IMMUNOASSAY OF CARBONIC ANHYDRASE III IN RAT TISSUES

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1. Introduction

Relatively high levels (i.e., up to 1% wet muscle wt) of a low-activity sulfonamide-resistant carbonic anhydrase isozyme has been reported from skeletal muscle of chicken, cat and sheep [1], ox [2], rabbit [3], mouse [4], human [8,9] and gorilla [7]. It has been accepted that these isozymes are all homologous forms of an enzyme designated CAIII and essentially confined to skeletal muscle. Nevertheless, trace levels of CAIII have also been detected immunologically in sheep lung [4], rabbit liver [4], and human liver, smooth muscle, lung and cardiac muscle [8].

It now appears that high levels of a CAIII isozyme structurally indistinguishable from rat muscle CAIII are also present in mature rat liver [9]. Utilizing an electrophoretic immunoassay [8] we report here the levels of CAIII in different rat tissues.

2. Materials and methods

2.1. *Purification of carbonic anhydrase III*

CAIII isozymes were initially prepared from rat skeletal muscle and liver homogenates by affinity chromatography on sulphonamide columns equilibrated with 0.01 M Tris–SO_4 (pH 8.7) and eluted with a 0–0.4 M KCl gradient. Further purification was carried out on DEAE-32 cellulose columns equilibrated with 0.01 M Tris–HCl buffer (pH 8.7) and eluted with a 0–0.2 M NaCl gradient. Subsequent purification of the CAIII isozymes was carried out by passage through a Sephadex G-75 column equilibrated with 0.005 M Tris–HCl buffer (pH 8.0). All stages of purification for the CAIII isozymes contained 0.5 mM dithiothreitol, as CAIII from human and rabbit muscle is known to dimerize.

2.2. *Immunoassay*

Assay of carbonic anhydrase III was carried out as described for human CAIII [8]. Antiserum was prepared in New Zealand white rabbits using 3 sequential weekly injections of 1 mg CAIII in Freund's complete adjuvant, followed by an i.v. injection of 1 mg rat CAIII in 0.005 M phosphate buffer (pH 7.0).

Tissue extracts (20%, w/v) were prepared in distilled water and spun at 20 000 × *g* for 15 min. Extracts from soleus, anterior tibialis (AT), extensor digitorum longus (EDL), prostate, white fat and liver were made as above.

2.3. *SDS gel electrophoresis*

Protein subunits were separated in 12% acrylamide gels which were stained with Coomassie blue.

3. Results

Rat muscle carbonic anhydrase III was analysed both qualitatively and quantitatively. The protein profiles after SDS gel electrophoresis and staining with Coomassie blue are shown in fig.1. The mean levels of CAIII in extracts of different muscles are shown in fig.2. The range of values for the same muscles from different male rats showed consistent differences, e.g., for EDL, AT and soleus the mean values (±SD) were: (a) EDL 298 μg CAIII/g wet wt ±63; (b) AT 1070 μg CAIII/g ±247; (c) Soleus 12 925 μg CAIII/g ±248. Similar values were obtained from female muscle (fig.2).

Extracts of liver were similarly analysed and the mean differences are summarised in fig.2. The mean CAIII level for male liver was 6975 μg CAIII/g wet wt ±1435 and for female liver 256 μg CAIII/g wet wt ±28. CAIII was also detected in white fat and a mean female value of 552 μg/g and a male value of 357 μg/g

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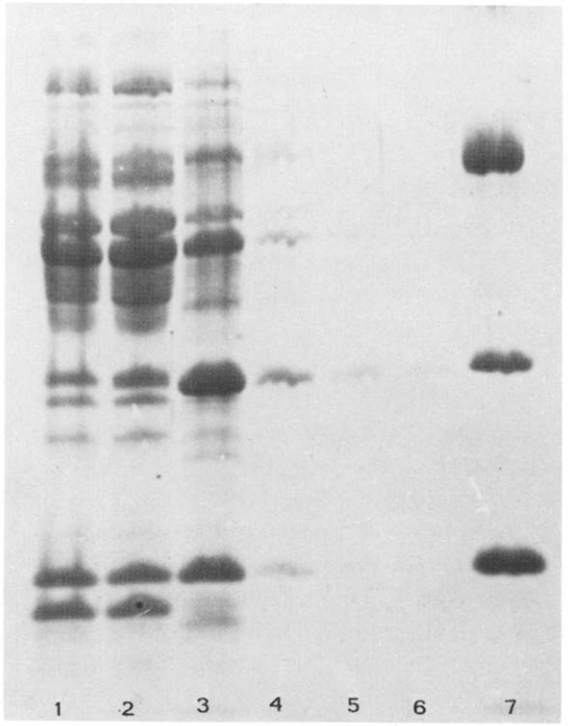

Fig.1. 12% Acrylamide/SDS gel stained with Coomassie blue containing: (1) EDL homogenate (see text); (2) AT homogenate; (3) soleus homogenate; (4) 1 in 10 dilution of soleus homogenate, (5) 1 in 50 dilution of soleus homogenate, (6) 1 in 100 dilution of soleus homogenate, [these 3 dilutions indicate predominance of CAIII component]; (7) protein markers (myoglobin, bovine carbonic anhydrase, albumin).

wet wt were found. Trace levels of CAIII were also found in several other tissues, detectable only by radioimmunoassay (unpublished).

4. Discussion

The evidence for a single structure gene locus coding CAIII in most rat tissues is strong [9], and the variable expression of this locus in different muscle types and in male *vs* female liver is established by these data. If we compare male liver with female liver and soleus muscle (red, slow) with EDL (fast, white) muscle, the CAIII level is ~30-times higher in the former tissue of each pair (fig.2). Whilst the levels of CAIII in male and female liver were found to be markedly different, homologous muscles from male and female gave similar levels (fig.2).

In man, the CAIII levels in soleus and gastrocnemius are similar (in contrast to the rat) and CAIII in liver is present only at trace levels [8]. In the mouse, CAIII is present in both male and female liver as well as muscle, but the male/female ratio in liver is only 3:1. A final paradox is the lack of induction of liver CAIII in castrated rats treated with testosterone, in contrast to other male related rat liver proteins such as α2u globulin [11], which are coupled to testosterone control.

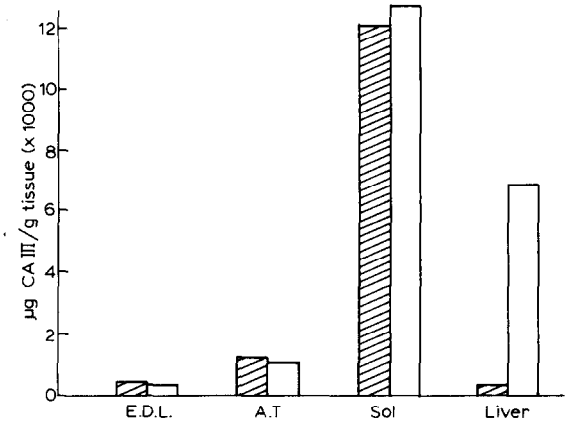

Fig.2. Histogram showing distribution of CAIII in male and female rat tissues.

Because of the high level of CAIII in liver and muscle it seems likely that the CO_2 hydrase activity of this isozyme is important. However the specific level of catalysis is in the order of 1% of carbonic anhydrase I, the low activity red cell isozyme. Enzymic functions, such as esterolytic or phosphatase activity have not yet been backed by any in vivo function of muscle or liver and the true rôle of this isozyme awaits explanation.

References

[1] Holmes, R. S. (1977) Eur. J. Biochem. 78, 511–520.
[2] Tashian, R. E., Hewett-Emmett, D., Stroup, S. K., Goodman, M. and Yu, Y.-S. L. (1980) in: Biophysics and Physiology of Carbon Dioxide (Bauer, C. et al. eds) pp. 164–176, Springer, Berlin, New York.
[3] Register, A. M., Koester, M. K. and Noltmann, E. A. (1978) J. Biol. Chem. 253, 4143–4152.
[4] Holmes, R. S. (1976) J. Exp. Zool. 197, 289–295.
[5] Carter, N., Jeffery, S., Shiels, A., Edwards, Y., Tipler, T. and Hopkinson, D. A. (1979) Biochem. Genet. 17, 837–854.
[6] Hewett-Emmett, D. and Tashian, R. E. (1979) Am. J. Human Genet. 31, 50A.
[7] Hewett-Emmett, D. and Tashian, R. E. (1981) Am. J. Phys. Anthropol. 54, 232–233.
[8] Jeffery, S., Edwards, Y. and Carter, N. (1980) Biochem. Genet. 18, 843–849.
[9] Carter, N. D., Hewett-Emmett, D., Jeffery, S. and Tashian, R. E. (1981) FEBS Lett. 128, 114–118.
[10] Roy, A. K. (1979) in: Biochemical Actions of Hormones, vol. 6, pp. 481–517, Academic Press, New York.