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LIVER PEROXISOMAL FATTY ACID OXIDIZING SYSTEM DURING AGING IN CONTROL AND CLOFIBRATE-TREATED MICE.

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SUMMARY

We have previously described an aging-related decrease in the peroxisomal polyunsaturated fatty acid oxidizing system in mouse liver. In order to determine whether peroxisome synthesis is involved in this phenomenon, we focused our work on different peroxisomal enzyme activities during aging in the liver of mice fed for 5 days with either a control or a clofibrate supplemented diet which enhanced peroxisome biogenesis. Liver peroxisomal acyl-CoA oxidase (AOX), catalase (CAT) and urate oxidase (UOX) activities per gram of liver were determined.
In control mice, UOX activity was not affected by aging whereas CAT and AOX activities were significantly decreased. At day 300 the clofibrate treatment increased all activities although UOX was not significantly increased. Thereafter, enzyme activities after clofibrate treatment were severely depressed at day 680. CAT and UOX were not induced in very old clofibrate-treated animals, whereas AOX was induced 7 fold in such mice compared to an 11 fold induction in day 300 animals.
The present results suggest that: 1- Aging decreased the peroxisomal polyunsaturated fatty acid oxidizing system. 2- This took place via a specific decrease in AOX activity. 3- Since clofibrate treatment triggers the peroxisomal proliferation, the aging-related decrease in peroxisomal activities might be due to an alteration in peroxisome synthesis.

INTRODUCTION

Biological membranes contain large amounts of long-chain and very long chain fatty acids (LCFA and VLCFA) that affect membrane activities [1,2]. Membrane LCFA and VLCFA composition results in part from a balance between fatty acid biosynthesis, occurring in the endoplasmic reticulum [3], and fatty acid degradation. LCFA and VLCFA are degraded by both mitochondria and peroxisomes [4,5]. First estimations attributed a minor role to peroxisomal

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oxidation of LCFA in rat hepatocytes [6], but recent evidence has demonstrated that peroxisomes play a major role in liver fatty acid oxidation [7]. Previous studies have reported an aging-related decrease in Δ6-desaturase, the key enzyme in VLCFA biosynthesis [8-10] and it was proposed that this decrease could be a key mechanism in the aging process [8] through alterations in membrane fatty acid composition and therefore in membrane functions. However, a relationship between membrane fatty acid composition and Δ6-desaturase activity is not yet clearly demonstrated. Thus, the aging-related decrease in Δ6-desaturase activity is not sufficient to explain alterations in membrane fatty acid composition occurring during aging [8,11,12].

Modifications in membrane polyunsaturated fatty acid content reported in human peroxisomal disorders emphasize the role of peroxisomes in determination of membrane fatty acid composition [13,14,15].

We have previously shown that peroxisomal oxidation activity for different polyunsaturated fatty acids parallels the endoplasmic reticulum Δ6-desaturase activity during development and aging, including the dramatic aging-related decrease [16]. In order to determine which mechanism is involved in this aging-related decrease in peroxisomal activity, we have investigated whether mouse liver peroxisomal proliferation is affected by aging.

MATERIALS AND METHODS

Animal treatments. Female OF1 mice of different ages were fed ad libitum for 5 days with either a control or a 0.5% w/w clofibrate-supplemented diet (APAE-INRA, Jouy-en-Josas, France). Animals were then killed, weighed and the liver was immediately removed and weighed prior to homogenization. The liver was homogenized in buffer containing 0.25 M sucrose, 5 mM Mops, 1 mM EDTA, 0.1% (v/v) ethanol, pH7.2. The homogenate was centrifuged 12 min at 1700 X g at 4°C. The supernatant (S1) was preserved, the pellet was homogenized in the same buffer, centrifuged 12 min at 1700 X g and the resulting supernatant was combined with S1, and adjusted to 10 ml/g of liver prior to enzyme assays.

Enzyme assays: Catalase activity was determined as described by Aebi [17]. Urate oxidase activity was measured according to Mahler [18]. Palmitoyl-CoA oxidase activity was determined as recommended by Lazarow [19] and protein content was assayed using the BioRad protein assay kit (BioRad, France).

RESULTS

All activities are given in figure 1. At days 300, 560 and 680, the number of animals was respectively 5 and 8 in control groups and 6, 4 and 4 in clofibrate-treated groups. At day 560, activity was significantly decreased by

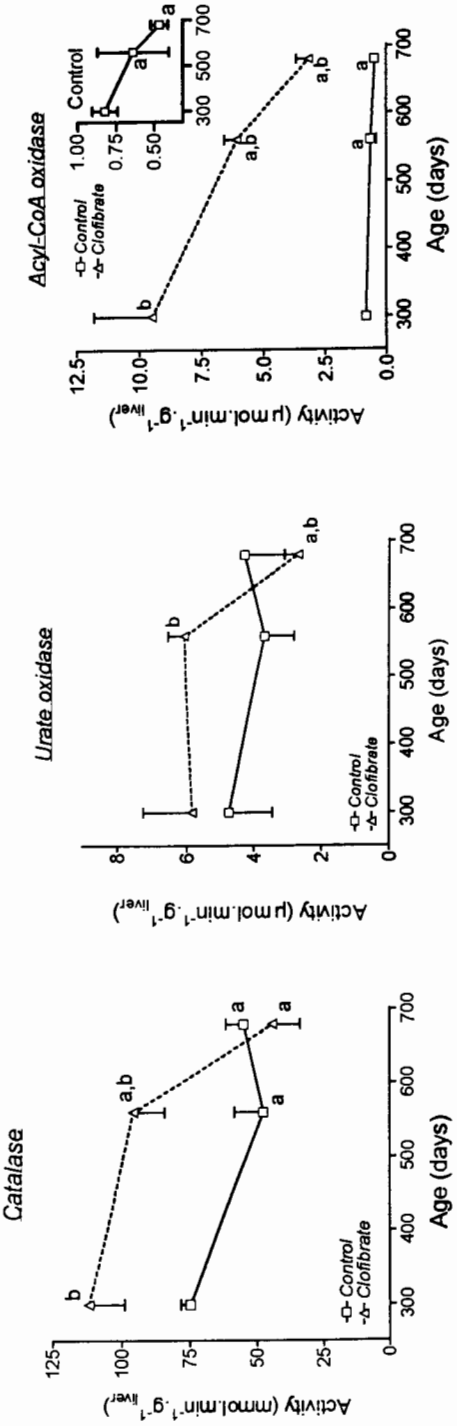

FIG. 1. Effect of aging on different peroxisomal activities in the liver of OF1 mice fed a control or clofibrate (0.5%) diet. For Acyl-CoA oxidase, control activity is shown on a larger scale in inset. ^{a}, significantly lower than day 300 value. ^{b}, significantly higher than control value. All statistical analyses were done using an unpaired one-tailed *t*-test with $p<0.05$

37% for catalase and by 22% for acyl-CoA oxidase in animals fed the control diet. Urate oxidase was not affected in control animals from day 300 to 680. After clofibrate treatment at day 560, activity was significantly decreased by 15% for catalase and 36% for acyl-CoA oxidase. Urate oxidase was not affected during this period. At day 680, all activities in clofibrate-treated animals decreased dramatically and significantly (at least 50%), including urate oxidase activity. In clofibrate-treated animals, catalase activity was induced 1.5 fold at day 300, 2 fold at day 560 and was not induced at day 680. Acyl-CoA oxidase activity was induced 11 fold at day 300, 10 fold at day 560 and 7 fold at day 680.

DISCUSSION

The aim of the present study was to investigate whether peroxisomal proliferation is affected by aging. Day 300 was selected as the first time point for this experiment based on our previous work where the aging-related decrease in peroxisomal activity started at about this age. The present results for catalase and acyl-CoA oxidase activity in control groups are consistent with our previous work [16] and Bieir's work [20]. Using immunoblot analysis he also showed a decrease in catalase and acyl-CoA oxidase protein content in purified peroxisomes. Thus, the present results strongly support the hypothesis that the aging-related decrease in peroxisomal fatty acid degradation takes place, in part, through a specific decrease in acyl-CoA oxidase.

After clofibrate treatment, we found the known significant increase in both catalase and acyl-CoA oxidase activity at day 300 [21]. In our study, urate oxidase activity was significantly increased by clofibrate treatment only at day 560. Other authors have reported a 2-3 fold induction in young animals treated with clofibrate [22]. This difference may be due toboth animal age and treatment duration which was only 5 days in the present study.

Peroxisome biogenesis takes place via a dynamic course described by Fahimi et al. [23]. Under clofibrate induction the peroxisome turnover is altered since synthesis of new peroxisomes is strongly enhanced. This results in a marked accumulation of liver peroxisomes. If the aging-related decrease in peroxisomal activity in control animals involved an impaired synthesis of new peroxisomes then peroxisome proliferation should be impaired too. Our results showed marked decreased activities for both control and clofibrate treated animals during aging. Since the extent of the aging process should be different in control and clofibrate-treated very-old animal groups it can not be concluded

that peroxisome synthesis was suppressed by aging since all control old animals do have a peroxisomal activity, but peroxisome synthesis was undoubtely dramatically decreased. Thus, among the processes that could lead to a decreased peroxisomal activity during aging, impairment in the synthesis of new peroxisomes might be the key mechanism.

The impact of peroxisome defects caused by aging on health is unknown and remains to be investigated but these aging-related peroxisome defects and especially in the fatty acid oxidizing system raise problems similar to those encountered in inherited peroxisome disorders. The aging-related decrease in peroxisomal fatty acid oxidation activity could lead to accumulations of VLCFA in plasma or in brain as occurs in severe peroxisomal diseases [24]. Thus, peroxisome might have an unexpected impact on aging process.

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