E_1^j , A quantitative variant at cholinesterase locus 1: immunological evidence

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Summary. Sera of various phenotypes at serum cholinesterase locus 1, including the newly recognized phenotypes $E_1^aE_1^j$, $E_1^uE_1^j$, and $E_1^fE_1^j$, were studied by immunodiffusion and rocket immunoelectrophoresis. The sera containing the E_1^j allele show reduced numbers of immunologically active cholinesterase molecules. This finding is consistent with the previously advanced hypothesis that E_1^j results in reduced numbers of circulating 'usual' (E_1^u) molecules. Whether this reduction is the result of the low rate of synthesis or of an increased rate of degradation of the cholinesterase remains to be determined.

In the immediately preceding paper a family was presented whose genetic and biochemical data strongly suggest the presence of a rare allele (E_1^j) at cholinesterase locus 1 (Garry *et al.*, 1976). The data are consistent with the hypothesis that the E_1^j gene causes reduced numbers of circulating usual (E_1^u) enzyme molecules. In the present paper we offer immunological evidence in support of this hypothesis.

Methods

Sera

All sera containing the E_1^j allele were from the H-J pedigree (Garry *et al.*, 1976). The remainder of the sera were from an extensive well-characterized library of sera maintained at -20°C .

Cholinesterase activity

The cholinesterase activity of all sera was determined by the method previously described (Dietz *et al.*, 1972; Dietz, Rubinstein, and Lubrano, 1973).

Immunological methods

The antiserum to purified serum cholinesterase was previously described (Rubinstein *et al.*, 1970). Antigen-antibody reactions were carried out in two ways.

a) Gel immunodiffusion. Rabbit antiserum, 100 μl , was incorporated into 100 ml of 1% agarose in

1% NaCl. After the gel had cooled to 47°C , microscope slides were covered with 3.3 ml of the gel. After the gel formed, 2 mm wells which held 5 μl serum were punched. When 10 μl sample was used, the initial application of 5 μl serum was allowed to diffuse into the gel before a second 5 μl was added. After diffusion for 24 hours at room temperature the reaction was visualized by staining for cholinesterase with α -naphthyl butyrate (Stern and Lewis, 1962). The stained slides were rinsed with 7% acetic acid and washed several times with water over a period of several hours to remove the salt and then dried at room temperature. The diameter of the cholinesterase activity can be measured without drying, but the precision is better when measurements are made on the dried slides.

b) Rocket electrophoresis. Electrophoresis of the antigen in antibody-containing agarose was carried out by the method of Laurell (1966). Rabbit antibody to cholinesterase, 100 μl , was added to 100 ml of 1% agarose in 0.025 mol barbitonium buffer pH 8.6. After the mixture had cooled to 47°C , 15 ml was poured onto an 8×10 cm lantern slide. Two or three rows of wells of 2 mm diameter were punched across the narrow dimension of the slide and the sera, usually 5 μl , added to the wells. Electrophoresis ran 7 hours at 18 v/cm with the electrode vessels filled with 0.05 mol barbitonium buffer of pH 8.6. Staining for cholinesterase was performed as given in a) and the distance of migration was measured.

Results and discussion

Fig. 1 and 2 show typical results for several different classes of sera (U, UA, and A phenotypes) by the immunodiffusion and rocket methods,

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FIG. 1. Immunodiffusion of serum cholinesterase in antibody-containing agarose. The cholinesterase activities of the sera used were: U phenotype 8.75 units/ml per min, UA phenotype 5.29, and A phenotype 2.39.

FIG. 2. Rocket electrophoresis of serum cholinesterase in antibody-containing agarose. The sera were the same as used for Fig. 1.

FIG. 3. Quantitation of serum cholinesterase by immunodiffusion method. Abscissa: log of activity. Ordinate: diameter of the antigen-antibody rings. The points and derived lines are based on the data shown in Fig. 1. The equations of the lines calculated by least squares: U, $y = 1.31 + 4.44x$, $r = 0.998$; UA, $y = 2.73 + 3.67x$, $r = 0.994$; A, $y = 4.32 + 3.65x$, $r = 0.996$.

respectively. The application of these methods to the H-J family and to sera of other phenotypes is summarized in Fig. 3-5.

Fig. 1 and 3 show that, by the immunodiffusion method using dilutions of U, UA, and A sera of average activity, the log of the cholinesterase activity plotted against the diameter of the antigen-antibody ring gives a straight line (Berne, 1974).

Using the same plot, but with larger numbers of sera of each class, Fig. 4 shows that equal volumes of U, UA, and A sera give approximately the same reading on the ordinate. This allows the conclusion that the E_1^* allele results in production of about the same number of immunopotent cholinesterase

FIG. 4. Immunodiffusion of the serum cholinesterase variants. In every case 5 μ l serum was used. The lines shown are those calculated by the method of least squares from 5 dilutions of sera indicated (see Fig. 1).

FIG. 5. Rocket immunoelectrophoresis of the serum cholinesterase variants. Abscissa: log of activity. Ordinate: migration distance of the antigen-antibody precipitate. The derived lines are based on the data shown in Fig. 2. The equations for these lines are: U, $y = -81.9 + 85.9x$, $r = 0.997$; UA, $y = -55.5 + 74.5x$, $r = 0.997$; A, $y = -29.4 + 82.9x$, $r = 0.994$. Each point corresponds to 5 μ l serum.

molecules as the E_1^u allele though the two differ, on the average, fourfold in their enzymatic activity when propionylthiocholine is the substrate (Dietz *et al*, 1972; 1973).

Fig. 4 also shows that alleles which result in diminished serum cholinesterase activity yield less immunologically reactive material. This has previously been demonstrated for the E_1^s allele (Rubinstein *et al*, 1970) and is confirmed here. In the case of the E_1^f allele, a reduction in the number of immunologically reactive cholinesterase molecules is also evident. The five $E_1^aE_1^f$ sera from the H-J family are located in a distinctive portion of the plot and do not overlap with any other phenotype. Similarly, two of the three points (III-9 and IV-9) representing the obligatory $E_1^uE_1^f$ sera occupy a region of the plot displaced towards the lower left, a region that U sera with diminished cholinesterase activity would occupy. Not excluded is the possibility, which we consider unlikely, that the J allele results in production of a variant molecule equally reduced in enzyme activity and immunological reactivity.

Fig. 2 and 5 show similar results by rocket immunoelectrophoresis.

In Fig. 4 and 5, it is seen that equal volumes of U and UJ sera, which have indistinguishable dibucaine inhibitions, occupy different areas of the graph. This suggests that these two phenotypes might be distinguished with fair certainty by measuring both enzymatic activity and immunological reactivity. This would be particularly useful when a pedigree analysis is inconclusive. However, a U serum showing an unusually low enzyme activity as the result of intercurrent disease (hepatic, renal, malignant, etc) or anticholinesterase medication would occupy the same portion of the plot as UJ sera.

Analysis of variants containing the E_1^f allele ($E_1^uE_1^f$, $E_1^fE_1^f$, $E_1^fE_1^s$, and $E_1^fE_1^f$) by gel immunodiffusion gives similar results.

Thus, we have two alleles at cholinesterase locus 1 resulting in decrease of circulating E_1^u molecules. The silent (E_1^s) allele, which apparently exists in two different forms (Rubinstein *et al*, 1970), results in 97 to 100% reduction. In the present case, according to data given in the preceding paper and confirmed in a nonquantitative fashion in this paper, the E_1^f allele causes 65 to 70% reduction. Further investigation of families with anomalous dibucaine or fluoride inhibitions may possibly reveal other alleles with differing quantitative effects.

It is not yet known if the E Cynthiana gene, which produces increased serum cholinesterase activity, is allelic at cholinesterase locus 1 (Neitlich, 1966; Yoshida and Motulsky, 1969). This will only be determined when a family is found in which E Cynthiana (so far only reported in one family) is segregating along with a known variant (A, F, or S) at locus 1.

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