Animal Model for Hyperprolinaemia: Deficiency of Mouse Proline Oxidase Activity

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New reports are described each year on the occurrence of human metabolic diseases due to specific enzyme deficiencies (Harris, 1970; McKusick, 1971). For the biomedical scientist interested either in the mode of genetic transmission of these biochemical traits or in the mammalian control mechanisms of gene-enzyme expression it would be desirable to be able to supplement the research on human beings with investigations on appropriate animal models. With regard to human hyperprolinaemia two variants of this metabolic disorder have been described (Efron, 1966). Shafter *et al.* (1962) reported a familial hyperprolinaemia associated with cerebral dysfunction and renal anomalies occurring in a family with hereditary nephropathy and deafness. A second case of familial hyperprolinaemia, reported by Efron (1965), was associated with congenital renal malformation, hereditary haematuria and mild mental retardation. The metabolic disorder in the second case was further associated with a deficiency of liver proline oxidase activity. Hyperprolinaemia caused by a deficiency of liver proline oxidase activity has been classified (Efron, 1966) as type I hyperprolinaemia (Scheme 1). Efron (1966) also described a second type of hyperprolinaemia, possibly caused by a deficiency of the enzyme Δ^1 -pyrroline-5-carboxylate dehydrogenase, although the metabolic block was not established. In type II hyperprolinaemia there was no evidence of renal disease as assessed by intravenous pyleography, blood urea N and creatinine clearance tests. Chromatograms of amino acids in the urine showed a spot with the chromatographic position and staining characteristics of Δ^1 -pyrroline-5-carboxylate. A deficiency of the second enzyme in the degradation pathway of proline, Δ^1 -pyrroline-5-carboxylate dehydrogenase, would be expected to result in accumulation of Δ^1 -pyrroline-5-carboxylate as well as proline.

Previously in this laboratory we reported on the discovery of an unusual biochemical trait occurring in the PRO/Re inbred strain of mice, namely a marked hyperprolinaemia and concomitant prolinuria (Blake & Russell, 1972). Since the proline concentration was elevated in both the blood and the urine, the possibility of an enzyme defect in the liver was considered. The present communication describes the finding of a markedly decreased proline oxidase activity in the liver of PRO/Re mice. Proline oxidase activity was also measured in the livers of C57BL/6J, 129/ReJ,

DBA/2J and ST/bJ inbred strains of mice and in the F₁ hybrid (PRO/Re $\times$ ST/bJ).

Proline oxidase was isolated from liver and assayed by the method of Strecker (1971). Livers were homogenized in 0.25 M-sucrose, pH 7.4, containing 1 mM-EDTA with Thomas tissue grinders of the Teflon-pestle model (3431-E20) at a 1:9 (w/v) ratio of tissue to sucrose solution. The homogenate was centrifuged at 2000 rev./min (480g) for 10 min in an SS-34 rotor of the Sorvall RC-2 refrigerated centrifuge. The S-1 supernatant fluid, derived from the first centrifugation, was centrifuged at 9200 rev./min (10000g) for 10 min. The S-2 supernatant fluid was centrifuged at 9200 rev./min for 10 min. The pellets P-2 and P-3 were combined and resuspended in the 0.25 M-sucrose solution to approx. 0.4 times the volume of the original homogenate. This suspension was centrifuged at 12000 rev./min (17300g) for 10 min to form a firm pellet. After the supernatant fluid had been decanted off, the pellet was stored overnight at -30°C . Immediately before assay for proline oxidase activity, the pellet was thawed, suspended in 2 vol. (per g wet wt. of whole liver) of the sucrose-EDTA solution and homogenized in the Thomas tissue grinder. This homogenate was utilized directly for enzyme activity measurements. Protein concentration was determined by the biuret method of Layne (1957).

The results presented in Table 1 compare the liver proline oxidase activity in the mitochondrial preparations of PRO/Re mice with that of several other inbred strains including the two original parental lines, the C57BL/6J and 129/ReJ mice (Blake & Russell, 1972). The specific activity of liver proline oxidase in the mitochondrial preparations of PRO/Re mice averaged about 0.4 enzyme unit/mg of protein. The liver proline oxidase activity of PRO/Re mice was assayed in amounts varying from 0.1 to 1.0 ml of the mitochondrial homogenate. In the C57BL/6J and 129/ReJ mice, enzyme activity was approx. 25-30 times as high, averaging about 9.3 and 11.3 units/mg of mitochondrial protein respectively. Similar enzyme activities were also determined in the ST/bJ and DBA/2J mice, although the activity was slightly higher in the DBA/2J strain.

For determination of proline oxidase activity in an F₁ hybrid, PRO/Re mice were bred with ST/bJ mice. Matings were made in both directions. Previous studies had shown that ST/bJ mice possessed apparently normal concentrations of proline in blood and

Scheme 1. Inborn errors and metabolic pathways of proline

Key to enzymes: (1) proline oxidase; (2) Δ^1 -pyrroline-5-carboxylate reductase; (3) Δ^1 -pyrroline-5-carboxylate dehydrogenase.

Table 1. Liver proline oxidase activity in inbred strains of mice

Enzyme activity is expressed as nmol of Δ^1 -pyrroline-5-carboxylate produced/min per mg of protein. The results are presented as the means $\pm$ S.E.M. for six animals.

Strain	Enzyme activity
C57BL/6J	9.3 $\pm$ 1.2
129/ReJ	11.3 $\pm$ 0.9
PRO/Re	0.4 $\pm$ 0.03
ST/bJ	10.7 $\pm$ 1.1
Hybrid (PRO/Re $\times$ ST/bJ) F ₁	6.0 $\pm$ 0.9
DBA/2J	12.6 $\pm$ 1.7

no detectable proline in the urine. By comparison, the blood proline concentration in PRO/Re mice was elevated about 7-fold above normal. Analyses of the proline concentration in blood of the F₁ hybrids demonstrated a normal value. However, an intermediate liver proline oxidase activity occurred in the F₁ hybrid mice. Apparently this enzyme activity is sufficient for a normal metabolism of proline derived either from the diet or from endogenous sources.

Comparative studies were also undertaken between the PRO/Re and ST/bJ mice to determine whether the deficiency of proline oxidase activity occurred only in liver or was manifest in other tissues such as kidney and brain. We found that the proline oxidase activity was very low in both kidney and brain of PRO/Re mice. In the kidney proline oxidase activity of ST/bJ and PRO/Re mice was 13.8 and 0.03 units/mg of protein respectively. In the brain proline oxidase activity was 1.5 and 0.02 units/mg of protein for the ST/bJ and PRO/Re mice. Since proline oxidase activity is deficient in both the liver and the kidney as well as the brain of PRO/Re mice, this inbred strain may serve as an excellent animal model for studies on aminoacidurias and the development of biochemical defects causing mental retardation. The administration of proline to neonatal and young PRO/Re mice when their central nervous system is still in the process of development and maturation might cause severe and persisting behaviour abnormalities.

We have no evidence as yet on the mechanism by which proline oxidase deficiency occurs in PRO/Re mice. A number of possibilities exist of both the quantitative and qualitative mechanisms. A gene mutation might affect the turnover rate of the enzyme. Enzyme activity may be deficient because of a decrease in the number of enzyme molecules as a consequence of either a low rate of enzyme synthesis or an increased rate of degradation. Alternatively the enzyme population might be normal in quantity but the catalytic activity of the enzyme might be defective. The PRO/Re strain should provide an excellent opportunity for the

investigation of control mechanisms of mammalian enzyme regulation in a mutant bearing an inborn error of metabolism.

With regard to the specificity of the enzyme defect we assayed livers of PRO/Re mice for Δ^1 -pyrroline-5-carboxylate dehydrogenase activity, since a defect in the regulation of this enzyme could also result in hyperprolinaemia. However, no significant differences were found between PRO/Re and C57BL/6J mice. Duplicate analyses demonstrated activities of 5.5 and 5.9 and of 5.6 and 5.8 nmol of Δ^1 -pyrroline-5-carboxylate oxidized/min per mg of protein at 25°C for PRO/Re and C57BL/6J mice respectively. Thus the inborn error of proline metabolism in PRO/Re mice appears to be specific for a deficiency of proline oxidase activity.

In summary, the hyperprolinaemia occurring in the highly inbred PRO/Re strain of mice is associated with a marked deficiency of proline oxidase activity. Enzyme activity is deficient in liver, kidney and brain. F₁ hybrid mice exhibit an intermediate liver proline oxidase activity. The PRO/Re strain may serve as an

animal model for biomedical investigations on type I hyperprolinaemia.

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