Endogenous Synthesis of Taurine and GABA in Rat Ocular Tissues

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The endogenous production of taurine and γ-aminobutyric acid (GABA) in rat ocular tissues was investigated. The activities of taurine-producing enzyme, cysteine sulfinic acid decarboxylase (CSAD), and GABA-synthesizing enzyme, glutamic acid decarboxylase (GAD), were observed in the retina, lens, iris-ciliary body and cornea. The highest specific activity of CSAD was in the cornea and that of GAD in the retina. The discrepancy between CSAD activity and taurine content within the ocular tissues indicates that intra- or extraocular transport processes may regulate the concentration of taurine in the rat eye. The GAD activity and the content of GABA were distributed in parallel within the rat ocular tissues. The quantitative results suggest that the GAD/GABA system has functional significance only in the retina of the rat eye.

It has been established that taurine is the most abundant free amino acid in the rat eye.[1] Taurine content is high not only in the retina but also in other ocular tissues.[1,2] The high content of taurine[1] and differences in the capacity of various ocular tissues to accumulate taurine[3] led to this study of the origin of taurine in the rat eye. We measured the cysteine sulfinic acid decarboxylase (CSAD) activity, which is an index of endogenous synthesis of taurine,[4,5] in the retina, lens, iris-ciliary body and cornea of the rat eye, and compared the CSAD activity to the content of taurine in these ocular tissues. γ-Aminobutyric acid (GABA) is another neuroactive compound which has been observed in the ocular tissues of some species.[1,2,6–8] The relationships between the activity of the GABA-producing enzyme, glutamic acid decarboxylase (GAD), and GABA content in rat ocular tissues have also been determined in this study. The endogenous production of taurine and GABA in the rat eye had not been quantified extensively prior to this study.

Experimental

Chemicals. L-Cysteine sulfinic acid (CSA) was obtained from Sigma, DL-[1-^{14}C]cysteine sulfinic acid (specific activity 407 MBq mmol^{-1}) from *Centre d'Energie Atomique* (Seclay, France), pyridoxal-5'-phosphate (PLP) and L-glutamic acid (Glu) from Fluka, L-[1-^{14}C]glutamic acid (specific activity 1.85 GBq mmol^{-1}) from the Radiochemical Centre, Amersham, 2-aminoethylisothiouronium bromide (AET) and dithiothreitol (DTT) from Calbiochem, ethylenediaminetetraacetic acid (EDTA) from Merck, and the amino acid standards from Pierce Eurochemie.

Enzyme assays. The technique employed for the assay of decarboxylation was exactly as previously described.[9] The reaction mixture for the CSAD assay contained: 50 mM (final concentration) sodium phosphate buffer at pH 7.2, 10 mM L-CSA, 4.652 kBq DL-[1-^{14}C]CSA, 0.1 mM PLP 1.0 mM DTT, 1.0 mM AET, 0.1 mM EDTA and 0.2–1.2 mg of tissue protein. The volume of the mixture was 200 μl. The GAD assay was identical with that for CSAD except that the substrates were replaced with 10 mM Glu and 4.652 kBq L-[1-^{14}C]Glu. The conditions of the reaction mixtures were approximately optimal for both enzymes.

Tissue samples. Adult Fischer strain 344 albino rats were used. After decapitation of the animals

the eyes were enucleated, and the retina, lens, iris (together with the ciliary body) and cornea were removed as previously described.[1] The tissues were individually suspended in 50 mM sodium phosphate buffer, pH 7.2, containing 0.1 mM PLP, 1.0 mM DTT, 1.0 mM AET and 0.1 mM EDTA. The suspensions were homogenized in an ILAB 1020 homogenizer at 8000 rev min^{-1} for 45–90 s at 0°C. Whole homogenates were used for the enzyme assays.

Protein determination. The tissue homogenates were diluted with 0.1 M NaOH. The suspensions were used for protein concentration determinations by the method of Lowry *et al.*[10] Bovine serum albumin (0.1 mg protein per ml of 0.1 M NaOH) was used as the standard.

Amino acid analysis. Taurine and GABA were determined using an automatic analyzer (Liquimat III, Kontron Instruments). A Pico-Buffer System IV (Pierce Eurochemie) was used for elution. The samples for amino acid analysis were prepared as previously reported.[1]

Results

All ocular tissues studied, viz. the retina, lens, iris-ciliary body and cornea of the rat eye, exhibited some CSAD activity. The highest specific activity of CSAD was observed in the cornea, where it was more than double that in the retina. Only a little CSAD activity was observed in the lens. The ratio of the CSAD activity to the concentration of taurine was not the same in all the ocular tissues of the rat eye (Fig. 1). The retina, lens, iris-ciliary body and cornea also exhibited GABA-producing GAD activity. The highest specific activity of GAD was observed in the retina. The GAD activity approximately paralleled GABA content within the ocular tissues (Fig. 2).

Discussion

In this study the taurine-producing enzyme CSAD was observed to be present in the retina, iris-ciliary body and cornea of the rat eye; the lens, however, showed only minimal CSAD activity. The highest specific activity of CSAD was observed in the cornea. The results clearly show that in rat eye retina, iris-ciliary body and cornea there is an endogenous taurine-producing system, which is at least partly responsible for the high taurine content of these ocular tissues. In the lens, which also has a quite high taurine concentration,[1,2,11,12] taurine may be the result of active transport processes occurring in a manner similar to that in the retina and iris-ciliary body.[13,14] The results of these *in vitro* experiments seem to indicate that the content of taurine in the

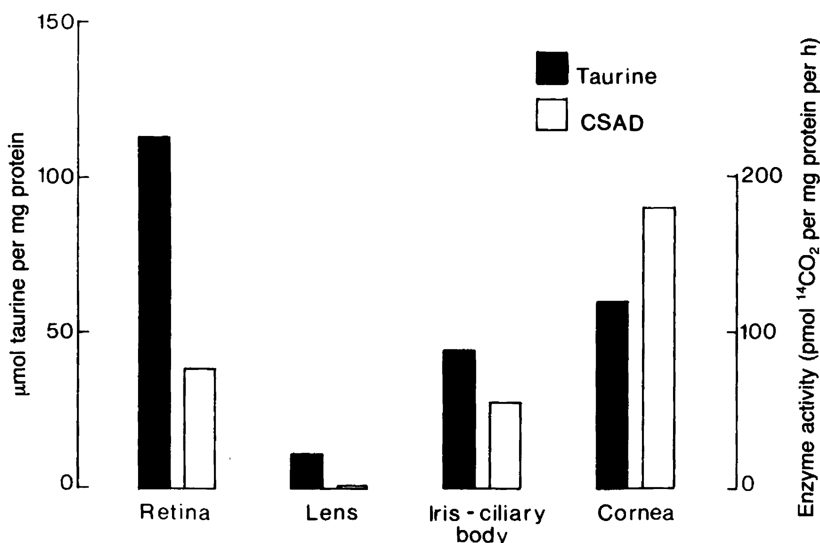

Fig. 1. Taurine concentration and cysteine sulfinic acid decarboxylase (CSAD) activity in rat ocular tissues.

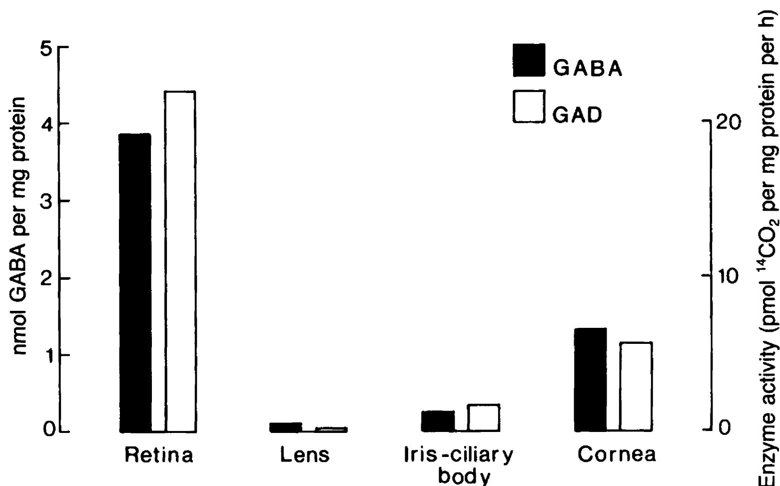

Fig. 2. GABA concentration and glutamic acid decarboxylase (GAD) activity in rat ocular tissues.

rat eye is not totally dependent upon extraocular transport, but that taurine is also produced via cysteine sulfinic acid by CSAD *in situ*. The discrepancy between CSAD activity and the content of taurine indicate that the intraocular transport mechanism may also regulate taurine concentration within the ocular tissues of the rat eye. Taurine has been suggested to have several retinal functions: e.g., to act as an inhibitory neurotransmitter in the retina[15,16] and to have a protective effect on retina;[17] it is suggested[18,19] to have other roles also. The functions of taurine in other ocular tissues have not been elucidated to any significant extent.

Glutamic acid decarboxylase (GAD) activity, which was present to only a small extent in the lens, iris-ciliary body and cornea of the rat eye, was found to be significant in the retina. GABA concentration was also remarkably high only in the retina. GAD activity and GABA content were distributed in a roughly parallel manner between the ocular tissues, which may mean that GABA is a product of endogenous synthesis in individual rat eye ocular tissues. It seems in the light of quantitative studies that the GAD/GABA system has a functional significance only in the retina of the rat eye. In this neural tissue, GABA is a well established inhibitory neurotransmitter.[20,21] However, in some primates GABA content has been found to be high also in the lens.[2] It is yet to be elucidated whether GABA has functions in non-neural ocular tissues of some species.

References

1. Heinämäki, A. A., Muhonen, A. S. H. and Piha, R. S. *Neurochem. Res. 11* (1986) 535.
2. Reddy, D. V. N. *Invest. Ophthalmol. 6* (1967) 478.
3. Baskin, S. I., Cohn, E. M. and Kocsis, J. J. In: Huxtable, R. and Barbeau, A., Eds., *Taurine,* Raven Press, New York 1976, p. 201.
4. Awapara, J. and Wingo, W. J. *J. Biol. Chem. 203* (1953) 189.
5. Jacobsen, J. G., Thomas, L. L. and Smith, L. H. *Biochim. Biophys. Acta 85* (1964) 103.
6. Reddy, D. V. N. and Kinsey, V. E. *Invest. Ophthalmol. 1* (1962) 635.
7. Davis, J. M., Himwhich, W. A. and Agrawal, H. C. *Developmental Psychobiol. 2* (1969) 34.
8. Pasantes-Morales, H., Klethi, J., Ledig, M. and Mandel, P. *Brain Res. 41* (1972) 494.
9. Heinämäki, A. A. *Biochem. Int. 3* (1981) 573.
10. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. *J. Biol. Chem. 193* (1951) 265.
11. Gupta, K., Mathur, R. L. and Agrawal, L. P. *Exp. Eye Res. 32* (1981) 793.
12. Gupta, K. and Mathur, R. L. *Exp. Eye Res. 34* (1982) 835.
13. Pasantes-Morales, H., Klethi, J., Urban, P. F. and Mandel, P. *Exp. Brain. Res. 19* (1974) 131.
14. Reddy, D. V. N. *Biochim. Biophys. Acta 158* (1968) 246.

15. Mandel, P., Pasantes-Morales, H. and Urban, P. F. In: Bonting, S. L., Ed., *Transmitters in the Visual Process,* Pergamon Press, New York 1976, p. 89.
16. Neal, M. J. *Gen. Pharmacol.* 7 (1976) 321.
17. Pasantes-Morales, H. and Gruz, C. In: Kuriyama, K., Huxtable, R. J. and Iwata, H., Eds., *Progress in Clinical and Biological Research,* Alan R. Liss, Inc., New York 1983, Vol. 125, p. 263.
18. Lombardini, J. B. In: Kuriyama, K., Huxtable, R. J. and Iwata, H., Eds., *Progress in Clinical and Biological Research,* Alan R. Liss, Inc., New York 1983, Vol. 125, p. 251.
19. Nishimura, C., Ida, S. and Kuriyama, K. In: Kuriyama, K., Huxtable, R. J. and Iwata, H., Eds., *Progress in Clinical and Biological Research,* Alan R. Liss, Inc., New York 1983, Vol. 125, p. 233.
20. Voaden, M. J. In: Bonting, S. L., Ed., *Transmitters in the Visual Process,* Pergamon Press, New York 1976, p. 107.
21. Lopez-Colome, A. M., Salceda, R. and Pasantes-Morales, H. *Neurochem. Res.* 3 (1976) 431.

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