UREA INHIBITION OF HUMAN PSEDOCHOLINESTERASE
A New Method of Detecting Atypical Pseudocholinesterase in Homozygotes

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SUMMARY
A method for differentiating between the usual and atypical pseudochoUnesterases in serum using urea is described. The method can be used for detecting suxamethonium-sensitive individuals. The results from studies of urea inhibition indicate that the atypical pseudochoUnesterase is less stable in its tertiary protein structure than is the usual enzyme.

Acylcholine acyl-hydrolase, E.C. 3.1.1.8, the plasma enzyme still most often referred to as pseudochoUnesterase exists as genetic variants. The atypical pseudochoUnesterase, which can cause suxamethonium sensitivity in some indi-viduals, can be differentiated from the usual enzyme by means of inhibitory substances. The principle of differentiation by inhibitors is based upon the use of an inhibitor at a concentration at which the difference in inhibition between the usual and the atypical enzyme is the largest possible.

Different substances have been used to detect suxamethonium-sensitive individuals and to characterize genetic variants of the atypical pseudochoUnesterase. The inhibitors most frequently used have been dibucaine (Kalow and Genest, 1957), fluoride (Harris and Whittaker, 1961), chloride (Whittaker, 1963), and substance Ro 2-0683 (Liddell, Lehmann and Davies, 1963).

In addition to the above-mentioned substances, urea can be employed to detect atypical pseudochoUnesterase. During investigation of two Danish families with atypical pseudochoUnesterase we met difficulties in the classification of the genotypes of the family members employing the above inhibitors (Hanel and Viby Mogensen, 1970). We therefore tried to inhibit the enzyme with urea, which appeared to be a valuable aid in our case.

MATERIAL AND METHODS

Laboratory investigations.

Blood samples from members of the two men- tioned Danish families, in which we previously studied the distribution of atypical pseudochoUnesterase, were used. In addition, blood sam-
RESULTS

The percentage urea inhibitions of pseudocholinesterase for 10 normal homozygotes, 6 heterozygotes, and 3 atypical homozygotes are shown in figure 1. The curves in figure 1 represent means of each homozygous group (mean ± 2SD). The pseudocholinesterases of the patients were found by inhibition studies to be of the genotypes, E₁⁺E₁⁺, E₁⁺E₁⁻, and E₁⁺E₁⁻, according to the nomenclature proposed by Motulsky (1964).

![Graph showing urea inhibition of typical and atypical pseudocholinesterases.]

A 50 per cent inhibition of the pseudocholinesterase of the genotype E₁⁺E₁⁺ was achieved at about 5 molar, and of the genotype E₁⁺E₁⁻ at about 2 molar urea concentration as shown in figure 1.

DISCUSSION

Urea inhibition can be used for differentiation between usual and atypical pseudocholinesterases. As can be seen from figure 1, at a 2–3 molar urea concentration, the inhibition for individuals of the genotype E₁⁺E₁⁻ was about 55–60 per cent, whereas for individuals of the genotype E₁⁺E₁⁺, the inhibition was about 20–32 per cent. Individuals of the genotype E₁⁺E₁⁻ were between these two values, but they cannot be distinguished from the normal group.

Higher urea concentrations would give greater differences between the atypical and the typical enzymes but would increase the technical difficulties in the measurement of the low activity, especially of the atypical pseudocholinesterase.

It has been shown that urea can cause an unfolding of the chains in the protein structure, which gives rise to an increased volume of the protein molecule, because its polypeptide structure becomes less compact (Nozaki and Tanford, 1963; Tanford, 1964). Olesen and Pedersen (1963) found by gel filtration of human albumin at different urea concentrations an increase of a part of the protein molecules, probably due to stepwise unfolding or swelling of albumin, indicating a stepwise denaturation of the protein. The urea inhibition of pseudocholinesterase activity may thus be caused by the same mechanisms. As the effect is more pronounced on the atypical than on the usual enzyme, this might indicate that the usual enzyme is more stable in its tertiary protein structure than is the atypical.

Similar results were obtained by Brandt and Hanel (1971) in an abnormal pyruvate kinase of erythrocytes from a family with hereditary non-spherocytic anaemia.

ACKNOWLEDGEMENTS

This study was supported by a grant from King Christian den X's Fond.

The authors wish to thank Mrs Grethe Nørgaard for skilful technical assistance.

REFERENCES


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