STRAIN DIFFERENCES IN LIVER DT DIAPHORASE ACTIVITIES

Christina LIND, Bitte RASE and Lars ERNSTER
Biokemiska Institutionen, Kungl. Universitet i Stockholm, Stockholm, Sweden

and

Michael G. TOWNSEND and A. David MARTIN
Pest Infestation Control Laboratory, Ministry of Agriculture, Fisheries and Food, Tolworth, Surbiton, Surrey, U.K.

Received 14 September 1973

1. Introduction

The involvement of vitamin K in the biosynthesis of certain blood-clotting factors and its inhibition by the indirect anticoagulants is well documented. It has recently been shown that vitamin K is metabolized to vitamin K oxide in the liver [1], the oxide being rapidly reconverted to vitamin K by a warfarin-sensitive reductase [2]. It has been suggested that the reduction of vitamin K oxide may involve the anticoagulant-sensitive flavoprotein, DT diaphorase [3, 4], and that a distinctive decrease in the activity of this enzyme may be responsible for the resistance of wild rat populations to warfarin [5] and the related anticoagulant rodenticides that was first described by Boyle [6]. These findings, apart from their intrinsic interest to the enzymologist were of potential practical value in that they might form the basis of a diagnostic test to identify warfarin resistance in wild rat populations. The warfarin-resistant rats used in the previous study [5] were obtained by cross breeding wild animals with a Wistar-derived strain of laboratory rat [7]. Although these animals possessed 98% of the genetic complement of the parent laboratory strain [7], the possibility remained that the observed variation in DT diaphorase activity between the resistant animals and the Sprague–Dawley-derived strain with which they were compared [5] could have resulted from a difference between the strains that was independent of resistance.

In the present study evidence in support of this hypothesis has been obtained by comparing the DT diaphorase activity of both Wistar and Sprague–Dawley-derived animals from a number of sources with that of two different strains of warfarin-resistant rats. The activity of this enzyme has also been determined in both susceptible and resistant rats caught in the wild.

2. Materials and methods

Wistar-derived rats were obtained both from a randomly bred warfarin-susceptible strain maintained in the laboratory at Tolworth and from an inbred strain of Wistar rats at the National Institute of Public Health in Stockholm. The Sprague–Dawley-derived animals were obtained from commercial suppliers (A. Tuck and Sons, Rayleigh, U.K. and Eklund Brothers, Taby, Sweden). Wild susceptible rats were trapped in a resistance-free area of England. Two distinct colonies of resistant rats have been developed in the laboratory from wild resistant animals originally trapped in Scotland and Wales [7]. The wild resistant animals were trapped in the field and screened for resistance in the laboratory prior to being used.

All animals were maintained on standard laboratory diets ad libitum for at least 2 weeks and starved for 24 hr prior to sacrifice at 150–250 g body weight. The dicoumarol-sensitive DT diaphorase activity of liver post-microsomal supernatants was assayed in duplicate using NADH and 2, 6-dichlorophenolindophenol as electron donor and acceptor respectively as previously described [5].

Table 1
Liver DT diaphorase activities.

Strain	Identification	Micromoles DCPIP reduced per gram liver per minute	
		Male	Female
Sprague–Dawley	Tuck	38.3 ± 6.4 (12)^{X}	–
	Eklund	61.1 ± 4.8 (10)^{X}	–
	Eklund	68.4 ± 3.4 (12)$^{\oplus}$	74.4 ± 4.4 (4)$^{\oplus}$
Wistar	Tolworth	4.1 ± 0.3 (18)^{X}	20.6 ± 2.0 (11)^{X}
	Tolworth	5.8 ± 0.2 (5)$^{\oplus}$	20.1 ± 1.8 (5)$^{\oplus}$
	Stockholm	24.6 ± 0.9 (3)$^{\oplus}$	24.8 ± 1.5 (3)$^{\oplus}$
	Scottish resistant	5.2 ± 0.3 (7)^{X}	21.7 ± 1.4 (3)^{X}
	Welsh resistant	4.1 ± 0.3 (6)^{X}	12.6 ± 0.5 (6)^{X}
		5.3 ± 0.1 (10)$^{\oplus}$	13.5 ± 2.7 (4)$^{\oplus}$
Wild	Susceptible	39.0 ± 7.0 (5)$^{\oplus}$	28.6 ± 5.3 (5)$^{\oplus}$
	Resistant	77.3 ± 16.7 (5)$^{\oplus}$	–

Results are expressed as mean ± S.E.M. with the number of animals in each set in parentheses. Enzyme activities were determined in Stockholm$^{\oplus}$ and Tolworth^{X}.

3 . Results and discussion

The results obtained are given in table 1. The Sprague–Dawley rats from both sources showed a high level of DT diaphorase activity. In contrast all the Wistar animals investigated showed a lower enzyme activity. In both sexes of rats from the Swedish source and in females from England, the enzyme activity was reduced to approximately the same level. The activity of the English males was even lower, being only about 20% of that found in females. Similar results were obtained in both Stockholm and Tolworth with animals from the same sources. The level of DT diaphorase activity in wild warfarin-resistant rats was no lower than that found in wild susceptible animals.

It would thus appear likely that the observed activities of DT diaphorase recorded in the previous [5] and present investigations reflect differences both between and within the various strains of rats used. Supporting genetic evidence is published in an accompanying paper [8]. Whether or not the reported variations in the activity of this enzyme are related to different vitamin K requirements in these strains of rats remains to be determined.

References

[1] Matschiner, J., Bell, R.G., Amelotti, J.M. and Knauer, T.E. (1970) Biochim. Biophys Acta 201, 309–315.
[2] Bell, R.G. and Matschiner, J.T. (1972) Nature 237, 32–33.
[3] Ernster, L., Danielson, L. and Ljunggren, M. (1962) Biochim. Biophys. Acta 58, 171–188.
[4] Hall, J.M., Lind, C., Golvano, M.P., Rase, B. and Ernster, L (1972) in: Structure and Function of Oxidation Reduction Enzymes (Akeson, A. and Ehrenberg, A., eds), p. 433–443, Pergamon Press, Oxford and New York.
[5] Ernster, L., Lind, C. and Rase, B. (1972) Eur. J. Biochem. 25, 198–206.
[6] Boyle, C.M. (1960) Nature 188, 517.
[7] Greaves, J.H. and Ayres, P. (1967) Nature 215, 877–878.
[8] Greaves, J.H., Lind, C. and Rase, B. (1973) FEBS Letters 37, 144–146.