RELATIVE AND COMBINED EFFECTS OF PROPYLTHIOURACIL, ETHANOL AND PROTEIN DEFICIENCY ON MUSCLE


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Abstract — A hypermetabolic state with increased oxygen consumption has been described in alcoholic hepatitis, playing a major role in ethanol-induced liver damage. Based on its ability to decrease oxygen consumption, propylthiouracil (PTU) has been proposed as a therapeutic agent in this context. On the other hand, several muscle changes have been described in hypothyroidism, including both atrophy and hypertrophy of muscle fibres. The aim of this experimental study was to analyse the effects of PTU on the alcohol-induced changes in muscle fibre size and proportion, also taking into account the presence or absence of protein deficiency. The study was performed on 64 male Wistar rats divided into eight groups, fed with: (1) Lieber-DeCarli control diet: (2) an isocaloric 36% ethanol-containing diet: (3) an isocaloric 2% protein-containing diet: (4) an isocaloric 36% ethanol 2% protein-containing diet, without and with PTU, respectively. Right gastrocnemius muscle was removed 2 months later and histochemical and morphometric studies were performed. Type IIb fibre atrophy was observed both in the alcoholic and protein-deficient animals, but not in the PTU-treated animals. The combination of protein deficiency and ethanol led to a more marked type IIb atrophy, with PTU reversing this effect. Malnutrition led to a decrease in type I fibre diameter: ethanol and PTU caused an increase in its size and PTU reversed the effect of protein deficiency. Proportion of type IIb fibres decreased in the three experimental groups without PTU with respect to the control, especially in the alcoholic protein-deficient animals. PTU-treated animals, especially those fed a low-protein diet, showed a more marked reduction in type IIb fibre proportion than that presented by the groups without PTU. However, an increase in type I fibre proportion was observed in the PTU-treated animals, especially marked in those fed a low-protein diet. Thus, PTU seems to ameliorate ethanol-induced changes on type IIb muscle fibres.

INTRODUCTION

Ethanol consumption may lead to muscle damage, including an acute form (Fahlgren et al., 1957), and a more common chronic myopathy (Ekbom et al., 1964). In the latter, muscle fibre atrophy occurs, especially regarding type IIb fibres (Slavin et al., 1983; Martin and Peters, 1985; González-Hernández et al., 1989; Preedy et al., 1989). Accompanying protein deficiency and/or marasmus-type malnutrition (a common feature among alcoholics) seems to play an additive role (Conde-Martel et al., 1992), although controversy exists regarding this matter (Urbano-Márquez et al., 1989; Romero et al., 1989).

Antithyroid drugs, such as propylthiouracil, have been proposed as a therapeutic approach in acute alcoholic hepatitis (Orrego et al., 1987), although some results point against its true efficacy (Halle et al., 1982; Pierrugues et al., 1989). In any case, it is important to keep in mind that hypothyroidism is associated with muscle changes, type II fibre atrophy (McKeran et al., 1975) and also fibre hypertrophy (Raju et al., 1971; Khalaeel et al., 1983) having been described. It is therefore important to identify and analyse the combined and relative effects of ethanol and propylthiouracil on muscle, also taking into account the presence or absence of associated protein deficiency. These aspects formed the aim of the present investigation.

ANIMALS AND METHODS

Thirty-two male Wistar rats were divided into four groups of eight animals each. The control rats received the Lieber-DeCarli control liquid diet (Lieber and DeCarli, 1989: Lieber et al.,...
1989) (Dyets Inc., Bethlehem, Pennsylvania, USA) containing 1 kcal/ml: a second group consisted of another eight animals fed an isocaloric, 36% ethanol-containing diet; the third, an isocaloric, 2% protein-containing diet; and the fourth, an isocaloric, 2% protein- and 36% ethanol-containing diet. 6-n 2-Propylthiouracil (PTU) (Sigma, St Louis, MO, USA) was added to these diets at a concentration of 0.05% (w/w) (i.e. ~30 mg/day), following dosage used in other studies (Kalland et al., 1978; Childs et al., 1991). This amount of PTU leads to the development of hypothyroidism; indeed, serum T3 of the PTU-treated animals was undetectable, and serum T4 ranged from undetectable to 0.53 ng/dl, with a mean value of 0.07 and a median of 0.01 ng/dl (Radioimmunoanalysis, Behring, Marburg, Germany).

Another four groups of eight animals each were fed respectively the same diets mentioned before, but without PTU. All these diets (those for the eight groups of animals) were prepared weekly by dissolving the nutrient mixture and the separate vitamin mix in water. Those rats receiving the alcohol, protein-deficient diet were allowed dietary consumption ad libitum, and the same amount consumed by these animals was then given to the other groups. This pair-feeding process was repeated every 2 days, always adjusting the amount of liquid diet received by the other groups to that consumed by the animals fed the protein-deficient, ethanol-containing diet. The mean daily amount of diet consumed was (in ml or kcal, means ± SD): (1) animals without PTU: 61.7 ± 4.2 ml the controls, 59.6 ± 5.0 ml the alcoholic animals, 58.5 ± 7.0 ml the low-protein, ethanol-fed animals, and 60.4 ± 3.5 ml the low-protein-fed animals; (2) animals with PTU: 59.0 ± 3.0 ml the controls, 60.1 ± 2.0 ml the alcoholics, 58.2 ± 2.9 ml the low-protein, ethanol-fed animals, and 55.2 ± 2.0 ml the low-protein-fed animals.

No differences were thus noted between the amount of diet consumed by the animals of the eight different groups (F = 1.81, P = 0.10).

Two months later the animals were anaesthetized with pentobarbital, and killed. The right gastrocnemius muscle was carefully removed. This muscle was chosen because it contains a mixture of type I and II fibres (Preedy and Peters, 1990; Preedy et al., 1990). A 3–5-mm-thick cross-section was cut from the middle portion (belly) of the muscle and rapidly frozen in liquid nitrogen. Sections were cut at 8 μm using a cryostat at −40°C. Fibres were classified according to the acid–alkali sensitivity (pH 4.3, 4.6, 9.6) of the ATPases in the myosin isoenzymes: type I (slow oxidative) fibres; type Ila (fast oxidative glycolytic) fibres; and type IIb (fast glycolytic) fibres.

The morphometric study was performed by means of a Magiscan image analysis system, using the Genias program. Six randomly selected fields of 400 × 400 μm of each animal, at a magnification of ×200 (at least 300 fibres) were used for the study of the mean diameter of the fibres, and six fields of 770 × 770 μm, at a magnification of ×100 for the study of fibre proportions.

Blood samples were obtained before killing for serum albumin (Bromcresol green, automated BM/Hitachi 717, Boehringer Mannheim) and T3 and T4 determination (by radioimmunoassay).

The different parameters mentioned above were compared statistically between the eight groups using analysis of variance (ANOVA) and then Student–Newman–Keuls test. Single correlation studies were performed to determine the significance of the relation between two quantitative variables. Moreover, two-way analysis of variance was used to determine the relative weight of PTU, protein malnutrition, and ethanol in the differences observed, as well as the possible interactions between these three factors.

**RESULTS**

Results regarding the four groups without PTU have been reported elsewhere (Conde-Martel et al., 1992) and are also summarized in this work.

**Weight change and serum albumin**

Animals fed the control diet showed a significant weight gain (means ± SD) during the study period (323 ± 18 vs 392 ± 11 g); those fed the diet with 36% ethanol and 18% protein did not show changes in body weight (316 ± 20 vs 313 ± 32 g), whereas animals of the other two groups (low-protein without and with ethanol respectively) showed significant weight loss (322 ± 17 and 310 ± 28 g vs 259 ± 16 and 204 ± 33 g, respectively).

Animals of the groups treated with PTU lost weight, especially those fed low-protein diets,
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Table 1. Serum albumin and weight difference

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum albumin (g/dl)</th>
<th>Weight difference (g) (final weight – initial weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>3.25 ± 0.32</td>
<td>+ 67.5 ± 13.6</td>
</tr>
<tr>
<td>Group II (ethanol)</td>
<td>2.84 ± 0.21</td>
<td>– 31 ± 22.7</td>
</tr>
<tr>
<td>Group III (low protein)</td>
<td>2.70 ± 0.15</td>
<td>– 62.5 ± 14.9</td>
</tr>
<tr>
<td>Group IV (ethanol &amp; low protein)</td>
<td>2.16 ± 0.44</td>
<td>–106.0 ± 10.5</td>
</tr>
<tr>
<td>Group V (PTU control)</td>
<td>3.30 ± 0.32</td>
<td>– 54.7 ± 22.6</td>
</tr>
<tr>
<td>Group VI (ethanol + PTU)</td>
<td>3.28 ± 0.24</td>
<td>– 30.0 ± 10.0</td>
</tr>
<tr>
<td>Group VII (low protein + PTU)</td>
<td>2.36 ± 0.19</td>
<td>–121.3 ± 7.9</td>
</tr>
<tr>
<td>Group VIII (ethanol + low protein + PTU)</td>
<td>2.12 ± 0.25</td>
<td>–123.3 ± 30.8</td>
</tr>
</tbody>
</table>

Analysis of variance

Significance between groups

Main effects

<table>
<thead>
<tr>
<th>Low protein</th>
<th>Ethanol</th>
<th>PTU</th>
</tr>
</thead>
<tbody>
<tr>
<td>P &lt; 0.01, negative</td>
<td>P &lt; 0.01, negative</td>
<td>P &lt; 0.01, negative</td>
</tr>
</tbody>
</table>

Interactions

<table>
<thead>
<tr>
<th>Low protein</th>
<th>Ethanol-PTU</th>
<th>P &lt; 0.025, negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTU-2% protein</td>
<td>P &lt; 0.01, positive</td>
<td></td>
</tr>
</tbody>
</table>

either with or without ethanol: the control group with PTU had an initial weight of 308 ± 8 g and a final weight of 253 ± 25 g; the ethanol. 18% protein with PTU group, an initial weight of 294 ± 14 g and a final weight of 264 ± 15 g; the low-protein. PTU-treated group, an initial weight of 304 ± 14 and a final weight of 183 ± 13 g, and the ethanol. 2% protein group with PTU, an initial weight of 311 ± 31 and a final weight of 188 ± 15 g. Two-way ANOVA (Table 1) revealed that the three factors — ethanol, protein deficiency and PTU — played significant and independent roles on weight loss, a positive interaction (that is, a potentiation of the effect) existing between protein deficiency and PTU, and a negative one (that is, an opposite effect) between PTU and ethanol.

Regarding serum albumin (Table 1), both ethanol and protein deficiency decreased serum albumin levels, a negative interaction existing between ethanol and PTU.

As it can be seen, despite weight loss, serum albumin levels of the animals receiving either PTU and 18% protein-containing diet or PTU and the 36% ethanol and 18% protein-containing diet were normal, in contrast with the reduced levels observed in the ethanol-fed animals without PTU.

Serum albumin significantly correlated with final weight ($r = 0.61, P < 0.0001$).

Fibre diameter

Fibre diameter data are listed on Table 2. As a rule, treatment with PTU resulted in an increase in diameter of the three types of fibres, especially marked in type I fibres. Whereas no differences existed between the control animals with and without PTU, the other three groups treated with PTU showed an increase in type I fibre diameter. Protein deficiency exerted a negative effect on type I fibre diameter, reducing it, whereas PTU increased type I fibre size.

Only the ethanol- and PTU-treated animals showed an increase, and the animals fed the 2% protein diet without PTU showed a significant decrease, in type IIa fibre diameter. Indeed, protein deficiency independently reduced type IIa fibre size, whereas PTU increased it. A negative interaction existed between PTU and ethanol regarding type IIa, fibre size.

PTU-treated animals, either with or without ethanol or protein deficiency, showed similar type IIb fibre diameter to the control group, in contrast with type IIb fibre diameter of the animals treated with ethanol and/or protein deficiency without
Table 2. Mean fibre diameter

<table>
<thead>
<tr>
<th>Group</th>
<th>Type I (µm)</th>
<th>Type IIa (µm)</th>
<th>Type IIb (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>47.33 ± 3.84</td>
<td>58.19 ± 6.31</td>
<td>51.23 ± 6.44</td>
</tr>
<tr>
<td>Group II (ethanol)</td>
<td>50.29 ± 6.43</td>
<td>58.36 ± 5.04</td>
<td>40.79 ± 3.36</td>
</tr>
<tr>
<td>Group III (low protein)</td>
<td>45.49 ± 2.85</td>
<td>52.18 ± 5.82</td>
<td>35.56 ± 3.32</td>
</tr>
<tr>
<td>Group IV (ethanol &amp; low protein)</td>
<td>40.94 ± 4.05</td>
<td>43.92 ± 4.10</td>
<td>32.13 ± 2.15</td>
</tr>
<tr>
<td>Group V (PTU control)</td>
<td>53.19 ± 2.40</td>
<td>55.51 ± 4.79</td>
<td>52.98 ± 4.74</td>
</tr>
<tr>
<td>Group VI (ethanol + PTU) (low protein + PTU)</td>
<td>60.43 ± 3.12</td>
<td>64.22 ± 4.29</td>
<td>54.25 ± 5.63</td>
</tr>
<tr>
<td>Group VII</td>
<td>58.92 ± 4.88</td>
<td>54.93 ± 3.77</td>
<td>52.73 ± 3.58</td>
</tr>
<tr>
<td>Group VIII (ethanol + low protein + PTU)</td>
<td>55.24 ± 9.37</td>
<td>55.72 ± 6.12</td>
<td>51.26 ± 7.41</td>
</tr>
</tbody>
</table>

Analysis of variance

- IV vs all the others
- III vs II–VIII
- IV vs I, II, v–VIII

Significance between groups (SNK)

Main effects

- Low protein
  - Ethanol: P = 0.022, negative
  - PTU: P = 0.0001, positive

Interactions

- 2% protein–ethanol: P = 0.001, positive
- 2% protein–ethanol: P = 0.003, positive
- PTU–ethanol: P = 0.01, negative
- PTU–ethanol: P = 0.02, negative

DISCUSSION

Ethanol is metabolized mainly in the liver to acetaldehyde and acetate. This process increases oxygen consumption and leads to an intracellular accumulation of reduced equivalents. The normal difference in oxygen tension between periportal and pericentral hepatocytes becomes exaggerated, due to the increased oxygen consumption by the former. Thus, hepatocytes of the pericentral area, in which oxygen tension is low, are unable to oxidize this excess of reduced equivalents and become damaged early in alcoholic
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Table 3. Proportion of the three fibre types

<table>
<thead>
<tr>
<th>Group</th>
<th>Type I (%)</th>
<th>Type II (%)</th>
<th>Type IIb (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>12.70 ± 3.26</td>
<td>37.98 ± 4.19</td>
<td>48.65 ± 3.15</td>
</tr>
<tr>
<td>Group II (ethanol)</td>
<td>10.77 ± 4.61</td>
<td>49.89 ± 2.01</td>
<td>39.27 ± 2.79</td>
</tr>
<tr>
<td>Group III (low protein)</td>
<td>14.88 ± 4.78</td>
<td>42.09 ± 4.88</td>
<td>43.00 ± 2.43</td>
</tr>
<tr>
<td>Group IV (ethanol &amp; low protein)</td>
<td>14.28 ± 3.33</td>
<td>48.51 ± 3.20</td>
<td>37.17 ± 2.68</td>
</tr>
<tr>
<td>Group V (PTU control)</td>
<td>21.01 ± 2.24</td>
<td>54.00 ± 4.02</td>
<td>24.98 ± 2.38</td>
</tr>
<tr>
<td>Group VI (ethanol + PTU)</td>
<td>25.16 ± 10.12</td>
<td>46.24 ± 3.09</td>
<td>28.60 ± 7.46</td>
</tr>
<tr>
<td>Group VII (low protein + PTU)</td>
<td>33.41 ± 3.07</td>
<td>51.42 ± 2.93</td>
<td>15.16 ± 2.19</td>
</tr>
<tr>
<td>Group VIII (ethanol + low protein + PTU)</td>
<td>14.83 ± 2.53</td>
<td>60.39 ± 6.45</td>
<td>25.50 ± 3.22</td>
</tr>
</tbody>
</table>

Analysis of variance
Significance between groups (SNK)

Main effects
Low protein
- P < 0.01, positive

Ethanol
- P < 0.01, negative

PTU
- P < 0.01, positive

Interactions
- Ethanol-PTU, P = 0.01, negative
- Ethanol-2% protein, P < 0.01, negative

Liver disease (Lieber, 1980). PTU may exert a protective effect in this setting, mainly by reducing oxygen consumption (Orrego et al., 1979).

Muscle fibres lack both the MEOS and alcohol dehydrogenase pathways, so a direct protective effect of PTU on muscle fibres, similar to that observed in hepatocytes, should not be expected. However, our study shows that PTU seems to ameliorate the effects of ethanol and protein deficiency on muscle.

Our results regarding the effect of PTU on control animals are in accordance with those obtained by some authors who have studied hypothyroid myopathy. This entity affects both proportion and diameter of muscle fibres, although results obtained by different authors are not fully concordant. Reduced type II fibre proportion with an increase in type I fibre proportion was observed by McKeran et al. (1975) and Wiles et al. (1979), and an increased type I fibre proportion has also been reported by Montgomery (1992); thus, these results regarding fibre proportion are similar to those obtained in our study. And also with the observation that hypothyroidism leads to a trans-formation of type II into type I fibres (Ianuzzo et al., 1977). Impairment of the activity of several enzymes involved in the glycolytic pathway (Dimitriades et al., 1989; Esteller et al., 1994) and glycogen breakdown (Czech et al., 1980; Taylor et al., 1992) may account for the reduction in type IIb fibre proportion, although mitochondrial impairment, affecting oxidative metabolism, has also been described (Argov et al., 1988; Kaminsky et al., 1992).

Type I fibre atrophy has been described by Spiro et al. (1970), whereas others (Emser and Schimrigk, 1977; Del-Palacio et al., 1990) have found type II fibre atrophy; other authors reported either atrophy (Cheek et al., 1966), hypertrophy (Raju et al., 1971) or a mixture of normal, atrophied and hypertrophied muscle fibres, without specifying the fibre type (Aström et al., 1961; Bergouignan et al., 1967; Fessel, 1968; Afifi et al., 1974; Bahillo-del-Rio et al., 1984; Monforte et al., 1990), and others, type I fibre hypertrophy (Khlaaeeli et al., 1983). Although increased muscle bulk has been widely described (Norris and Panner, 1966; Afifi et al., 1974; Klein...
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The underlying mechanism of muscle hypertrophy remains unclear. In our study, diameters of the three fibre types were similar in the control animals receiving PTU and in the controls without PTU, in accordance with some of the cases reported by other authors (Norris and Panner, 1966; Fessel, 1968; Afifi et al., 1974). Therefore, in our study, the addition of PTU to the control animals led to a change of the fibre types from type IIb to type I, without significantly altering fibre size.

Ethanol may cause muscle damage by several mechanisms, e.g. a direct effect on membrane function and fluidity, impairment of free radical scavenger mechanisms, increased lipid peroxidation and alterations in muscle content of some trace elements (Preedy and Peters, 1990). Both ethanol and/or acetaldehyde by themselves, or ethanol-derived systemic metabolic disturbances may affect muscle fibre atrophy. The end product of ethanol metabolism, acetate, is promptly consumed by muscle fibres, whereas glucose uptake is reduced (Juhlin-Dannfelt et al., 1977). This fact, together with impairment of glycogen breakdown (Cussó et al., 1989) may alter contraction of those fibres whose metabolism depends on anaerobic glycolysis, as is the case of type IIb fibres, especially if we consider that some enzymes involved in the glycolytic pathway are also inhibited by ethanol (Langohr et al., 1983). Because contraction is a stimulus for protein synthesis, low activity of type IIb fibres may lead to its atrophy. Moreover, acetaldehyde forms adducts with actin (Xu et al., 1989), thus enhancing atrophy of these fibres.

As expected, fibre atrophy is also more marked if protein supply is limited. In our study, ethanol caused a slight increase in type I fibre diameter, and a significant type IIb fibre atrophy; type IIb fibre diameter was further reduced in the animals...
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F = 36.77, p < 0.0001

Fig. 2. Type II fibre proportions.
Symbols are as in Fig. 1

Animals treated with either ethanol or protein deficiency and PTU showed an increase in type I and IIb fibre diameter, compared with the control animals and with the animals treated with ethanol and/or a 2% protein-containing diet without PTU. The increase in fibre diameter is especially striking in the ethanol. 18% protein-fed animals. Indeed, this group also showed a significant increase in type IIa fibre diameter. PTU also counteracts the type I fibre atrophy observed in the protein-deficient animals.

Fibre atrophy reflects a decreased protein content, either due to decreased synthesis or increased catabolism. All the three factors considered in this study (ethanol, PTU and protein deficiency) may affect protein metabolism. Ethanol reduces protein synthesis (Preedy and Peters, 1990), but also reduces slightly protein catabolism (Martin and Peters, 1985). Protein deficiency inhibits protein synthesis more markedly than catabolism, with a net result of muscle atrophy. Hypothyroidism reduces both protein synthesis and catabolism, (Carter et al., 1981). Perhaps reduction of muscle catabolism underlies muscle hypertrophy described in hypothyroid patients. In any case, from our study, it follows that hypothyroidism affects protein metabolism in a different way than do ethanol and protein deficiency. PTU exerting a paradoxical counteraction on net protein breakdown mediated by ethanol and protein deficiency, ameliorating alcoholic myopathy in our experimental model.

REFERENCES


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10. 501-510.


