d; 90:755–761. [PubMed: 15987733]
- Burniston JG, Tan L-B, Goldspink DF. β_2 -Adrenergic receptor stimulation *in vivo* induces apoptosis in the rat heart and soleus muscle. J Appl Physiol. 2005e; 98:1379–1386. [PubMed: 15591297]
- Communal C, Colucci WS. The control of cardiomyocyte apoptosis via the beta-adrenergic signaling pathways. Arch Mal Coeur Vaiss. 2005; 98:236–241. [PubMed: 15816327]
- Communal C, Singh K, Sawyer DB, Colucci WS. Opposing effects of beta1- and beta2-adrenergic receptors on cardiac myocyte apoptosis. Circulation. 1999; 100:1210–1217.
- Communal C, Singha K, Pimentel DR, Colucci WS. Norepinephrine stimulates apoptosis in adult rat ventricular myocytes by activation of the beta-adrenergic pathway. Circulation. 1998; 98:1329–1334. [PubMed: 9751683]

- De Angelis N, Fiordaliso F, Latini R, Calvillo L, Funicello M, Gobbi M, Mennini T, Masson S. Appraisal of the role of angiotensin II and aldosterone in ventricular myocyte apoptosis in adult normotensive rat. *J Mol Cell Cardiol.* 2002; 34:1655–1665. [PubMed: 12505063]
- Duchanue, D. Underground steroid handbook (II) update: 1992. Venice, CA: HLR Technical books; 1992.
- Engelhardt S, Hein L, Wiesmann F, Lohse MJ. Progressive hypertrophy and heart failure in beta1-adrenergic receptor transgenic mice. *Pharmacology.* 1999; 96:7059–7064.
- Goldspink DF, Burniston JG, Ellison GM, Clark WA, Tan LB. Catecholamine-induced apoptosis and necrosis in cardiac and skeletal myocytes of the rat in vivo: The same or separate death pathways? *Exp Physiol.* 2004; 89:407–416. [PubMed: 15131072]
- Goldstein DR, Dobbs T, Krull B, Plumb VJ. Clenbuterol and anabolic steroids: a previously unreported cause of myocardial infarction with normal coronary arteriograms. *South Med J.* 1998; 91:780–784. [PubMed: 9715231]
- Hoffmann C, Leitz MR, Oberdorf-Maass S, Lohse MJ, Klotz KN. Comparative pharmacology of human beta-adrenergic receptor subtypes--characterization of stably transfected receptors in CHO cells. *Naunyn Schmiedebergs Arch Pharmacol.* 2004; 369:151–159. [PubMed: 14730417]
- Hon JK, Yacoub MH. Bridge to recovery with the use of left ventricular assist device and clenbuterol. *Ann Thorac Surg.* 2003; 75:S36–41. [PubMed: 12820733]
- January B, Seibold A, Whaley B, Hipkin RW, Lin D, Schonbrunn A, Barber R, Clark RB. beta2-adrenergic receptor desensitization, internalization, and phosphorylation in response to full and partial agonists. *J Biol Chem.* 1997; 272:23871–23879. [PubMed: 9295336]
- Jones SW, Baker DJ, Gardiner SM, Bennett T, Timmons JA, Greenhaff PL. The effect of the beta2-adrenoceptor agonist prodrug BRL-47672 on cardiovascular function, skeletal muscle myosin heavy chain, and MyoD expression in the rat. *J Pharmacol Exp Ther.* 2004; 311:1225–1231. [PubMed: 15243031]
- Kierzkowska B, Stanczyk J, Kasprzak JD. Myocardial infarction in a 17-year-old body builder using clenbuterol. *Circ J.* 2005; 69:1144–1146. [PubMed: 16127201]
- Liggett SB, Tepe NM, Lorenz JN, Canning AM, Jantz TD, Mitarai S, Yatani A, Dorn GW II. Early and delayed consequences of {beta}2-adrenergic receptor overexpression in mouse hearts : critical role for expression level. *Circulation.* 2000; 101:1707–1714. [PubMed: 10758054]
- Mann DL, Kent RL, Parsons B, Cooper G. Adrenergic effects on the biology of the adult mammalian cardiomyocyte. *Circulation.* 1992; 85:790–804. [PubMed: 1370925]
- Ng Y, Goldspink DF, Burniston JG, Clark WA, Colyer J, Tan L-B. Characterisation of isoprenaline myotoxicity on slow-twitch versus cardiac muscle. *Int J Cardiol.* 2002; 86:299–309. [PubMed: 12419570]
- Pack RJ, Alley MR, Dallimore JA, Wood KR, Burgess C, Crane J. The myocardial effects of fenoterol, isoprenaline and salbutamol in normoxic and hypoxic sheep. *Int J Exp Pathol.* 1994; 75:357–362. [PubMed: 7999636]
- Rajab P, Fox J, Riaz S, Tomlinson D, Ball D, Greenhaff PL. Skeletal muscle myosin heavy chain isoforms and energy metabolism after clenbuterol treatment in the rat. *Am J Physiol.* 2000; 279:R1076–R1081.
- Ryall JG, Gregorevic P, Plant DR, Sillence MN, Lynch GS. Beta 2-agonist fenoterol has greater effects on contractile function of rat skeletal muscles than clenbuterol. *Am J Physiol.* 2002; 283:R1386–1394.
- Sears MR, Taylor DR. The beta 2-agonist controversy. Observations, explanations and relationship to asthma epidemiology. *Drug Saf.* 1994; 11:259–283. [PubMed: 7848546]
- Sillence MN, Matthews ML, Moore NG, Reich MM. Effects of BRL-47672 on growth, beta 2-adrenoceptors, and adenylyl cyclase activation in female rats. *Am J Physiol.* 1995; 268:E159–167. [PubMed: 7840174]
- Soppa GK, Smolenski RT, Latif N, Yuen AH, Malik A, Karbowska J, Kochan Z, Terracciano CM, Yacoub MH. Effects of chronic administration of clenbuterol on function and metabolism of adult rat cardiac muscle. *Am J Physiol.* 2005; 288:H1468–1476.

- Tan L-B, Burniston JG, Clark WA, Ng Y, Goldspink DF. Characterisation of adrenoceptor involvement in skeletal and cardiac myotoxicity induced by sympathomimetic agents: towards a new bioassay for beta-blockers. *J Cardiovasc Pharmacol*. 2003; 41:518–525. [PubMed: 12658052]
- Xiao R-P, Zhang S-J, Chakir K, Avdonin P, Zhu W, Bond RA, Balke W, Lakata EG, Cheng H. Enhanced Gi signaling selectively negates beta2-adrenergic receptor (AR)-but not beta1-AR-mediated positive inotropic effect in myocytes from failing rat hearts. *Circulation*. 2003; 108:1633–1639. [PubMed: 12975249]
- Xydas S, Kherani AR, Chang JS, Klotz S, Hay I, Mutrie CJ, Moss GW, Gu A, Schulman AR, Gao D, Hu D, Wei C, Oz MC, Wang J. {beta}-2 Adrenergic Stimulation Attenuates Left Ventricular Remodeling, Decreases Apoptosis, and Improves Calcium Homeostasis in a Rodent Model of Ischemic Cardiomyopathy. *J Pharmacol Exp Ther*. 2006
- Yacoub MH. A novel strategy to maximise the efficacy of left ventricular assist devices as a bridge to recovery. *Eur Heart J*. 2001; 22:534–540. [PubMed: 11259141]
- Zaugg M, Xu W, Lucchinetti E, Shaiq SA, Jamali NZ, Siddiqui MAQ. beta adrenergic receptor subtypes differentially affect apoptosis in adult rat ventricular myocytes. *Circulation*. 2000; 102:344–350. [PubMed: 10899100]

Figure 1.

Immunohistochemical detection of myocyte apoptosis.

Caspase 3-positive myocytes (brown staining) in the heart (A) and soleus muscle (B) after a single injection of 3 mmol kg⁻¹ fenoterol. No positive staining was observed in control muscles (C; heart and D; soleus) from animals administered saline only. Cryosections are counterstained with haematoxylin (blue); scale bar = 10 μ m (panels A and C) or 100 μ m (panels B and D).

Figure 2.

Dose dependent β -agonist-induced myocyte apoptosis.

Incidence of myocyte apoptosis detected in the heart (A) and soleus muscle (B) after a single injection of fenoterol, clenbuterol or isoproterenol over the range 0.003 mmol kg⁻¹ to 3 mmol kg⁻¹. Data are presented as Means $\pm$ SEM ($n = 6$, in each group). * $P < 0.05$, significantly different from the vehicle control group. † $P < 0.05$, significantly different from an equimolar dose of fenoterol or isoproterenol.

Dose response for myocyte apoptosis in the heart (C) and soleus muscle (D) after administration of β -agonists (fenoterol, clenbuterol or isoproterenol). Data are presented as percentages relative to the most myotoxic dose: 3 mmol kg⁻¹ fenoterol, in the heart; 0.03 mmol kg⁻¹ clenbuterol, in the soleus.

Figure 3.

Effect of selective β -AR subtype antagonism on β -agonist-induced myocyte apoptosis. Incidence of myocyte apoptosis in the heart (A) or soleus muscle (B) of independent groups of animals administered either bisoprolol (β_1 -AR antagonist) or ICI 118 551 (β_2 -AR antagonist) before being challenged with 3 mmol kg⁻¹ of fenoterol, clenbuterol or isoproterenol. Control animals received either 3 mmol kg⁻¹ agonist (fenoterol, clenbuterol or isoproterenol; positive control) or only the saline vehicle (negative control). Data are presented as Means $\pm$ SEM ($n = 6-8$, in each group). * $P < 0.05$, significantly different from administration of the respective β -agonist only (positive control).

Figure 4.

Blood pressure waveforms.

Representative blood pressure waveforms of conscious, unrestrained animals administered $0.03 \text{ mmol kg}^{-1}$ clenbuterol (left), saline only (middle) or $0.03 \text{ mmol kg}^{-1}$ fenoterol (right). Recordings were made via radiotelemetry 10 min after intraperitoneal injection of each agent.

Figure 5.

Time dependent changes in heart rate and blood pressure.

Heart rate (A), diastolic blood pressure (B) and systolic blood pressure (C) recorded after intraperitoneal injection of 3.0 mmol kg⁻¹ fenoterol, clenbuterol or saline vehicle only. Data presented as Means $\pm$ SEM ($n = 4$).

Figure 6.

Dose-dependent β -agonist-induced changes in heart rate and blood pressure. The effect of β -agonist administration on heart rate (A), diastolic blood pressure (B) and systolic blood pressure (C) measured via telemetry in conscious unrestrained animals. Data are presented as Means $\pm$ SD ($n = 4$). * $P < 0.05$, significantly different for saline only. † $P < 0.05$, significantly different from equimolar dose of fenoterol.