Differential Effects of 3 Beta Blockers on Lipid Peroxidation in Hyperthyroid Muscle

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Abstract

To determine whether beta blockade protects against the acceleration of lipid peroxidation in hyperthyroid rat soleus (slow-oxidative) muscle, in vivo chronic (3 weeks) effects of 3 beta blockers with different ancillary properties on mitochondrial oxidative enzymes, antioxidant enzymes, and thiobarbituric acid-reactive substances were investigated. The rats were rendered hyperthyroid by the administration of thyroxine and treated simultaneously with either carteolol (a nonselective blocker with partial agonist activity; 30 mg/kg/day), atenolol (a betal-selective blocker; 50 mg/kg/day), or arotinolol (a nonselective blocker with weak alpha-blocking action; 50 mg/kg/day) over a 3 week period. Hyperthyroidism induced tachycardia, an increase in the mitochondrial oxidative enzymes, manganese (mitochondrial) superoxide dismutase and thiobarbituric acid-reactive substances, and a decrease in the other antioxidant enzymes. The tachycardia was alleviated completely by either atenolol or arotinolol, but partially by carteolol. Arotinolol, but neither carteolol nor atenolol, inhibited the increase in oxidative enzymes and thiobarbituric acid-reactive substances. The levels of antioxidant enzymes were minimally affected by the beta-blocker treatment. Betal-, and possibly alpha- as well, but not betal-, blockade suppressed mitochondrial hypermetabolism and protected against peroxidative injury in the hyperthyroid soleus muscle. Partial agonist activity was not beneficial.

Mitochondrial hypermetabolism associated with accelerated respiratory electron transport has been postulated to result in increased production of superoxide anion radical (Boveris \textit{et al.} 1976). This species can initiate free radical chain reactions eventually leading to peroxidation of mitochondrial membrane lipid unless detoxified by the coordinated action of superoxide dismutase (superoxide oxidoreductase, EC 1.15.1.1., SOD), glutathione peroxidase (glutathione: \textit{H}_2\textit{O}_2 oxidoreductase, EC 1.11.1.9, GPX), and catalase (\textit{H}_2\textit{O}_2: \textit{H}_2\text{O}_2 oxidoreductase, EC 1.11.1.6, CAT). We previously demonstrated that hyperthyroidism accelerated mitochondrial oxidative metabolism in rat soleus (slow oxidative) muscle,
leading to an increase in the level of thiobarbituric acid-reactive substances (TBARS) (Asayama et al., 1987). This increase was associated with decreased activity of GPX and CAT, and induction of mitochondrial (manganese) SOD (MnSOD), but not cytosolic (copper zinc) SOD (CuZnSOD).

Beta blockers alleviate cardiac symptoms and certain metabolic abnormalities in hyperthyroidism (Gelfand et al., 1987) independently of the thyroid hormone status. Beta-adrenergic action has also been demonstrated to be enhanced in hyperthyroid skeletal muscle (Karlberg et al., 1974), suggesting a therapeutic benefit of beta blocking agent on thyrotoxic myopathy. Currently, beta blockers with different ancillary properties are available. The present study evaluated the effects of 3 different beta blockers. Carteolol is a non beta1 selective blocker with partial agonist activity (PAA), and is more than 10 times as potent as propranolol in terms of both beta1 and beta2 effects (Yabuuchi and Kinoshita, 1974). Arotinolol is a nonselective blocker possessing a weak alpha-blocking action (alpha/beta=1/8), but no PAA (Hara et al., 1978). Its relative potency compared to propranolol is 9 times as a beta1-blocker and 25 times as a beta2-blocker. Atenolol is a beta1-selective blocker without PAA (Barrett et al., 1973). Most beta blockers are proved to be effective in hypertensive animals. On the other hand, their effect on the oxidative stress induced by thyroxine (T4) in skeletal muscle (Asayama et al., 1987) has not yet been studied. For the treatment of hypertension, the properties of beta selectivity and PAA are generally considered to reduce the untoward effects of beta blockers (Frishman, 1987). However, it has not been determined whether such properties are beneficial or not in hyperthyroid skeletal muscle.

To determine whether adrenergic beta-blockade modifies T4-induced oxidative stress in skeletal muscle, the in vivo effects of chronic administration of these drugs on the level of mitochondrial oxidative enzymes, antioxidant enzymes, and TBARS in hyperthyroid rat soleus muscle were investigated.

**Materials and Methods**

**Drugs**

Carteolol was dissolved in distilled water at concentration of 15 mg/ml. Atenolol was dissolved at a concentration of 25 mg/ml by titration with 2N HCl and pH was adjusted to 8.0. Arotinolol was suspended in 0.5% methyl cellulose at a concentration of 25 mg/ml.

**Animal treatments**

Five-week-old male Sprague-Dawley rats (Shizuoka Laboratory Animal Center, Shizuoka, Japan) were used for the present study. Details of the experimental design have been reported elsewhere (Asayama et al., 1989). Two sets of experiments (Exp) were performed. The study groups in Exp I consisted of controls, and the groups treated with either T4, T4 + carteolol (30 mg/kg/day sc), or T4 + atenolol (50 mg/kg/day sc); those in Exp II were controls, T4, and T4 + arotinolol (50 mg/kg/day po). The group for each experiment consisted of 7 animals. Heart rate measured one day before sacrifice in the T4 groups in Exp I and II increased to a similar extent (Asayama et al., 1989). On the other hand, the heart rate of the animals was completely normalized in the groups treated with either atenolol or arotinolol throughout the interval of the drug administration. Carteolol partially alleviated the tachycardia. The rats were killed under pentobarbital anesthesia (50 mg/kg ip), and their sera and bilateral soleus muscles were obtained.

**Biochemical analysis**

Serum T3 and T4 in the rats given T4 were well in the hyperthyroid range and were similar to those reported previously (date not shown). The tissue homogenate was prepared as described previously (Asayama et al., 1987). The rat MnSOD and CuZnSOD were assayed by specific radioimmunoassays (Asayama and Burr, 1985). Cytochrome c oxidase, fumarase and glutathione peroxidase were assayed spectrophotometrically,
catalase polarographically, and TBARS fluorimetrically, also as described previously (Asayama et al., 1987). Protein was measured by the technique of Lowry et al. (1951).

**Statistical analysis**

The data are presented as the means±SE. Statistical significance was determined by the method of least significant difference, calculated after one way analysis of variance.

**Results**

**Body weight and soleus muscle weight**

The final body weight for the controls, T4, T4 + carteolol and T4 + atenolol groups in EXP I was 288±6, 252±3, 254±6 and 262±8 g, respectively, and was somewhat less in the T4-treated groups (p<0.01) than in the controls. That for the controls, T4, and T4 + arotinolol groups in Exp II was 280±5, 277±4 and 274±4 g, respectively. The weight of the bilateral soleus muscles in the controls, T4, T4 + carteolol and T4 + atenolol groups in Exp I was 223±6, 202±4, 212±10 and 202±11 mg, respectively, not significantly different from one another. Similarly, that in the controls, T4, and T4 + arotinolol groups in Exp II was 213±3, 208±8 and 210±4 mg, respectively.

**Activity of mitochondrial marker enzymes in soleus muscle**

Cytochrome c oxidase activity in soleus muscle was increased in the T4 groups in Exp I and II (Fig. 1). Carteolol did not modify this increase, and atenolol appeared to even intensify the increase (Exp I). On the other hand, arotinolol normalized the T4-induced increase in cytochrome c oxidase (Exp II). The fumarase activity was in-

![Fig. 1. Cytochrome c oxidase activity in soleus muscle. Data are means±SE (no. 7). □: controls, ■: T4, ■: T4 + carteolol, ■: T4 + atenolol, and ■: T4 + arotinolol groups. Statistical significance: a. p<0.05, b. p<0.01, c. p<0.001 (vs. controls), and d. p<0.001 (vs. T4).](image)

![Fig. 2. Fumarase activity in soleus muscle. Data are means±SE (no. =7). Each bar represents the group indicated in the legend to Fig. 1. Statistical significance: a. p<0.001 (vs. controls), and b. p<0.01 (vs. T4).](image)
creased in the T4, T4+carteolol, and T4+atenolol groups in Exp I to a similar extent (Fig. 2). The activity in the T4+arotinolol group was significantly increased, but less markedly than that in the T4 group in Exp II.

**Antioxidant enzymes in soleus muscle**

MnSOD in all T4-treated groups in Exp I and II was increased markedly (Fig. 3). In the T4+atenolol group in Exp I, and in the T4+arotinolol group in Exp II it was further increased compared to the respective T4 groups. Table 1 lists the levels of CuZnSOD, GPX, and CAT in the soleus muscle. CuZnSOD in the T4 group in Exp I was unaltered, but slightly decreased in the T4+carteolol and T4+atenolol groups, and was also slightly decreased in the T4, and T4+arotinolol groups. GPX activity was markedly decreased in all T4-treated groups, and the treatment with beta-blockers did not modify the activity. CAT activity was unaltered in the T4 group in Exp I, but decreased significantly in the rest of the T4-treated groups in Exp I and II.

**TBARS level in soleus muscle**

TBARS in the T4 groups in Exp I and II was increased markedly (Fig. 4). Carteolol did not suppress the increase significantly.

### Table 1. Copper zinc superoxide dismutase, glutathione peroxidase and catalase in soleus muscle

<table>
<thead>
<tr>
<th></th>
<th>CuZn-superoxide dismutase</th>
<th>Glutathione peroxidase</th>
<th>Catalase</th>
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<tbody>
<tr>
<td><strong>Ex I</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>1.34±0.06</td>
<td>700±18</td>
<td>1.03±0.07</td>
</tr>
<tr>
<td>T4</td>
<td>1.27±0.04</td>
<td>301±13</td>
<td>1.08±0.07</td>
</tr>
<tr>
<td>T4+carteolol</td>
<td>1.14±0.045a</td>
<td>311±6d</td>
<td>0.79±0.0666f</td>
</tr>
<tr>
<td>T4+atenolol</td>
<td>1.20±0.04a</td>
<td>291±16d</td>
<td>0.91±0.04</td>
</tr>
<tr>
<td><strong>Exp II</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>1.44±0.04</td>
<td>592±19</td>
<td>0.99±0.02</td>
</tr>
<tr>
<td>T4</td>
<td>1.32±0.03a</td>
<td>266±9d</td>
<td>0.75±0.03c</td>
</tr>
<tr>
<td>T4+arotinolol</td>
<td>1.32±0.02d</td>
<td>297±6d</td>
<td>0.67±0.0256c</td>
</tr>
</tbody>
</table>

Data are means±SE of 7 observations. Values are expressed as μg/mg protein for CuZn-superoxide dismutase, mU/mg protein for glutathione peroxidase, and μmolO2/min/mg protein for catalase. Statistical significance is as follows: a. p<0.05, b. p<0.02, c. p<0.01, d. p<0.001 (v. controls), and e. p<0.05, f. p<0.01 (vs. T4).
Fig. 4. Level of thiobarbituric acid-reactive substances in soleus muscle. Data are means ±SE (no.=7). Each bar represents the group indicated in the legend to Fig. 1. Statistical significance: a. p<0.001 (vs. controls), and b. p<0.01, c. p<0.001 (vs. \(T_4\)).

The TBARS level in the \(T_4+\)atenolol group was even higher than that in the controls. On the other hand, arotinolol suppressed the increase almost completely.

**Discussion**

The induction of SOD is considered to afford protection against oxidant challenge (Freeman and Crapo, 1981). However, Scott et al. (1987) reported that genetically transformed bacteria in which SOD was exclusively increased without a concurrent increase in other antioxidant enzymes were more susceptible to oxidant toxicity. They concluded that the activities of antioxidant enzymes must be balanced in order to be effective. In the hyperthyroid soleus muscle, the mitochondrial oxidative metabolism was accelerated, and the MnSOD was increased presumably as an adaptive response to enhanced oxidative stress. Conversely, the other antioxidant enzymes were decreased or tended to be decreased, and, as a defense system, they did not appear to protect against the acceleration of lipid peroxidation. This lack of protection may be due to the unbalanced regulation of the antioxidant enzymes in the hyperthyroid soleus muscle.

Arotinolol suppressed the acceleration of both oxidative metabolism and lipid peroxidation in the skeletal muscle, while the other blockers tested did not. In the preliminary experiment, arotinolol affected neither oxidative enzymes nor antioxidant enzymes in euthyroid animals, and therefore the suppression brought about by the arotinolol treatment observed here appeared to be specific to hyperthyroid skeletal muscles (Asayama et al., 1989). Thus, the parallel change in these two systems in response to both hyperthyroidism and beta blocker treatments observed here supports the view that mitochondrial superoxide generation is increased in response to enhanced oxidative metabolism (Boveris et al., 1976) induced by hyperthyroidism, and that this species is an initiator of the free radical reactions leading to peroxidation of membrane lipid. Although an increase in the MnSOD in response to arotinolol, and also to atenolol, treatment was observed, there was no increase in GPX or CAT. Therefore, such a change did not appear to contribute to the suppression of lipid peroxidation observed in the group treated with arotinolol. The modifications of the activities of oxidative enzymes appeared to be of primary importance, as was also suggested by the results obtained in the cardiac muscle model (Asayama et al., 1989).
The beta-adrenergic mechanism is known to play an essential role in modulating the oxidative metabolism in skeletal muscle. A chronic injection of the beta-adrenergic stimulant isoprenaline could induce the activities of mitochondrial oxidative enzymes in skeletal muscle (Harri and Valtola, 1975). Furthermore, the enzymatic adaptation to endurance exercise training (i.e., increased activities of oxidative enzymes) did not take place in rats receiving propranolol (Harri, 1980). More recently, Ji et al. (1986) demonstrated that this increase was inhibited by a nonselective, but not by a beta1 selective, blocker, suggesting that the beta2 effect mediates the adaptive response of oxidative enzymes.

Sympathetic blockade has been reported to reduce the increased oxygen consumption caused by T4, although some authors failed to detect a significant effect (Howitt and Rowlands, 1966). Beta blockers possessing membrane stabilizing activity, drugs not used in the present study, have recently been recognized to inhibit the conversion of T4 to T3 (Heyma et al., 1980). The metabolic suppression by such drugs in hyperthyroidism has also been considered to be at least partly attributable to the reduction in the circulating T3 per se (Saunders et al., 1978). On the other hand, Nilsson et al., (1979) showed that the basal metabolic rate was reduced to the same extent by propranolol and atenolol, with the latter not affecting the serum T3 concentration. Of particular interest, Stout et al. (1969) reported that combined alpha-(phenoxybenzamine)- and beta-(propranolol) blockade significantly reduced the oxygen consumption and increased the body weight in hyperthyroid patients without affecting the resin T3 uptake. However, the implication of alpha blockade is debatable in hyperthyroidism in which the function of alpha adrenoceptor in tissues is attenuated (Williams and Leffkowitz, 1979) independently of the blockade.

Effects of various beta-blockers on oxidative enzymes in hyperthyroid soleus muscle have not been studied previously. The beta-blockers possessing PAA have been reported to reduce the heart rate to a lesser extent than those without PAA in hyperthyroidism (Gibberd and Staffurth, 1973). The lack of suppression by cartelol was likely due to inadequate beta-blockade because of PAA. This notion is supported by our previous report that cartelol has failed to suppress the T4-induced acceleration of lipid peroxidation in cardiac muscle (Asayama et al., 1989). The present observation that atenolol suppressed the heart rate but not the oxidative metabolism in the soleus muscle can be explained by the theory that the oxidative enzymes in the skeletal muscle are predominantly modulated by the beta2 mechanism (Ji et al., 1986). In contrast, lipid peroxidation was significantly suppressed by atenolol in our cardiac muscle model in which the beta1 mechanism is predominant (Asayama et al., 1989). Thus, sartindol was considered to suppress the oxidative metabolism in the soleus muscle by a potent beta2-blockade (Hara et al., 1978). Additionally, a synergistic effect of alpha-blockade may contribute to the suppression (Stout et al., 1969).

The effect of beta-blockers on thyrotoxic myopathy is still in debate. Angeras et al. (1987) and Hasselgren et al. (1984) found no effect of beta blockers on the protein degradation induced by T4 in human and rat skeletal muscles. Angeras et al. (1986) also reported that beta blockers did not modify T3-induced morphologic changes in rat skeletal muscles. On the other hand, beta blockade was found to improve the nitrogen balance in hyperthyroid patients (George et al., 1975), and the muscle power in severely affected patient (Murchison et al., 1979). Beta2 blockade is also known to improve finger tremors (McDevitt and Nelson, 1978).

The present study demonstrated that
beta\textsubscript{2} blockade, and possibly alpha blockade as well, but not beta\textsubscript{1} blockade alone protected against oxidative stress in hyperthyroid soleus muscle. PAA was not beneficial in terms of such a biochemical process. Thus, beta\textsubscript{1} selectivity and PAA, which are generally considered to afford safety for the treatment of hypertension, do not appear to be beneficial for the protection of skeletal muscle against oxidative stress in hyperthyroidism.

**Acknowledgement**

The authors gratefully acknowledge Teiko Niitsu and Hajime Obi for their technical assistance. This work was supported in part by Grants-in-Aid 62770644 and 63570431 from the Ministry of Education (Japan).

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