Isoproterenol ameliorates workstress-induced rat skeletal muscle degeneration

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β-Agonists though have been widely studied for their protein anabolic effects in skeletal muscles, but the lipid status under work stress and agonist treatment have not been understood well in the skeletal muscles and heart of rat. In the present study, adult male Wistar rats were subjected to work overload stress and β agonist isoproterenol treatment (2 mg kg⁻¹ day⁻¹ intraperitoneally) to examine, whether it ameliorates work stress-induced changes or not. Simultaneously, β₂ antagonist butoxamine (2 mg kg⁻¹ day⁻¹ intraperitoneally) was administered to another isoproterenol-treated group. Work stress led to myofibrillar degeneration as well as rapid utilization of lipid to meet increased energy demands and for muscle repair, which was reflected through histochemical localization of lipids and biochemical estimation of cholesterol and triglycerides. Significantly decreased cholesterol levels in skeletal muscles and heart muscles were noticed. As expected, isoproterenol reversed the conditions by raising cholesterol and triglyceride levels significantly in the skeletal muscles and also by ameliorating the degenerative changes in muscle fibres as induced by work overload. However, severe accumulation of lipids in the heart infers towards deleterious effects of isoproterenol on heart and thus remains a limiting factor for its immediate clinical application. Further research is needed to separate desirable effects of β agonists on skeletal muscles from any undesirable effects on the heart, so as to optimize their therapeutic potential.

Keywords: Isoproterenol, butoxamine, lipids, work stress.

β Adrenoceptor (βAR) agonists have been widely used in the treatment of bronchoconstriction in patients with asthma or chronic pulmonary disease, because of their ability to relax smooth muscles. However, at higher doses they result in a rapid and marked increase in skeletal muscle growth and reduction in body fat. These agonists are also known to revert muscle weaknesses, due to muscle disorders like denervation atrophy, muscular dystrophy as well as exercise stress and ageing. Though the precise mechanism responsible for the action of different β agonists remains unclear, but it has been postulated that they in vivo activate the βARs and produce the effects, as produced by the stimulation of sympathetic nervous system. β Agonists, because of their ability to mimic actions of sympathetic nervous system are also sometimes referred to as sympathomimetic substances. These substances include clenbuterol, isoproterenol, salbutamol, cimaterol, fenoterol and many others which possess similar muscle anabolic capabilities. βAR agonists are also known to markedly increase the catabolic and decrease the anabolic lipid metabolic processes in the adipocytes, thereby decreasing adipocyte hypertrophy, which consequently lead to decreased fat deposition.

βAR’s have been categorized into β₁, β₂ and β₃ sub-types, therefore, βAR agonists are also βAR-type specific. Clenbuterol, a β₂AR-specific agonist promotes skeletal muscle growth that has been attributed to accelerate protein turn-over rate. Isoproterenol, another β AR agonist, though not βAR type-specific (known to act on both β₁ and β₂ receptors) relaxes the smooth muscles, particularly those of bronchi as well as gastrointestinal tract and is also reported to retard denervation-induced muscle atrophy, similar to clenbuterol. Prolonged isoproterenol administration to growing chicks results in their increased live weight, largely due to increased dry muscle mass and total proteins of hypertrophied muscle fibers. In addition to skeletal muscle hypertrophy, isoproterenol administration also induces cardiac hypertrophy and oxidative stress in the myocardium, similar to clenbuterol. On the other hand, β AR blocking agents (agonists) selectively and competitively block the actions of catecholamines and βAR agonists, thereby reducing the stimulatory effects of sympathetic nervous system, as mediated through βAR activation. Butoxamine, a...
βAR antagonist, specific for β2ARs blocks the vasodilator and metabolic effects of β2AR stimulation. It also reverts clenbuterol-induced slow to fast fibre transformation of skeletal muscles in rats and is efficient in inhibiting isoproterenol-stimulated fat cell adenylate cyclase enzyme in humans, when compared to another cardioselective β blocking agent practolol.

The present study was undertaken to determine the clinical potential of βAR agonist isoproterenol in attenuating muscle atrophy under stress with following objectives: (i) to see the effects of work overload stress on the skeletal muscles (gastrocnemius and pectoralis) and heart of rat, (ii) whether isoproterenol is able to recover the animals from such a stress, and (iii) whether β2AR blockade by butoxamine is effective in suppressing undesirable effects of isoproterenol especially on heart.

Materials and Methods

Materials

Isoproterenol and butoxamine hydrochlorides were obtained from Sigma Chemical Co., USA. All the other chemicals used were of highest purity and analytical grade. Adult male rats of Wistar strain weighing (120-150 g) were obtained from Central Research Institute (CRI), Kasauli, Himachal Pradesh and maintained in the animal house of the department under suitable hygienic conditions. Proper arrangement of light (16 hours daylight) and temperature (24 ± 2°C) was ensured. Animals were provided standard food (Hindustan Lever Ltd.) and water ad libitum. All the procedures conducted were in accordance with the guidelines of the Institutional Animals Ethics Committee.

Animals and treatment

Animals were randomly divided into six groups, each group having six animals. Group I animals were normal and served as control; group II animals were denervated (sciatic nerve of left hind limb was cut ~ 1 cm) as per the method described elsewhere. Denervation resulted in paralysis of one of the hind limbs as a result of which the contralateral limb was subjected to continuous work overload stress. Because of imbalance created in its movements due to paralyzed hind limb, the pectoralis muscle was also subjected to work stress. Group III included rats administered isoproterenol daily intraperitoneally (2 mg/kg body wt for 15 days). Group IV animals were also administered isoproterenol, similar to group III and also received butoxamine hydrochloride (2 mg/kg body wt for 15 days). Groups V and VI included denervated rats that received similar treatments as groups III and IV, respectively. The rationale for dose was decided on the basis of previous study, according to which β-agonist in the range of 1-2 mg/kg/day was effective in inducing muscle hypertrophy. Also, the maximum protein anabolic effects were obtained within a fortnight, without any significant difference by prolonged administration.

Biochemical, histological and histochemical study

Rats of all the groups were maintained under similar experimental conditions for a period of 30 days and were sacrificed on day 7 and 30 of post-denervation by cervical dislocation. At least 4-6 animals from each group were sacrificed at each stage. Gastrocnemius from the contralateral limb, pectoralis and heart were excised immediately and processed for biochemical, histological and histochemical studies. Tissues used for histological study were fixed in Bouin’s fixative and the tissues for histochemistry were stored at 4°C till further use, whereas the tissues for biochemical study were immediately employed for lipid extraction. Lipid extract was then used to estimate cholesterol quantitatively using sulphuric acid and acetic anhydride. Triglycerides were estimated as described previously. Total lipids were histochemically localized in the cryostat cut thin sections (7 μm) of gastrocnemius and pectoralis using Sudan Black B, whereas the fatty acids were localized by sulphuric nile blue staining technique. Haematoxylin-eosin staining was done to study the histopathological changes.

Statistical analysis

Statistical significance was determined by Student’s t-test to find out significance of main differences among the groups. Differences were assumed significant at P<0.01 and P<0.001.

Results and Discussion

Muscles display a remarkable ability to adapt to altered conditions. Lipids are an important source of metabolic energy for sustained work in skeletal muscles. Present study is focussed at understanding the alterations occurring in the cholesterol and triglyceride levels of skeletal muscles and heart under
stress. Histochemical methods showed that the muscles contain three different types of muscle fibres as type I, IIA and IIB. Both gastrocnemius and pectoralis muscles from normal rats show differential staining pattern with Sudan stain, forming a checker board (Fig. 1a), with type I (slow twitch, oxidative) fibres taking up the maximum stain, whereas type IIB (fast glycolytic) fibres are least stained and third type stained intermediately, depending upon the amount of lipids present in them. Gastrocnemius and pectoralis muscles from the rats, subjected to work overload revealed slightly varied staining pattern, as compared to the normal. Lesser lipid content is demonstrated by the type I fibres of exercised muscles on day 7, as reflected through lightly stained fibres (Fig. 1b), suggesting that due to work overload, the muscles depend on lipids for energy. As slow and sustained contractions are brought about by narrow red (type I) fibres (via oxidative phosphorylation), the fats in them get utilized.

Isoproterenol treatment to the animals under stress reversed the lipid distribution pattern back to normal on day 30 (Fig. 1c), suggesting its positive influence towards recovery. On the other hand, isoproterenol-treated normal rats show lipid accumulation in the fibres, indicating that the fats, lipolyzed by isoproterenol in the adipose tissue are mobilized to the muscular tissue, where they are much needed. Lesser lipid content is seen in the butoxamine-supplemented animals on day 30 (Fig. 1d), suggesting that the butoxamine reversed the isoproterenol effects to some extent, during the later stages. Since exercise leads to hypertrophy of certain muscle fibres, therefore, most of the lipids are used in the formation of new membrane systems.

Histochemical localization of free fatty acids (FFA) in the skeletal muscles of normal animals show very less amount of FFA (Fig. 2a), a slight amount of which is also noticed in the exercised animals (Fig. 2b). Work stress leads to sarcolemmal disruptions, resulting in fatty acid leakage into the extra-cellular spaces. The availability of fatty acids from the adipose tissue can be limiting during exercise and that within skeletal muscles, there is an upper limit to fatty acid oxidation, probably occurring at the mitochondrial level. Isoproterenol treatment to the animals under stress, however, shows a large amount of fatty acids and neutral fats accumulated in the perimysial spaces on day 30, as is evident from Fig. 2c. This is in agreement with an earlier study, where β agonist salbutamol administered in lambs resulted in significant increase in fatty acid content of longissimus dorsi muscle than control. As expected, the butoxamine treatment to the isoproterenol-treated animals seems to sustain the conditions near normal (Fig. 2d), thereby suggesting antagonistic effect of butoxamine.

Histopathological study revealed hypertrophied fibres, depicting large and increased number of nuclei forming longitudinal chains in the muscles under work overload stress, when compared to the normal on day 7 (Fig. 3a and b). Exercise stimulates the anabolic processes, which results in muscle

Fig. 1—Histochemical localization of lipids showing three main types of fibres – slow oxidative, fast glycolytic and intermediate oxidative as well as glycolytic fibres (I, IIA, IIB) in gastrocnemius of rats (a) normal (b) under work stress at day 7, showing less lipids, (c) recovery with isoproterenol treatment is seen on day 30 and (d) butoxamine supplementation antagonizes isoproterenol effects on day 30. (SBBx 200).
exercise does not affect the amount of free fatty acids (FFA), very small amounts of FFA are present in the brain. During stress, the amount of FFA is increased, leading to the accumulation of blue-colored acidic fats and pink fatty acids in perimysial spaces on day 30, as seen in rats under work stress. Butoxamine treated animals show reversal on day 7.

Fig. 2—Histochemical localization of free fatty acids in pectoralis muscle exhibiting negligible amount of pink-colored FFA in (a) normal and (b) rats under work stress (c) accumulation of blue-colored acidic fats and pink fatty acids in perimysial spaces on day 30 is seen and (d) butoxamine treated animals showing reversal on day 7. (SNBx 200).

Fig. 3—Haematoxylin-eosin stained (a) normal gastrocnemius, (b) gastrocnemius subjected to work stress demonstrating hypertrophied fibres (↗) with different shapes of nuclei ( vật) on day 7 and (c) degenerating fibres (↗) with pinhead foci (↗) on day 30. Pectoralis muscle treated with isoproterenol and butoxamine (d) showing largely hypertrophied fibres (↗) and proliferated connective tissue (↗) on day 30. (HEx 900).

During later stages, i.e., day 30, degenerating muscle fibres show pin-head foci and fibre merging at certain places (Fig. 3c). β-Agonists are known to induce muscle hypertrophy, a great deal of evidence for which is provided by the histological preparation of pectoralis from isoproterenol and butoxamine-treated animals, where the hypertrophied fibres as well as proliferated connective tissue are clearly seen (Fig. 3d). Here, butoxamine seems to have least effect in suppressing isoproterenol effects, which is contrary to the earlier finding, where it was found to reverse slow to fast fibre transformation as induced by clenbuterol.

Results of quantitative estimation of cholesterol and triglycerides are presented in Table 1. The cholesterol content showed a decrease on day 30, when compared to day 7, in all the muscles, except the heart of isoproterenol-treated animals, where increase was noticed. This observation is in agreement with previous studies on chicks and rats. The cholesterol level of all the three muscles decreased in the animals subjected to work overload, decrease being significant in the gastrocnemius muscle on day 30, suggesting that work stress leads to increased utilization of lipids. Isoproterenol
administration to normal and denervated animals, however, increased the cholesterol level in all the muscles, increase being significant in heart and pectoralis, which infers towards its deleterious effects on heart34,35. Butoxamine was unable to repress isoproterenol effects during the early stages, but the antagonist was able to block the thermogenic effects induced by β agonist5,9,11,35. Butoxamine treatment to the isoproterenol-treated denervated animals initially increased the triglyceride level with a subsequent significant decrease on day 30 in all the muscles, which confirmed that the antagonist was able to block the β3 ARs completely by this time and isoproterenol at this stage was acting exclusively via β1 receptors.

It could be concluded from the present study that the work stress led to myofibrillar degeneration, sarcolemmal breakdown and lipid utilization in the muscles to meet increased energy requirements. β Agonist though led to muscle hypertrophy and mobilized the fats to muscles, thereby recovering the normal conditions, yet at the same time increased lipids in the heart indicates that these agonists may affect cardiac function deleteriously9,11,35. Butoxamine administration to isoproterenol-treated group, however, inhibited isoproterenol-induced changes to some extent in the skeletal muscles but not in the heart. Whether any deleterious effects on heart can be prevented effectively with β3 AR antagonist, deserves further att

### Table I—Effect of butoxamine (2 mg kg⁻¹ day⁻¹) and isoproterenol (2 mg kg⁻¹ day⁻¹) on cholesterol and triglyceride levels (mg/g fresh tissue wt) of pectoralis, gastrocnemius and heart muscle of rats under work overload stress

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cholesterol</th>
<th>Triglycerides</th>
<th>Pectoralis</th>
<th>Cholesterol</th>
<th>Triglycerides</th>
<th>Gastrocnemius</th>
<th>Cholesterol</th>
<th>Triglycerides</th>
<th>Heart</th>
<th>Cholesterol</th>
<th>Triglycerides</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV (CIB)</td>
<td>3.986± 0.458</td>
<td>3.220± 0.051</td>
<td>156.77± 5.174</td>
<td>141.64± 5.492</td>
<td>3.548± 0.189</td>
<td>2.988± 0.055</td>
<td>256.92± 3.969</td>
<td>224.56± 6.101</td>
<td>3.216± 0.282</td>
<td>2.477± 0.019</td>
<td>88.76± 1.979</td>
</tr>
<tr>
<td>V (WI)</td>
<td>3.662± 0.126</td>
<td>3.564± 0.042</td>
<td>255.98± 5.386</td>
<td>246.65± 4.837</td>
<td>2.886± 0.126</td>
<td>2.662± 0.284</td>
<td>245.23± 3.265</td>
<td>218.14± 2.838</td>
<td>1.054± 0.029</td>
<td>1.365± 0.027</td>
<td>176.27± 3.926</td>
</tr>
<tr>
<td>VI (WIB)</td>
<td>3.243± 0.327</td>
<td>2.651± 0.179</td>
<td>242.32± 8.409</td>
<td>235.89± 15.706</td>
<td>3.289± 0.270</td>
<td>2.359± 0.018</td>
<td>238.90± 4.540</td>
<td>163.03± 2.511</td>
<td>3.242± 0.328</td>
<td>1.259± 0.216</td>
<td>183.76± 3.328</td>
</tr>
</tbody>
</table>

C= control; W= work overload; I= isoproterenol; B= butoxamine

P values: *< 0.01; **< 0.001
further attention. Nevertheless, the findings of this study indicate that β agonists have a definite therapeutic potential in treating various myopathies. However, further investigation into β agonist stimulated pathways, leading to cardiac hypertrophy and its malfunctioning, is essential for the continued development of this approach for tackling muscular diseases.

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References