Carbonic anhydrase II isoenzyme in rat liver is under hormonal control

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1. A specific and sensitive radioimmunoassay for rat carbonic anhydrase II (CAII) was developed. 2. Rat livers were perfused with Ringer's solution and the hepatic CAII was measured. 3. Concentrations of CAII in female liver were significantly higher than those in male liver. Castration increased the concentration in male liver, though not to that in females, and diethylstilboestrol treatment of castrated males gave values higher than those in females.

CA occurs as a number of isoenzymes with greatly differing activities towards CO₂ (Sanyal *et al.*, 1982). In decreasing order of activity these isoenzymes are CAII, CAI and CAIII. It has been shown recently that the low-activity isoenzyme, CAIII, occurs at much higher concentrations in male liver than in female liver, and the regulation of this isoenzyme is at least partially dependent on testosterone (Garg, 1974; King *et al.*, 1974; Carter *et al.*, 1981; Shiels *et al.*, 1983*a,b*). A report in the literature has suggested that a high-activity isoenzyme is also under hormonal control (Garg, 1975), but this isoenzyme was never identified. In a continuation of our study on hormonal control of rat liver CA (Shiels *et al.*, 1984), we have developed a sensitive and specific assay for CAII to discover whether it is this high-activity isoenzyme that is subject to hormonal regulation.

Materials and methods

Preparation and treatment of animals

Wistar rats (Bantin and Kingman, Hull, Humberside, U.K.) were kept in a fixed lighting regime of 12 h light/12 h dark at 22°C and allowed food *ad libitum*. Castration and sham operation were performed under halothane/N₂O anaesthesia, when hormone implants were placed under the skin. Implants were as previously described (Shiels *et al.*, 1983*b*). Animals were killed by decapitation 20 days after the operations. Blood was collected from the cervical cut, centrifuged at 400*g* for 10 min at 5°C, and the resulting plasma obtained was stored at –20°C until assayed for lutropin by

radioimmunoassay by the method of Naftolin & Corker (1971); the details of the assay have been described previously (Kendle *et al.*, 1978).

Tissue collection

Livers from the Wistar rats were perfused with 200 ml of Ringer's solution (147 mM-NaCl, 4 mM-KCl, 2 mM-CaCl₂) *in situ* and then removed and weighed, and homogenized (1 g/2 ml) in distilled water for 30–60 s with an Ultra-Turrax homogenizer.

Liver homogenates were centrifuged at 30000*g* for 20 min at 4°C, and the supernatants were stored at –70°C. Ventral prostates and seminal vesicles were also removed and weighed at autopsy.

Preparation of anti-CAII serum

Rabbit antiserum to purified rat CAII was prepared as described previously (Carter *et al.*, 1981, 1982) and stored at –70°C.

Preparation of radioiodinated CAII

Purified rat CAII was radioiodinated by conjugation labelling with the use of a commercially prepared Bolton and Hunter reagent (Amersham International) as described by Heath *et al.* (1982).

Radioimmunoassay

Samples were assayed directly for CAII (i.e. without extraction) in duplicate, with standards in triplicate, as described by Heath *et al.* (1982).

Determination of soluble protein

This was done by the method of Lowry *et al.* (1951).

Abbreviation used: CA, carbonic anhydrase (EC 4.2.1.1).

Results

Radioimmunoassay characteristics

The assay was the most sensitive so far developed for the CA isoenzymes, owing to the high titre of the antiserum, used at 1:20000 initial dilution, compared with 1:4000 for rat CAII (Shiels *et al.*, 1984) and 1:5000 for human CAII (Heath *et al.*, 1982). The minimum detection limit of the assay was taken as B/B_0 of 90%, where B was the radioactivity (c.p.m.) bound in the presence of unlabelled CAII (sample or standard) and B_0 that bound in the absence of competing unlabelled CAII. The sensitivity at B/B_0 of 90% was 250 pg/ml.

Concentration of CAII in perfused liver homogenates

The concentrations of CAII in male and female liver were examined, the liver homogenates being diluted 1:250 to lie within the range of the standard curve. The values are given in Table 1. Male rat liver contained 81 ng of CAII/mg of soluble protein, whereas female liver contained almost three times as much, the difference being significant to $P < 0.001$.

Groups of five or six castrated male rats were implanted with 5 α -dihydrotestosterone, diethylstilboestrol or empty implants (sham operated). The concentrations of hormone and drug in the circulation were not measured directly, but there was clear evidence that they exerted a physiological effect, since plasma gonadotropin concentrations were suppressed to values lower than those seen in the intact controls, and with 5 α -dihydrotestosterone the weights of the ventral prostates and seminal vesicle were maintained at control

values, whereas the weights of these organs in castrated and castrated + diethylstilboestrol animals were significantly lower ($P < 0.001$) than for controls (see Table 2).

The concentrations of CAII in the livers of all three groups are shown in Table 1. Castration leads to a doubling of CAII concentration in the male liver, the difference between normal and castrated animals being significant to $P = 0.001$. Castration does not increase the concentration of CAII in liver up to that found in females, the difference being significant at $0.01 < P < 0.002$. Treatment of castrated males with diethylstilboestrol increased hepatic CAII concentrations to above those found in females, although the difference was not statistically significant. When 5 α -dihydrotestosterone was used in the implants of castrated males, the hepatic CAII concentrations were not significantly different from those in control males.

Discussion

The presence of endogenous carbonic anhydrase in rat liver has been established for some years (King *et al.*, 1974), but the identity of the major isoenzyme in male liver as CAIII was established much more recently (Shiels *et al.*, 1983a,b; Carter *et al.*, 1981), and its synthesis was shown to be subject to hormonal regulation. Testosterone has been shown to promote synthesis of CAIII and oestrogen to inhibit it (Shiels *et al.*, 1983b).

We have shown in the present study that the high-activity erythrocyte isoenzyme CAII occurs in rat liver and is regulated by oestrogen in the opposite manner to CAIII, i.e. oestrogen induces CAII but inhibits CAIII, whereas testosterone

Table 1. *Effect of castration and subsequent hormone treatment on hepatic CAII concentrations*
Values are means $\pm$ S.E.M. for the numbers of rats (age-matched Wistars) shown in parentheses.

Group of animals	CAII (ng/ml of soluble hepatic protein)
Males (4)	81 $\pm$ 5.15
Females (9)	222 $\pm$ 10.65
Castrated males (6)	160 $\pm$ 14.4
Castrates males plus diethylstilboestrol (5)	274 $\pm$ 14.3
Castrated males plus 5-dihydrotestosterone (5)	85 $\pm$ 27.9

Table 2. *Effect of castration and subsequent hormone treatment on lutropin serum concentrations and organ weights*
Values are means $\pm$ S.E.M. for the numbers of rats (age-matched Wistars) shown in parentheses.

Group of animals	Lutropin (ng/ml)	Ventral-prostate wt. (mg)	Seminal-vesicle wt. (mg)
Sham (6)	3.62 $\pm$ 0.77	623.7 $\pm$ 75	865 $\pm$ 71
Castrated (6)	59.9 $\pm$ 0.36	46.5 $\pm$ 3.5	179 $\pm$ 20.2
Castrated + diethylstilboestrol (5)	1.13 $\pm$ 0.12	58.5 $\pm$ 15	169 $\pm$ 29.2
Castrated + 5-dihydrotestosterone (5)	1.02 $\pm$ 0.10	517 $\pm$ 53	523 $\pm$ 114

promotes CAIII synthesis, and inhibits that of CAII. Previous work (Garg, 1975) suggests that total carbonic anhydrase activity in male and female liver is the same, and that this remains constant on castration, even though the relative concentrations of the two isoenzymes alter, as we have shown in the present paper. As well as providing a unique system of reversed sexual dimorphism for two isoenzymes of the same protein, it seems possible that rat liver might also exhibit a feedback mechanism which keeps total carbonic anhydrase activity constant, even though this involves the regulation of two different isoenzymes with such disparate activities. Further investigation into the gene structure of these two isoenzymes is required, with the aim of investigating possible upstream regulation sites. Development of a radioimmunoassay for the third isoenzyme, CAI (S. Jeffery & N. D. Carter, unpublished work), will enable investigations into possible hormonal control of this isoenzyme to be carried out.

In view of the influence of growth hormone (somatotropin) on the sexual dimorphism exhibited by certain rat liver proteins (Gustafsson *et al.*, 1983), it would be of interest to discover what effect, if any, hypophysectomy has on the CAII concentrations in male and female rat liver.

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