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SHORT COMMUNICATIONS

Disappearance of sex difference in rat liver drug metabolism in old age

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It has been known that age and sex are two important determinants of hepatic microsomal monooxygenase activities in rats [1, 2]. The presence of markedly higher monooxygenase activities in male rat liver microsomes has been widely documented [3]. As an explanation for the sex-associated difference of hepatic microsomal drug metabolizing enzyme activities, Fujita *et al.* [4, 5] reported the evidence indicating that the relative abundance of multiple species of hepatic microsomal cytochrome P-450 is different between male and female rats. Furthermore, Kamataki *et al.* [6] have recently purified male and female specific forms of P-450 in rat liver microsomes which were electrophoretically and immunologically distinct from each other. Despite the fact that large sex differences in drug metabolism exist in rats, and that relative sex hormone levels are altered with old age [7, 8], the consequence of sex difference in old age has not been studied. In fact, most of the past studies concerning old age-associated alterations of hepatic drug metabolism in rats have been limited to male animals only. Most of these data indicate a general trend of decrease in drug metabolism in aging rats [9–12]. Age-associated alterations of drug metabolism may be caused by age-associated qualitative changes in either microsomal membrane [13, 14] or cytochrome P-450 [12, 15, 16]. The qualitative changes in the microsomal cytochrome P-450, in this context, mean changes in the relative population of cytochrome P-450 in microsomes. In the present study, we report evidence for the latter possibility utilizing the fact that the change in the peak–height ratio of the ethylisocyanide difference spectra of reduced cytochrome P-450 reflects the change in quality of cytochrome P-450 [17].

Materials and methods

Wistar rats of both sexes (originally from Shizuoka Jikken Dobutsu, Hamamatsu) were bred and maintained in the aging facility of the Tokyo Metropolitan Institute of Gerontology in an SPF condition. The husbandry conditions, survivals and age-associated pathologies on this strain are reported elsewhere [18, 19]. All chemicals used were of analytical grade. Ethylisocyanide was prepared according to the method of Jackson and McKusic [20]. Cytochromes P-450 and b_5 contents in liver microsomes, ethylisocyanide difference spectra of reduced microsomes and APD, HBH, AH, and p-NAD* activities were determined according to the previously described methods with slight modifications [21].

Results and discussion

Figures 1(A–D) indicate age-associated alterations in activities or quantities of enzymes of the microsomal electron transport system which leads electrons to cytochrome P-450 from NADPH or NADH. NADPH cytochrome *c* reductase activity decreased with age after 3 months in both sexes (Fig. 1A), which is in agreement with the observations in the past reports including our own previous study using Fischer 344 rats [21]. In contrast, NADH cytochrome *c* reductase activities increased with age (Fig. 1B). It is noteworthy that females had a higher NADH cytochrome *c* reductase activity than males in all age groups which also agrees with our earlier observation [21]. Compared with

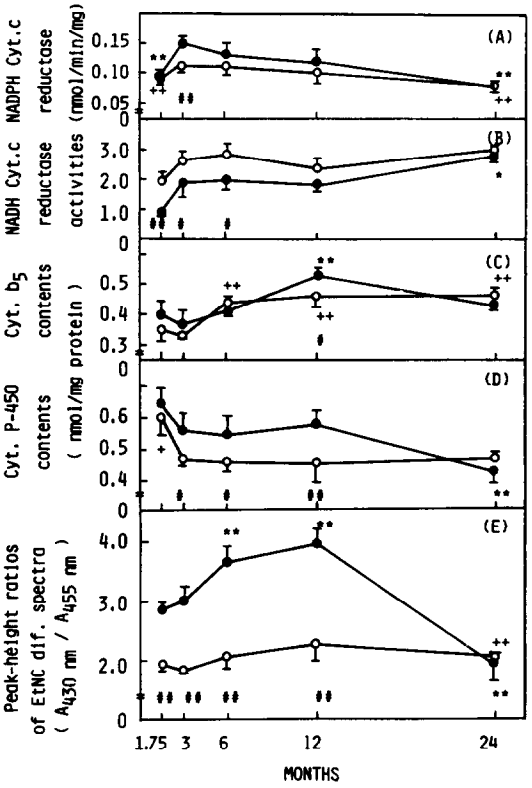

Fig. 1. Age-associated changes in activities, contents, and/or quality of the components of the hepatic microsomal electron transport system in male and female rats. (A) NADPH cytochrome *c* reductase, (B) NADH cytochrome *c* reductase activities expressed in nmol cytochrome *c* reduced/min/mg microsomal protein, (C) cytochrome b_5, (D) cytochrome P-450 contents in microsomes expressed in nmol per mg microsomal protein. The peak–height ratio of the ethylisocyanide difference spectra of the dithionite reduced microsomes at pH 7.0 (E) were obtained by taking the ratio between two absorption maxima of the spectra at or near 430 nm (A_{430nm}) and 455 nm (A_{455nm}). Each point represents the mean ± S.E. of data collected from 6 animals. Open circles represent data points for females, closed circles represent male animals. *† Significantly different from 3-month-old male and female values, respectively ($P < 0.05$). **††Significantly different from 3-month-old male and female values respectively ($P < 0.01$). #, ##Male values significantly different from the corresponding female values ($P < 0.05$ and $P < 0.01$, respectively).

* Abbreviations: APD, aminopyrine N-demethylase; HBH, hexobarbital hydroxylase; AH, aniline hydroxylase; p-NAD, p-nitroanisole O-demethylase.

corresponding 3-month-old values, cytochrome b_5 levels were significantly higher at 12 months of age in males and at 6, 12 and 24 months in females (Fig. 1C).

Figure 1(D) indicates the age-associated alterations in cytochrome P-450 levels. In both male and female rats, liver microsomes from the youngest animals (1.75 months) had the highest content of P-450. In males, the P-450 level at 24 months was significantly lower than the 3-month-old value, while in females the P-450 levels did not change after the first 3 months.

Figure 1(E) shows the age-associated alteration of the peak–height ratio (A_{430nm}/A_{455nm}) of the ethylisocyanide difference spectra of microsomes at pH 7.0. Significant differences were found in the ratio between male and female microsomes of the corresponding age groups in all age groups but 24-month-old rats, and patterns of age-associated changes were clearly different between male and female rats. In males, the value gradually increased with age, and at 12 months it reached the maximum value. Then it began to decrease drastically, getting very close to the corresponding female level at 24 months. In females, the value did not significantly differ at any age. This clearly indicates that qualitative differences exist between male and female liver microsomal cytochrome P-450 and that the relative quantity of multiple species of cytochrome P-450 in male liver is altered with age. This marked alteration in the peak–height ratio in male liver microsomes was entirely due to the decrease in 430 nm absorbing species of ferrous P-450-ethylisocyanide complex at pH 7.0, while 455 nm absorbing species did not show any significant age-associated alteration.

Figure 2 indicates the age-associated changes in AH, p-NAD, HBH and APD activities in male and female rat liver microsomes. Male activities for HBH and APD were markedly higher than female values until 12 months, after which the male values drastically decreased, approaching the corresponding female levels at 24 months. In contrast such a large sex difference as seen for HBH and APD activities was not observed for AH and p-NAD activities in any age group. In males, the activities of these 4 enzymes showed the highest values at 12 months and the lowest values at 24 months, while in females, age-associated changes in activities were insignificant and showed no general pattern. These patterns of age-associated alterations of monooxygenase activities are for the most part in good agreement with our similar studies on Fischer 344 rats [21].

None of the age-associated changes in cytochrome *c* reductase activities and in contents of cytochromes b_5 and P-450 did closely parallel any changes in monooxygenase activities. This may suggest that the direct influence of these parameters of microsomal electron transport system on changes in monooxygenase activities is insignificant. On the other hand, the patterns of age-associated alteration in HBH and APD activities closely resembled the patterns of age-associated changes in the peak-height ratio of ethylisocyanide difference spectra (Figs. 1E and 2). Furthermore, the correlation coefficient between the values of the peak-height ratio and these monooxygenase activities exceeded 0.9 (Fig. 3). Therefore it seems most likely that age-associated alteration in relative abundance of P-450 isozymes is responsible for the age-associated alterations in hepatic microsomal monooxygenase activities. This agrees with our recent observation that a male predominant species of P-450 in solubilized microsomes separated by HPLC column decreases in old age to female levels [22].

In consequence with above observations on drug metabolisms, we confirmed that large sex differences (male > female) in the plasma testosterone to 17β-estradiol level ratio as well as in testosterone level existed in young ages. Furthermore, such sex differences totally disappeared at the age of 24 months due to the rapid decrease with age in testosterone level in male rats [19]. In addition, age-associated alteration in synthesis of testosterone by testicular microsomes from the same groups of rats closely paralleled the alterations in drug metabolism (data not shown). Thus, it is likely that the marked decrease in some of the monooxygenase activities and peak–height ratios in old (24 months) male rats may be related to the plasma sex hormone changes (the decrease in testosterone level) at this age.

In summary, the patterns of age-associated alterations in rat liver microsomal monooxygenase activities were different depending on the sex of the animals and the substrates used. With the activities which were greater in young males than in females, the sex difference disappeared in old age due to the decrease in male activities. The pattern of decrease in monooxygenase activities in male rats correlated well with that of peak–height ratio of reduced ethylisocyanide difference spectra. It is concluded that most, if not all, of the age-associated alterations of monooxygenase activities appear to be explained by the age-associated alterations of relative abundance of P-450 species without assuming the qualitative alteration of microsomal membrane structure. Furthermore, age-associated alterations of relative abundance of P-450 species in liver microsomes are suggested to be phenomena secondary to the changes in humoral factors, and probably not the direct manifestation of hepatocyte aging.

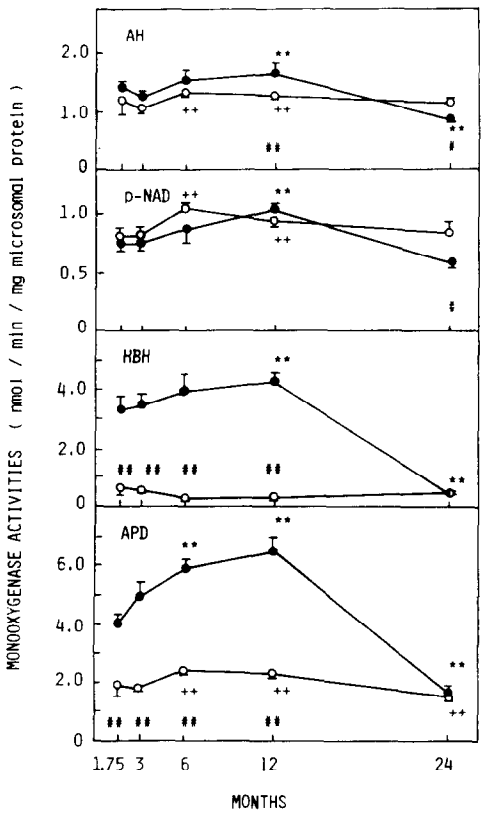

Fig. 2. Age-associated changes in monooxygenase activities in male and female rats. Aniline hydroxylase (AH) and p-nitroanisole O-demethylase (P-NAD) activities were expressed in nmol product formed/min/mg microsomal protein. Hexobarbital hydroxylase (HBH) activity was expressed in nmol hexobarbital disappeared/min/mg microsomal protein. Aminopyrine N-demethylase (APD) activity was expressed in nmol formdehyde formed/min/mg microsomal protein. Each point represents the mean ± S.E. of data collected from 6 animals. The symbols are the same as in Fig. 1.

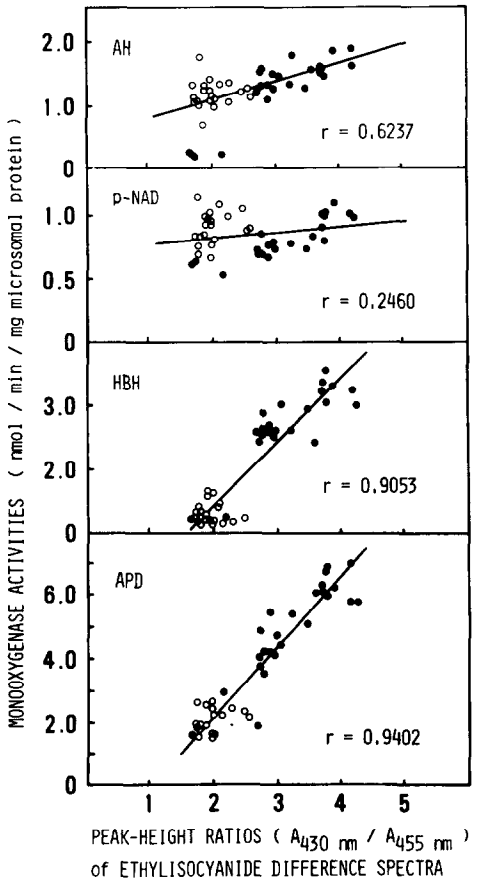

Fig. 3. Relationship between monooxygenase activities and peak-height ratios of ethylisocyanide difference spectra. Aniline hydroxylase (AH), p-nitroanisole O-demethylase (p-NAD), hexobarbital hydroxylase (HBH) and aminopyrine N-demethylase (APD) activities of microsomes from aging rats were plotted against the peak-height ratios of ethylisocyanide difference spectra of the corresponding microsomes. Each point represents the average of the duplicate determinations for microsomes of a single rat. Open circles represent data points for female and closed circles represent male animals. The line passing through the data is a least squares fit to a linear equation of the form $Y = AX + B$, where Y is the monooxygenase activity and X is the peak-height ratio of the ethylisocyanide difference spectra, and indicates the proportionality between peak-height ratio and monooxygenase activity. For AH activity, the slope of the line was 0.288 and the correlation coefficient was 0.6237, for p-NAD activity, 0.0464 and 0.2460, for HBH activity, 1.02 and 0.9053, and for ADP activity, 2.20 and 0.9402.

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