Life Sciences 88 (2011) 892–897

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Regulation of contractility and metabolic signaling by the β_2-adrenergic receptor in rat ventricular muscle

Joaquín Pérez-Schindler [a,*], Andrew Philp [b,1], Keith Baar [b,1], Jesús Hernández-Cascales [a]

[a] Department of Pharmacology, Faculty of Medicine, University of Murcia, Murcia, Spain
[b] Division of Molecular Physiology, University of Dundee, Dundee, UK

ARTICLE INFO

Article history:
Received 7 September 2010
Accepted 25 March 2011

Keywords:
β_2-adrenergic receptor
Cardiac contractility
Cardiac metabolism
AMPK
PI3K

ABSTRACT

Aims: Cardiac function is modulated by the sympathetic nervous system through β-adrenergic receptor (β-AR) activity and this represents the main regulatory mechanism for cardiac performance. To date, however, the metabolic and molecular responses to β_2-agonists are not well characterized. Therefore, we studied the inotropic effect and signaling response to selective β_2-AR activation by tulobuterol.
Main methods: Strips of rat right ventricle were electrically stimulated (1 Hz) in standard Tyrode solution (95% O_2, 5% CO_2) in the presence of the β_1-antagonist CGP-20712A (1 μM). A cumulative dose–response curve for tulobuterol (0.1–10 μM), in the presence or absence of the phosphodiesterase (PDE) inhibitor IBMX (30 μM), or 10 min incubation (1 μM) with the β_2-agonist tulobuterol was performed.
Key findings: β_2-AR stimulation induced a positive inotropic effect (maximal effect = 33 ± 3.3%) and a decrease in the time required for half relaxation (from 45 ± 0.6 to 31 ± 1.8 ms, −30%, $p<0.001$) after the inhibition of PDEs. After 10 min of β_2-AR stimulation, p-AMPKα^{T172} (54%), p-PKB^{T308} (38%), p-AS160^{T642} (46%) and p-CREB^{S133} (63%) increased, without any change in p-PKA^{T197}.
Significance: These results suggest that the regulation of ventricular contractility is not the primary function of the β_2-AR. Rather, β_2-AR could function to activate PKB and AMPK signaling, thereby modulating muscle mass and energetic metabolism of rat ventricular muscle.

Introduction

The contractile and metabolic proprieties of cardiac muscle are critical factors in the regulation of heart performance and the susceptibility to cardiac dysfunction (Hill and Olson, 2008). Heart function is modulated by the sympathetic nervous system through β-adrenergic receptor (β-AR) activity and this represents the main regulatory mechanism for cardiac performance. In the heart, the β_1 and β_2-AR are the main receptors expressed (Salazar et al., 2007) and are each thought to activate alternate signaling pathways and regulate diverse cellular responses (Xiao et al., 2004). Stimulation of the β_2-AR exerts a cardioprotective effect, whereas β_1-AR stimulation has a negative effect on cardiac function (Bernstein et al., 2005; Xiao et al., 2004; Zhu et al., 2001).

The β_1-AR is the most abundant subtype in the heart and the main regulator of cardiac contractility (Salazar et al., 2007; Yoo et al., 2009). β_1-AR modulates cardiac function via the stimulatory G protein ($G_{\alpha s}$)/adenylyl cyclase (AC)/cyclic AMP (cAMP)/cAMP dependent protein kinase (PKA) signal pathway, leading to the phosphorylation of proteins involved in regulating excitation-contraction coupling in cardiac muscle (Salazar et al., 2007). β_2-AR is coupled not only to $G_{\alpha s}$ but to an inhibitory G protein ($G_{\alpha i}$), that can block AC and prevent PKA activation (Xiao et al., 1995). This dual coupling to $G_{\alpha s}$ and $G_{\alpha i}$ is consistent with the lower inotropic response reported in rat heart following administration of β_2 compared to β_1-AR agonists (McConville et al., 2005). Therefore, the regulation of cardiac muscle contractility does not seem to be the primary function of β_2-AR in rodent heart.

In addition to inhibiting $G_{\alpha s}$/AC/PKA, $G_{\alpha i}$ has other functions within the heart. Together with the $G_{\beta\gamma}$ heterodimer, $G_{\alpha i}$ can activate phosphatidylinositol 3-kinase (PI3K) (Jo et al., 2002). In fact, some β_2-agonists induce hypertrophy of cardiac and skeletal muscle (Lynch and Ryall, 2008; Pönicke et al., 2003) and prevent apoptosis in a $G_{\alpha i}$/PI3K dependent manner (Communal et al., 1999; Zhu et al., 2001).

In association with the increase in cardiac muscle mass, β_2-agonists also affect muscle metabolism. Soppa et al. (2005) demonstrated that chronic treatment with the β_2-agonist clenbuterol increased carbohydrate (CHO) contribution to the tricarboxylic acid (TCA) cycle in rat ventricular cardiomyocytes, whereas the stimulation of β_2-AR in skeletal muscle increases fatty acid (FA) oxidation while glucose uptake is diverted towards glycogen synthesis (Nevzorova et al., 2006; Ngala et al., 2008; Yamamoto et al., 2007).

* Corresponding author at: Focal Area Growth and Development, Biozentrum, University of Basel, Klingelbergstrasse 50/70, CH-4056 Basel, Switzerland. Tel.: +41 61 267 2237; fax: +41 61 267 2221.
E-mail address: j.perezschindler@unibas.ch (J. Pérez-Schindler).
[1] Current address: Neurobiology, Physiology and Behavior, University of California, Davis, CA, United States.

0024-3205/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.lfs.2011.03.020

Collectively, these studies provide evidence that the β_2-AR could play an important role in the regulation of cardiac metabolism, however, to date, the cellular signaling pathways involved in these responses are not well characterized.

Chronic obstructive pulmonary disease (COPD) is associated with the development of pulmonary hypertension, which impairs right ventricle function by inducing pathological hypertrophy (MacNee, 2010). Therefore, the assessment of the impact of β_2-agonists on right ventricle function has a particular interest for the treatment of COPD. Tulobuterol is a β_2-agonists used for long-term treatment of COPD in humans (Patel, 1985). Besides early pharmacological studies (Gonzalez-Sicilia et al., 1988; Laorden et al., 1985; Ruff et al., 1988), the effects of tulobuterol on cardiac function have not been examined. Therefore, the aim of the present study was to determinate the effect of selective β_2-AR stimulation with tulobuterol on contractility and intracellular signaling in rat right ventricle preparations ex vivo.

Materials and methods

The study was performed in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and approved by the Ethical Committee of the University of Murcia.

Animals and right ventricle isolation

Male adult Sprague–Dawley rats (250–300 g) were killed by a blow to the head followed by cervical dislocation. The heart was rapidly removed and placed in Tyrode solution (136.9 mM NaCl, 5 mM KCl, 1.8 mM $CaCl_2$, 1.5 mM $MgCl_2$, 0.4 mM NaH_2PO_4, 11.9 mM $NaHCO_3$, 5 mM D-glucose and 5 mM pyruvate) maintained at pH 7.4 and gassed with 95% O_2 and 5% CO_2. The external wall of the right ventricle was extracted and cut into strips ~1 mm wide, ~10 mm long and ~0.5 mm thick. Subsequently, the strips were mounted longitudinally between two platinum electrodes, placed in an organ bath (30 mL) with Tyrode solution (37 °C, pH 7.4, 95% O_2 and 5% CO_2) and electrically stimulated (Grass SD-9) against a preload of ~1 g at 1 Hz, 5 ms and supramaximal voltage (~25% above response threshold). Contraction amplitude and relaxation was measured with a force transducer (Grass FT-03) and amplified (Stemtech Inc., Houston, Texas) for future quantification. The strips were allowed to equilibrate with the experimental conditions for ~40 min before drug treatment.

Cumulative dose–response curve

After equilibration, a cumulative dose–response curve with the β_2-agonist tulobuterol (0.1–10 μM; Lab. Ferrer, Co. Spain) in the presence of the β_1-antagonist CGP-20712A (1 μM; Sigma) was performed. To determinate the selective β_2-AR activation, the β_2-antagonist ICI-118,551 (50 nM; Sigma) was used. The role of the cyclic nucleotide phosphodiesterase (PDE) was determined using the non selective PDE inhibitor IBMX (30 μM; Sigma). The β-antagonists and IBMX were added to the bath 15 min before tulobuterol addition. Drugs were added to the organ bath (30 mL) in a volume no higher than 0.1 mL. The experiments were finalized with the addition of 9 mM $CaCl_2$ to determinate the response capacity and maximal contractile amplitude of the preparations. Changes in contractile force induced by tulobuterol are expressed as a percentage of the control contraction amplitude (in the presence of the corresponding β-antagonist and IBMX, if applicable). The relaxation capacity of the strips was studied by the determination of the time required for half relaxation ($t_{1/2}$).

Incubation for protein phosphorylation measurement

To determine the metabolic signaling regulated in response to the selective β_2-AR activation, the strips were incubated after the equilibration with 1 μM CGP-20712A alone or in combination with a single dose of 1 μM tulobuterol for 10 min. On completion, cardiac strips were frozen in liquid nitrogen and stored at −80 °C until analysis.

Protein isolation

Samples were pulverized on dry ice, homogenized with a polytron in 200 μL of ice cold sucrose lysis buffer (50 mM Tris pH 7.5, 250 mM Sucrose, 1 mM EDTA, 1 mM EGTA, 1% Triton X 100, 50 mM NaF, 1 mM Na_3VO_4, 50 mM $Na_4P_2O_7$, 0.1% DTT) and shaken for 30 min at 4 °C. Samples were then centrifuged at 14 000 rpm for 10 min at 4 °C and the protein concentration of the supernatant determined by the DC protein assay (Bio-Rad, Hercules CA). Equal aliquots of protein were boiled for 5 min in Laemmli sample buffer (250 mM Tris–HCl pH 6.8, 2% SDS, 10% glycerol, 0.01% bromophenol blue, 5% β-mercaptoetanol).

Western blot

Samples were separated on an SDS-polyacrylamide gel (8.25%) for 50 min and then transferred to a Protran nitrocellulose membrane (Whatman, Dassel, Germany) for 60 min. Membranes were blocked for 1 h in 3% milk in Tris-buffered saline + 0.1% tween-20 (TBST) before overnight incubation at 4 °C with appropriate primary antibody in TBST (1:1000 dilution). Proteins were detected with a primary antibody to p-PKB^{T308} (Cell Signaling; 4056), p-$AMPK\alpha^{T172}$ (Cell Signaling; 2535), p-$CREB^{S133}$ (Rockland; 600-401-270), p-PKA^{T197} (Cell Signaling; 4781), the primary antibodies to p-$AS160^{T642}$ were kindly provided by Professor Grahame Hardie (Division of Molecular Physiology, University of Dundee). Since the tulobuterol treatments lasted only 10 min and therefore the total amount of the signaling proteins was unlikely to change, eEF2 (Cell Signaling; 2332) was used as a loading control for all western blots. Following incubation, membranes were washed 3 times with TBST before incubation with an appropriate peroxidase-conjugated secondary antibody in TBST (1:10000 dilution; Pierce, Rockford, IL). Antibody binding was detected using enhanced chemiluminescence HRP substrate detection kit (Millipore, Billerica, MA). Imaging and band quantification were carried out using a ChemiGenius Bioimaging Gel Doc System (Syngene, Cambridge, UK).

Statistical analysis

All values are expressed as mean ± S.E.M (n = 4–5 independent experiments). The log EC_{50} was calculated by the analysis of the non linear regression of the dose–response curve (Graph Pad Software, San Diego, CA). The statistical significance was determined using the Student *t*-test for paired and unpaired data. The significance difference was set at $p<0.05$.

Results

Inotropic and lusitropic effect of the selective β_2-AR stimulation

The β-antagonists and IBMX did not alter contraction amplitude compared to basal levels (data not shown). In addition to its effects on β_2-AR stimulation, tulobuterol has also been suggested to stimulate β_1-AR at high concentrations (Morin et al., 2000). To ensure that the effects of tulobuterol were β_2-AR specific, the β_1-antagonist CGP-20712A was used in combination with tulobuterol. Thus, tulobuterol in the presence of CGP-20712A did not produce a positive inotropic effect (n = 4; Fig. 1A and B). This data provides preliminary evidence that activation of the β_2-AR does not increase the contractile force of the heart. However, IBMX in combination with CGP-20712A produced a dose-dependent positive inotropic effect in response to tulobuterol, with a maximal effect of 33 ± 3.3% and a log EC_{50}: −6.9 ± 0.6 M (n = 5; Fig. 1A and B). Moreover, under these conditions, tulobuterol not only increased the contractile force but also induced a decrease in