The effects of aging on carbonic anhydrase concentrations in rat liver and skeletal muscle

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The isoenzymes carbonic anhydrase II (CAII) and III (CAIII) have been measured by radioimmunoassay in the livers of male and female rats aged from 21 to 800 days. No sexual dimorphism at 21 days was found, but from 50 to 400 days both isoenzymes show sexual differences. From 600 days onwards, these differences are less apparent. CAIII concentrations in two 'fast' fibre muscles and one 'slow' fibre muscle have been determined. There is no sexual dimorphism in muscle, but a wide variation between individuals was observed. Fast muscles show maximal CAIII levels at 800 days, whereas in slow muscle the concentration of the isoenzyme is declining at this time.

INTRODUCTION

Carbonic anhydrase (EC 4.2.1.1) has three well-characterized, genetically discrete, isoenzymes (Carter & Jeffery, 1985) and at least two other less-well-defined isoenzymes (Dodgson *et al.*, 1980; Wistrand & Kinne, 1977). The predominant isoenzyme in skeletal muscle of many species and in rat liver is carbonic anhydrase III (CAIII) (Holmes, 1977; Register *et al.*, 1978; Shiels *et al.*, 1983). Carbonic anhydrase II (CAII) is also expressed in rat liver, though at levels only 1% of that for CAIII (Jeffery *et al.*, 1984). Both CAII and CAIII exhibit sexual dimorphism in rat liver, CAIII being present in male liver at concentrations 10–20 times those in females, whereas CAII shows a reverse pattern, being 1.5–3 times higher in female liver than in that of males. A preliminary study had suggested that, in rat skeletal muscle, there is no difference in CAIII concentrations between male and female (Carter *et al.*, 1982). There is known to be a clear effect of age on CAIII concentrations in liver (Shiels *et al.*, 1983), but there is no information on age and CAIII in muscle. Similarly, the CAII concentrations in liver at different ages, and any changes in sexual dimorphism, are not at present known. To understand the control of tissue expression of rat CA isoenzymes, it is therefore necessary that any differences between males and females for the various isoenzymes are known, and that any effects of age on CAIII concentrations are fully recognized. To this end we have assayed the livers of a number of male and female Sprague–Dawley rats for CAII and CAIII concentrations from 21 days to 800 days, together with various muscles from the same rats for the assessment of CAIII concentrations.

MATERIALS AND METHODS

Rats (CFY strain) were obtained from the colony maintained at The Wolfson Institute, University of Hull. For the livers, three animals were used at each time point; for the muscle, five animals were employed. Livers were perfused as described previously (Jeffery *et al.*, 1984), then homogenized in distilled water (1:4, w/v),

and supernatants were prepared by centrifugation (Jeffery *et al.*, 1984). The supernatants were frozen at -70°C before CAIII determination. Hind limbs from the rats were frozen at -70°C until assays were performed. The limbs were then thawed and three muscles dissected for examination: soleus, extensor digitorum longus (EDL) and anterior tibialis (AT). Homogenates were prepared (1:5, w/v, with distilled water), and supernatants obtained as for the liver samples.

Statistics

Two-way analysis of variance with replicates was carried out, after checking for distributional assumptions, to determine whether there was a sex difference between male and female.

Determination of CAII and CAIII

The isoenzymes CAII and CAIII were determined by radioimmunoassay with unextracted samples, as described previously (Shiels *et al.*, 1983). Soluble protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

RESULTS

Liver

Figs. 1(a) and 1(b) show how the concentrations of CAIII in male and female rat liver vary with age. Care has to be taken in interpreting the data with three animals in each group, but it is evident that, for CAIII in male rats, the level is extremely low at 21 days and rises very quickly to reach maximum concentration at 50 days. The decline at 100 days, rising again by 200, is likely to be a result of the sample number, but there is evidently a continued decline in CAIII concentrations from 400 days, reaching, at 800 days, levels similar to those at 21 days. Analysis of variance shows a significant difference between sexes for CAIII concentrations, but there is an interaction when age and sex are considered, because the concentrations of CAIII at 21 and 800 days are not significantly different.

Abbreviations used: CAII and CAIII, carbonic anhydrase isoenzymes II and III; EDL, extensor digitorum longus; AT, anterior tibialis; GH, growth hormone.

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Fig. 1. Concentration of CAIII in male (a) and female (b) rat liver and of CAII in female (■) and male (□) rat liver (c) as a function of age

Results are means $\pm$ S.E.M.

For female rats, the CAIII levels over the age range are very similar, though the 21-day group gave the highest concentrations, in marked contrast with the males.

Fig. 1(c) shows the CAII profiles for male and female rats with age. The CAII differences in these rats are not as great as those we have reported for the Wistar rats used previously (Jeffery *et al.*, 1984), but are similar to those for Sprague-Dawley rats used in growth-hormone (GH)-manipulation experiments (Jeffery *et al.*, 1986a). Analysis-of-variance data showed a significant difference for CAII concentrations between males and females.

As with CAIII, concentrations are similar for males and females at 21 days, but a difference between the sexes is evident at 50 days.

Muscle

The three muscles chosen for examination were EDL, AT and soleus. EDL is predominantly a 'fast' muscle with few type I fibres; AT is more mixed, whereas soleus is composed mostly of 'slow' type I fibres. These muscles were chosen because CAIII is known to be found mainly in type I fibres in the rat (Jeffery *et al.*, 1986b).

Fig. 2. Concentration of CAIII in EDL (a), AT (b) and soleus (c) from male (□) and female (■) rats

Results are means $\pm$ S.E.M.

Figs. 2(a), 2(b) and 2(c) show the CAIII concentrations at different ages for EDL, AT and soleus respectively. The high standard error for these samples reflects the wide variation between individual rats in most age groups. EDL and AT were only sampled from 100 days onwards, whereas soleus was examined from 21 days, although the 21-day and 50-day samples were pooled. Both EDL and AT present very similar profiles, with CAIII concentrations showing no significant differences from 100 to 400 days, but then rising to reach maximum levels at 800 days. There are no significant differences between males and females at any age for EDL or AT.

Soleus shows a different pattern, with a rise from 21 days to 100 days, followed by a plateau up to 600 days. The male values reached a maximum at 400 days, but, again, there are no significant differences between the male and female age-matched groups. After 600 days, there is a decline in CAIII concentrations. At 800 days, the amount of CAIII in soleus is below the 50-day level. For soleus, AT and EDL the analysis of variance showed no sex difference for CAIII concentrations.

DISCUSSION

The previously recorded sexual differentiation of the rat liver isoenzymes CAII and CAIII has been shown to exist from post-puberty up to 800 days for CAIII and up to 600 days for CAII.

CAIII concentrations in males are correlated with the production of testosterone, which begins to rise in plasma at about 40 days, peaks at 60 days, then declines gradually from 100 to 800 days, (Merry & Holehan, 1981), reaching pre-pubertal levels between 800 and 900 days. Although removal of testosterone from male rats reduces hepatic CAIII concentrations (Shiels *et al.*, 1983), hypophysectomy has no effect on CAIII concentrations in liver; the same operation on female rats, however, brings the hepatic CAIII concentrations up to those found in males. It is known that patterns of release of GH into the plasma vary between male and female rats. Male rats secrete regular pulses, with low levels between peaks, whereas females release GH in irregular bursts and maintain a higher basal level (Eden, 1979). This difference in pattern of GH release begins at about 30 days (Eden, 1979). Since we have recently shown that hepatic CAIII concentrations in male rats can be partially 'feminized' by infusing continuously with GH (Jeffery *et al.*, 1986a), this evidence, taken with the results of hypophysectomy, suggests that it is the pattern of GH release which causes the male/female difference in hepatic CAIII concentrations. Unfortunately, there are no data to indicate whether the pulsatile release of GH in males continues throughout life. It is therefore not possible to say whether the decline in CAIII concentration after 400 days is related to a change in the pattern of GH release, though it does correlate with the decline in testosterone plasma levels, which in turn are known to affect patterns of GH secretion (Mode *et al.*, 1982).

The other tissue in the rat which contains large amounts of CAIII is skeletal muscle, specifically that with a high percentage of slow fibres such as soleus. To discover whether there was any sexual dimorphism in this tissue and how the CAIII content varied with age, three muscle types were examined that are known to have different amounts of slow fibres. Only soleus muscle, containing most CAIII, was examined at 21 and 50 days; the others were taken from 100 days onwards. CAIII concentrations in soleus of both male and female are relatively low at 21 days (though still above the adult levels of the fast muscles examined), reach a maximum at 100 days, then decline from 600 to 800 days, showing no difference between the sexes. At 800 days, CAIII concentrations are similar to those at 50 days. What is apparent for this muscle type and the other two examined is the wide variation in CAIII levels between individual animals, much more so than that in the liver. This may be related to the fact that muscle fibres change in type throughout the life of the rat and these changes do not appear to be the same for individual rats of the same age. In soleus, for example, type IIA fibres (which are low in CAIII), are absent at some ages, then reappear and then disappear again (S. Jeffery, unpublished work).

For the fast muscle (EDG and AT) CAIII concentrations from 200 to 400 days were relatively constant, then rose to maximal levels at 800 days. The profiles of

CAIII with age for the fast muscles are the reverse of that for the soleus, which shows the lowest adult concentrations for the enzyme at 800 days. It is of interest that, for this strain of rats, the CAIII concentrations in AT are only 1.5–2 times those in EDL, whereas Wistar and Sprague-Dawley rats from other sources show very low concentrations of CAIII in the EDL, and up to 8 times the amount in AT (Jeffery *et al.*, 1987).

It is clear from our results that both hepatic and muscle CAIII have a distinct age profile, but only the liver isoenzyme exhibits sexual dimorphism. The mechanisms which cause this difference are still unclear, but relate to GH patterns in the case of the hepatic CAII and CAIII. For muscle, CAIII levels are known to be drastically affected by denervation (Wistrand *et al.*, 1986) and thyroidectomy (Jeffery *et al.*, 1987), but whether neural or hormonal influences are involved in the changes in CAIII concentration seen at 800 days remains to be investigated. In this context, it is interesting that GH is known to restore protein synthesis in the skeletal muscle of old male rats (Sonntag *et al.*, 1985).

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