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REMODELING OF CARDIOMYOCYTE IN CHRONIC ISOPROTERENOL DOSE ADMINISTERED MURINE MODEL

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ABSTRACT
β_2-Adrenoceptor agonists such as isoproterenol are anabolic in skeletal muscle, and because they promote hypertrophy and improve force producing capacity, they have potential application for enhancing muscle repair after injury. Synthetic β_2-adrenoceptor agonists were initially developed for acute asthma treatment, to facilitate bronchiolar smooth muscle dilation. Isoproterenol has been used to treat asthma by opening up the airways in the lungs. Despite their muscle anabolic properties, β-agonists have also been associated with some undesirable side effects, including increased heart rate (tachycardia) and muscle tremor, which have so far limited their therapeutic potential. Since the early 1990s, the use of β-agonists for the purpose of enhancing sporting performance has become increasingly prevalent. In fact, many athletes are not aware of the deleterious cardiovascular effects of chronic high-dose β-agonist administration and in many cases rely on anecdotal information about these compounds from nonscientific sources. The intent of this study is to characterize the effect isoproterenol on healthy murine hearts. Cardiac hypertrophy was commonly observed in mice when were treated chronic doses of β-agonist isoproterenol. In adult mice treated daily with an oral dose of isoproterenol (1.5 mg/kg) for 4 wk, cardiac hypertrophy was observed. The cardiac hypertrophy in isoproterenol-treated mice was associated with an increase in midventricular collagen deposition. Furthermore, it is possible that similar damage can contribute to a deterioration of cardiac functions.

KEYWORDS: Isoproterenol, cardiac muscle, Hypertrophy.

INTRODUCTION

Studies on animals have shown that β_2–agonist such as clenbuterol affect skeletal muscles, but they also affect heart deleteriously as evidenced by tachycardia, cardiac hypertrophy, and decreased cardiac performance (Duncan *et al* 2000; Maggioni 2001; Lynch, 2002). Systemic administration of β_2-adrenoceptor agonists can improve skeletal muscle regeneration after injury. However, therapeutic application of β_2-agonists for muscle injury has been limited by detrimental cardiovascular side effects. Intramuscular agonist administration may obviate some of these side effects (Ryall *et al* 2008). The therapeutic potential of these β_2–agonists show tremendous positive aspects in case of skeletal muscle, but very little information is available regarding the effects of these drugs on cardiac muscle. These drugs may have deleterious effects on structure and function of cardiac muscle. Chronic uses of β_2-agonists have been reported to have toxic effects on heart (Kendall and Haffner, 1993). Severe myocardial lesions were found in the heart of the sheep when given intravenous doses of salbutamol, isoproterenol or isoprenaline (Pack *et al* 1994). The β_2–agonists affect the muscle proteins in a number of ways. Collagen is a major non-contractile structural protein that plays an important role in myocardial structure and function. About 55% of total extracellular matrix (ECM) is collagen. Collagen maintains not only the three-dimensional structure of the tissue, but also enable the myocardium to resist deformation. Collagens play a pivotal role in contraction-relaxation cycle and subsequently determine the viscous and elastic behaviour of the myocardium (Bishop, 1998). Different ischemic heart diseases are invariably coupled with a deposition of collagen (Schapher and Speiser, 1992). Collagen turn over cycle in myocardium is regulated by matrix metalloproteinases (MMPs), a family of Zn-dependent proteases. Synthesis and degradation of ECM are balanced and controlled in a normal myocardium (Weber *et al* 1994; Tyagi *et al* 1996). Differential expressions of MMPs have been documented in mammalian hearts under different pathophysiological conditions (Gunja-Smith *et al* 1996; Spinale *et al* 1999).

MATERIALS AND METHODS

Drug administration and tissue harvesting

A stock solution of isoproterenol (2 mg/ml) was prepared in distilled water. Normal healthy-looking mice showing no sign of morbidity were divided into two group:1st group served as normal (control) animals received only saline water and 2nd group were administered a daily dose of isoproterenol hydrobromide (1.5 mg/kg body weight) for 30 days. Body weight of animals was recorded every week for 30 days. Animals were sacrificed at 5, 10, 20 and 30 days of experiment by cervical dislocation. Heart was excised and weighed.

Dry muscle mass and total tissue proteins

Dry muscle mass of mice heart was calculated according to Heverberg and colleagues (1975) as described elsewhere (Agrawal *et al* 2003). A weighed amount of tissue was homogenized in nine volumes of ice-cold distilled water. Proteins were precipitated in 10% TCA at 4 ^{0}C for 15 min. Precipitated proteins were separated by centrifugation at 2000 x g for 20 min and washed twice with ice-cold 10% TCA. The precipitate was then washed successively in (i) ice-cold 95% ethanol, (ii) ethanol: chloroform = 3: 1, (iii) ethanol: ether = 3: 1 (twice), and (iv) ether. The residue was dried to a constant weight in vacuum-drying oven. The dried powder was then weighed to determine the dry muscle mass. Total protein of muscle homogenate was estimated according to Lowry *et al. (*1951).

RESULTS

Body weight of animals (Table. 1)

Administration of β-adrenergic agonist, isoproterenol (1.5 mg/kg body weight) results in an increase in the body weight of mice. At the beginning of the experiment, the average body weight of the animals was 25.5 ± 1.10 g and there was no significant difference between the groups. There was a progressive increase in the body weight of the animals during chronic treatment of drug from 5 to 30 days. The increase in body weight was found to be 6.48%, 8.66%, 9.09% and 10.85% at 5, 10, 20 and 30 days respectively which was significantly higher ($p<0.05$) than the normal mice. On day 30 of chronic treatment, weight of isoproterenol treated animals were significantly higher (32.7 ± 1.69 g) as compared to normal control animals (29.5 ± 1.58 g).

Table.1

Groups	Body weight (g) in Days			
	5	10	20	30
N	26.2±1.32	27.7±1.37	28.6±1.51	29.5±1.58
NI	27.9±1.51*	30.1±1.55*	31.2±1.59*	32.7±1.69*
% Increase	6.48	8.66	9.09	10.85

Table 1: Effects of Isoproterenol (I) on body weight of mice from 5-30 days. Values are presented as mean ± SEM; *$p<0.05$ (n> 8). Mean weight of mice at the beginning was 25.2±1.10 g. **N-normal; I- Isoproterenol**

Heart weight of animals

β-adrenergic agonist, isoproterenol caused cardiac hypertrophy in animals as evident from increase in heart weight of treated mice as compared to normal control ones. There was a continuous increase in the cardiac muscle mass at all the stages of chronic treatment. The mean increase in weight of heart muscle was pronounced at all stages of experiment. This increase expressed in percent, was significantly higher (10.27, 12.95, 13.86 and 14.56) at 5, 10, 20 and 30 days respectively (Table 2).

Table. 2.

Groups	Heart weight (mg) in Days			
	5	10	20	30
N	120.51±1.66	128.25±1.78	132.74±2.41	138.62±2.67
NI	132.89±1.68*	144.87±2.46*	151.14±2.62*	158.81±2.79*
% Increase	10.27	12.95	13.86	14.56

Table 2: Effects of Isoproterenol (I) on heart weight of mice from 5-30 days. Values are presented as mean ± SEM; *$p<0.05$ (n> 8). Mean weight of heart at the beginning was 118.23±1.80 mg.

Ratio of muscle weight to body weight

Cardiac muscle (Table 3)

There was a constant increase in heart weight/body weight ratio (%) at all four stages of treatment. The increase in heart weight/body weight ratio was reported as 3.47%, 4.11%, 4.31% and 3.62% at 5, 10, 20 and 30 days respectively, which confirmed the establishment of cardiac hypertrophy in treated animals.

Table 3

Groups	Cardiac muscle/body weight ratio (%) in Days			
	5	10	20	30
N	4.60± 0.018	4.62± 0.019	4.64± 0.012	4.69± 0.014
NI	4.76± 0.019*	4.81± 0.011*	4.84± 0.013*	4.86± 0.017*
% Increase	3.47	4.11	4.31	3.62

Table 3: Changes in cardiac muscle to body weight ratio (mg/g) after administration of Isoproterenol from 5-30 days. Values are presented as mean ± SEM; *p<0.05 (n>8).

Dry muscle mass and total tissue protein
Cardiac muscle (Table 4 Fig.1)
Dry muscle mass of heart showed a progressive increase at all stages of chronic isoproterenol treatment. There was an increase from 181.5±1.66 µg/mg fresh tissue weight of normal innervated cardiac muscle to 199.1±1.08 µg/mg fresh tissue weight of treated heart, showing a significant increase of 9.69% at day 5 of study. This increase was 13.02% at day 30 of chronic treatment (Table 4; Figure 1). These changes in dry heart muscle mass are also supported by the data on total tissue proteins. Total tissue proteins in normal innervated heart muscle exhibited an increase of 12.30% at day 30 of chronic isoproterenol treatment. This shows that isoproterenol treatment induces cardiac hypertrophy in mice.

Table 4

Groups	Dry muscle mass (µg/mg fresh tissue weight) in Days			
	5	10	20	30
N	181.5±1.66	193.4±1.84	198.4±2.11	203.4±2.96
NI	199.1±1.08*	215.7±2.06*	223.3±2.62*	229.9±2.90*
% Increase	9.69	11.53	12.55	13.02

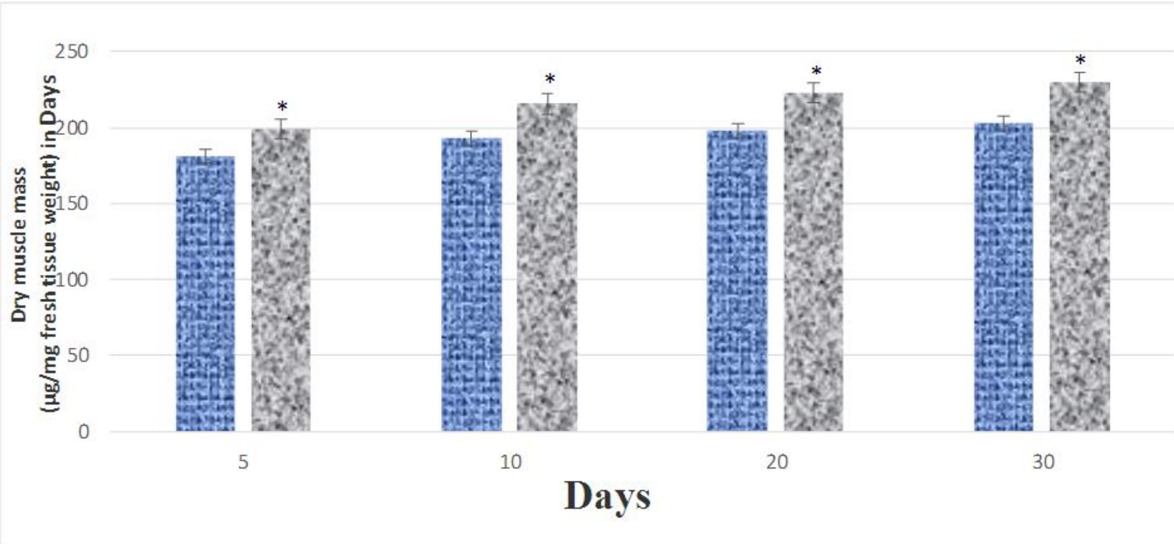

Fig.1

Table 4 Fig. 1: Changes in dry muscle mass of cardiac muscle (µg/mg fresh tissue weight) after administration of Isoproterenol from 5-30 days. Values are presented as mean ± SEM; *p<0.05 (n>8).

DISCUSSION
Cardiac hypertrophy leading to heart failure is one of the major causes of morbidity and mortality in the world. Cardiac hypertrophy is characterized by a chronic physiological increase in cardiac muscle mass resulting from systolic or diastolic wall stress. This often occurs normally during development, during pregnancy, and in response to sustained exercise (Lorell and Carabello 2000). Synthetic β_2-adrenoceptor agonists, such as isoproterenol and clenbuterol, were initially developed for acute asthma treatment, to facilitate bronchiolar smooth muscle dilation (Van Nolte *et al* 1974). The β_2-agonist isoproterenol has potent anabolic effects on rat skeletal muscle. Muscle mass and force-producing capacity of skeletal muscle has been attributed to a non-selective increase in the cross-sectional area of all muscle fiber types. Isoproterenol treatment causes a small increase in fatigability due to a decrease in oxidative metabolism in skeletal muscle fibers, with some cardiac hypertrophy (Larsson and Ramamurthy, 2000; Ryall *et al* 2004). Systemic administration of a β_2-adrenocepotor agonist has profound effect on global gene expression in skeletal muscle. In terms of hypertrophy,

β_2-adrenoceptor agonist treatment alters the expression of several genes associated with myostatin signaling (Pearen *et al* 2009). The repartitioning characteristics of β -agonists (increasing muscle mass and decreasing fat mass) have also made them attractive anabolic agents for use in livestock and by some athletes. However, potentially deleterious cardiovascular side-effects of beta-agonists need to be obviated in order for the therapeutic potential of beta-agonists to be realized (Koopman *et al* 2009). Chronic isoproterenol administration for 30 days (1.5 mg/kg body weight) resulted in an increase in the body mass of mice. On day 30 of chronic treatment, weight of innervated isoproterenol treated animals were significantly higher (32.7 ± 1.69 g) as compared to normal innervated control animals (29.5 ± 1.58 g), showing an increase of 10.85% in body weight of treated mice over the control animals. β-agonist treatment stimulates biosynthesis of proteins in skeletal muscles, therefore increase in the body mass of isoproterenol treated mice is due to the hypertrophy of muscle fibers. Similarly, these agonists have been reported to stimulate muscle growth by effectively increasing protein accretion in the cells (Costelli *et al* 1995). This increase in muscle mass is due to β_2-adrenoceptor-mediated protein accretion via increase in intracellular cAMP-promoting both an increase in protein synthesis and a decrease in degradation (Navegantes *et al* 2002). These β-agonists stimulate the β-adrenergic system in animals and humans, and have anabolic effects on muscles (Lynch and Ryall, 2008). Oral and intraperitoneal administration of beta-agonists results in increase in fiber diameter, dry muscle mass and protein content in cardiac and skeletal muscle of mice, thereby increases the body weight in treated animals (Sharma and Thakur, 2005). Present study revealed that there was a continuous increase in the cardiac muscle mass at all the stages of chronic treatment. The mean increase in weight of heart muscle was pronounced at all stages of experiment. This increase expressed in percent, was significantly higher (10.27, 12.95, 13.86 and 14.56) at 5, 10, 20 and 30 days respectively. Similarly, 4.31% increase in heart weight/body weight ratio was reported on day 30 of chronic administration. Similarly, dry muscle mass and total tissue protein in heart showed a progressive increase at all stages of chronic isoproterenol treatment. These findings are supported by some earlier studies which elucidate that administration of β-agonists induces cardiac hypertrophy in rats (Lynch *et al* 2007).

CONCLUSION

β-agonists despite their muscle anabolic properties, have also been associated with some undesirable side effects, including increased heart rate and muscle tremor, which have so far limited their therapeutic potential. Though isoproterenol induces muscle specific remodeling and increases the muscle performance in cardiac and skeletal muscle, but at the same time augments deposition of non-contractile protein collagen which induces some cardiovascular side effects. Therefore, present study aimed at effects of β-adrenergic stimulation using isoproterenol and its effects on structure and functions of cardiac and skeletal muscles have been explored in details. Therefore, a greater understanding of β-adrenergic signaling in cardiac and skeletal muscle is important for identifying its role in muscle growth, development and regeneration, and for identifying new therapeutic targets.

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