Hypercholinesterasemia with Isoenzymic Alteration in a Family

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A family with hypercholinesterasemia with isoenzymic alteration is reported. The propositus, a 55-year-old woman, was admitted to our hospital because of diabetes mellitus. Because her cholinesterase activity (ΔpH 3.2) was supranormal, with no other abnormal liver-function test result throughout the hospitalization period, and was independent of her disease state, we investigated whether this condition might be familial. We studied six of her 17 family members in three generations. All six had above-normal serum cholinesterase activity. Gradient gel electrophoresis on polyacrylamide gels showed that the normal control individuals had seven isoenzymes, but all the family members with hypercholinesterasemia had two additional isoenzymes. The enzymic properties of the affected members were similar to those of the normal individuals. Hypercholinesterasemia in this family seems to be the result of an increased number of enzyme molecules, but how this isoenzymic alteration emerged remains obscure.

Cholinesterase (ChE; acetylcholine acyl-hydrolase, EC 3.1.1.8, also called "pseudocholinesterase") activity has been routinely measured in serum as a liver-function test, although the significance of the enzyme in metabolism is rather obscure. Slightly high values are often associated with diabetes mellitus, fatty liver, nephrotic syndrome, obesity, hyperthyroidism, and other disorders (1–3). In contrast, serum ChE is decreased in various disorders of the liver (4), in carcinoma (5), and after administration of anticholinesterase drugs (3). Several genetically determined variants of ChE have also been found. At least five genes that determine serum ChE biosynthesis have been recognized (5). They are the usual (E), atypical (E), fluoride resistant (E), silent (E), and C (C) genes. E, E, and E are allelic to E, and these genes give rise to 10 genotypes, all of which have been recognized. E is non-allelic to E. The genotypes determined by the genes E, E, and E can be clearly differentiated by use of certain inhibitors, but the genotype determined by the C gene has been confirmed only by electrophoresis on starch-gel. Besides these variant forms of ChE, Neitlich (3) reported another genetic variant (E Cynthia), which is associated with a plasma ChE activity two to three times the normal mean, with production of an extra enzyme component. Here we describe a new type of familial hypercholinesterasemia with isoenzymic alteration, and some enzymic properties of the enzyme.

Materials and Methods

Reagents

Dibucaine hydrochloride, ethopropazine hydrochloride, dl-propranolol, and sialidase (from Clostridia perfringens;

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Received August 6, 1985; accepted September 1, 1985.

EC 3.2.1.18) were from Sigma Chemical Co., St. Louis, MO 63178. Acetylthiocholine iodide, butyrylthiocholine iodide, sodium fluoride, other chemicals, and the cholinesterase test kit were from Wako Pure Chemical Industries, Ltd., Osaka, Japan. Antiserum to human cholinesterase, produced in rabbits, was from DAkOPATT a/s, Copenhagen F, Denmark.

Source of Enzyme

Serum from the affected members illustrated in Figure 1 was kept at −20 °C until used.

Enzyme Assay

We assayed enzyme activity, using benzoylcholine 5 × 10⁻³ mol/L in phosphate buffer (100 mmol/L, pH 7.4) at 26 °C, by the method of Kalow and Lindsay (6). Another cholinesterase assay was done with the Wako cholinesterase test kit. In this kit, acetylcholine is the substrate, and acetic acid produced by the enzyme decreases the color of m-nitrophenol in proportion to the change of pH in the reaction mixture, the decrease being measured at 420 nm.

Electrophoresis in Polyacrylamide Gel

Electrophoresis in polyacrylamide gradient gels was as described previously (7) except for the gel concentration. Polyacrylamide gel with a continuous concentration gradient from 2 to 16 g/dL was used. After electrophoresis, the enzyme was stained by the method of Juul (8), with acetylthiocholine or butyrylthiocholine as substrate.

Case Report

The propositus, a 55-year-old woman, was admitted to our hospital because of diabetes mellitus. Her disorder was in a stable state until two months before admission, when she began to feel unduly thirsty.

On examination she was an obese woman who appeared well. Her height was 159 cm, body weight 79 kg. The blood pressure was 134/90 mmHg. Examination of the heart, lungs, and abdomen revealed no remarkable findings. Knee

Fig. 1. Family distribution of increased serum cholinesterase activity with isoenzymic alteration
and ankle jerks were absent. Sensory disturbances were bilaterally noted in the lower extremities. Babinski’s sign was not found.

The urine gave a ++ test for glucose but was negative for protein. The sediment was not remarkable. The hemocrit was 14.8 g/dL; the leukocyte count was 4600/mm³, with 36% neutrophils. The platelet count was 190,000/mm³. The erythrocyte sedimentation rate was 6 mm/h. Results of the serum test of liver-function were normal except for the high serum ChE activity, a ΔpH of 3.2 (normal range 0.8–1.1). The plasma glucose concentration was 2.38 g/L (specimen taken after a short fast).

She had a five-year history of diabetes mellitus, controlled by oral agents until two months earlier. Her parents were not consanguineous. There was no family history of diabetes mellitus or obesity. Her father died of cerebral infarction, her mother of encephalitis japonica.

Results

Family Study

The propositus’s ChE activity (ΔpH of 3.2) was about 3.4 times the normal mean. Because no disease could be found to explain this increase and because it remained almost constant for eight months, we examined her family for increased serum ChE. Six of the 17 family members in three generations could be examined. All had increased enzyme activity as shown in Figure 1 and Table 1. Not enough serum was obtained from case 6, a two-year-old boy, for the following examination except for electrophoresis.

Effect of Inhibitors

The in vitro sensitivity of serum ChE from the affected members was tested with dibucaine, fluoride, propranolol, ethopropazine, or physostigmine. Dibucaine numbers were determined according to Kalow and Genest (9). Fluoride numbers were measured by the method of Harris and Whittaker (10). The percentage inhibition produced by other compounds was measured in the same way as dibucaine determination except that each compound at the final concentration indicated in Table 2 was substituted for dibucaine in the test system.

Dibucaine numbers and fluoride numbers were within the normal range in all the members tested. The residual activity of the enzyme from the affected members, examined with use of the other inhibitory compounds listed in Table 2, were almost the same as those of the normal individuals.

Effect of Alkyl Alcohols

The effect of four alkyl alcohols on the enzyme activity at pH 7.4 was studied according to the method of Whittaker (11). Each alcohol was incorporated in the assay mixture so as to give the final concentration indicated. The activity remaining was expressed as the percentage obtained to the untreated control. Table 3 shows the effect produced by each alkyl alcohol on the enzyme activity. Each alcohol activated the enzyme activity, but no significant differences were observed between the affected members and normal controls.

Electrophoretic Properties

Figure 2 shows a zymogram of the polyacrylamide gradient gel electrophoretogram stained for ChE activity. Seven isoenzymes of ChE (I to VII, numbered from the anodic side to the cathodic) were observed in normal sera, but two additional isoenzymes—a faint one (A) located between II and III, and a distinct one (B) with a slower electrophoretic mobility than VII—were noted in the sera of all the family members we examined. A faint band sometimes appeared between III and IV, but it was excluded from the present numbering. Effects of inhibitors or activators and of heat treatment (56°C, 30 min) on each isoenzyme were investigated on the polyacrylamide gradient gel (not shown). All isoenzymes behaved quite similarly, and the effects on the two additional isoenzymes found in serum from the family members were not different from those on the common isoenzymes shared by the normal individuals.

Immunological Neutralization Test

To estimate the relative specific activity, we carried out an immunological neutralization test. The reaction mixture consisted of 50 ìL of serum from the propositus or a normal

Table 1. Serum Cholinesterase Activities in Cases of Hypercholinesterasemia with Isoenzyme Alteration

<table>
<thead>
<tr>
<th>Case</th>
<th>1</th>
<th>2*</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholinesterase activity, ΔpH (normal range 0.8–1.1)*</td>
<td>1.6</td>
<td>3.2</td>
<td>1.8</td>
<td>1.8</td>
<td>2.1</td>
<td>2.7</td>
</tr>
</tbody>
</table>

*The propositus.

*This value was obtained from the central laboratory room at our hospital.

Table 2. Effect of Inhibitors on Serum Cholinesterase at pH 7.4

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Conc. mol/L</th>
<th>Control (n = 10)</th>
<th>Case</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dibucaine</td>
<td>10⁻⁵</td>
<td>16.0–19.5</td>
<td>25.2</td>
</tr>
<tr>
<td>Fluoride</td>
<td>5×10⁻⁵</td>
<td>30.0–36.0</td>
<td>38.8</td>
</tr>
<tr>
<td>Propranolol</td>
<td>10⁻⁶</td>
<td>11.4–15.1</td>
<td>12.5</td>
</tr>
<tr>
<td>Phophonazine</td>
<td>10⁻⁶</td>
<td>38.6–53.2</td>
<td>55.5</td>
</tr>
<tr>
<td>Ethopropazine</td>
<td>10⁻⁷</td>
<td>37.5–63.1</td>
<td>50.0</td>
</tr>
</tbody>
</table>

*Figures are the percentage of enzyme activity found in the absence of inhibitor. Cases 1 to 5 were persons with hypercholinesterasemia with isoenzymatic alteration.

Table 3. Effect of Alcohols on Serum Cholinesterase at pH 7.4

<table>
<thead>
<tr>
<th>Alcohol</th>
<th>Conc., mEq/L</th>
<th>Control (n = 10)</th>
<th>Case</th>
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<tr>
<td>Methanol</td>
<td>150</td>
<td>128–143</td>
<td>129</td>
</tr>
<tr>
<td>Ethanol</td>
<td>100</td>
<td>165–203</td>
<td>163</td>
</tr>
<tr>
<td>Propanol</td>
<td>10</td>
<td>136–172</td>
<td>175</td>
</tr>
<tr>
<td>Butanol</td>
<td>10</td>
<td>155–190</td>
<td>191</td>
</tr>
</tbody>
</table>

*Figures are the percentage of enzyme activity found in the absence of alcohol. Cases 1 to 5 are persons who have hypercholinesterasemia with isoenzymatic alteration.
individual, an appropriate volume of variously diluted antisem, and Tris HCl (20 mmol/L, pH 7.4) in a total volume of 200 μL. The mixture was incubated at 4 °C overnight and centrifuged at 10 000 × g for 10 min. The supernatants were carefully separated from the antigen–antibody precipitate. The enzyme activity in the supernatant fluid was assayed. Figure 3 shows that the amount of antibody required to inhibit the original activity of the propositus by 50% was about three times that for the normal control. Because the enzyme activity of the propositus was about three times the normal mean value, the propositus thus seemed to have more enzyme molecules, the specific enzyme activity of which were similar to that for the normal controls.

Discussion

Few papers deal with hypercholinesterasemia associated with abnormal electrophoretic bands. First, Harris et al. (12) reported that at least four distinct zones (C₁ to C₄) with properties of serum ChE were demonstrated by starch-gel electrophoresis of normal serum or plasma. Some individuals showed another zone (C₅) with properties of serum ChE. C₅ had a slightly slower mobility than C₄ in starch-gel electrophoresis. The mean serum ChE in C₅ individuals was about 30% greater than that of C₅ individuals of the usual phenotype. Family studies suggested that the extra band (C₅) is due to a gene that is non-allelic to Eₚ. Neitlich reported another type of hypercholinesterasemia associated with a novel electrophoretic band (3). He found one person with high serum ChE activity (3.6 times the normal mean) out of 1029 male volunteers. This subject’s serum showed an additional band of ChE on disc electrophoresis, and in an in vivo study, he was more resistant to succinylcholine than were the normal subjects.

Our cases, with ChE activity 1.7 to 3.4 times the normal mean and two additional electrophoretic bands, differ from the cases reported by Harris et al. (12). The family members also had a different number of electrophoretic bands and sensitivity to dibucaine from those reported by Neitlich; the percentage inhibition of our cases by dibucaine was normal, but perhaps his case was highly sensitive to dibucaine, judging from the resistance to succinylcholine.

We electrophoretically examined the sera of 30 subjects with various diseases, including diabetes mellitus, obesity, and fatty liver. None showed the abnormal isoenzymes detected in our cases.

The family study suggests that the inheritance in our cases is autosomal dominant, but X-linked dominant inheritance can not be ruled out.

As judged from the effect of inhibitors and activators on the enzyme, the catalytic properties of it in the family members seem to be similar to those of the normal control subjects. As none of the family members in our study have taken succinylcholine as a muscle relaxant, it is not clear if they are more resistant to the drug than are the normal subjects, as was observed in Neitlich’s patients.

Theoretically, the increased serum ChE activity in the family members could result from a structural abnormality producing a more active enzyme molecule, from increased rate of production of a structurally abnormal enzyme with normal activity, from the increased rate of production of a structurally normal enzyme due to a regulatory disturbance, from a decreased degradation rate, or from some combination of these effects.

In our cases, the immunological neutralization test (Figure 3) showed that the amount of antibody required to inhibit the original ChE activity of the propositus by 50% was about three times that required for the normal control. Also, in the Ouchterlony double-diffusion test, the antigen–antibody complex of the normal subjects did not produce a precipitin line stained with Amido Black 10. After concentration of the normal serum, the antigen–antibody complex produced a visible protein band; the antigen–antibody complex of our propositus produced a clear precipitin line stained with Amido Black 10 without being concentrated (not shown). Evidently the increase of enzyme activity in our propositus may be due to an increase in the number of enzyme molecules, just as was reported by Yoshida and Motulsky (13), who examined plasma provided by Neitlich (E Cynthia).

All the serum ChE isoenzymes of the family members remained at the origin on the gradient gel after treatment with antibody to human ChE, so the antigenic sites of the two additional isoenzymes found in the family members seemed to be the same as those of the common isoenzymes found in normal individuals (not shown).

The effects of the inhibitors and storage at −20 °C on the enzyme activity and the heat stability (56 °C, 30 min) of the enzyme in our cases were the same as in the normal individuals.

Judging from the staining intensity of ChE, the enzyme activity of the two additional isoenzymes we observed was about 15% of the total activity, insufficient to explain the increased serum ChE activity in the propositus’s family. In fact, to obtain a similar staining intensity, only a half or one-third of the amount required with normal serum was necessary for the family members. Therefore, each isoenzyme of serum ChE in the family subjects may have an abnormally high enzyme activity, due mainly to their increased concentration. In addition, as enzyme properties of the two additional isoenzymes examined on the polyacrylamide gel were not different from properties of the common isoenzymes, the catalytic properties of the two isoenzymes seem to be the same as those of the common enzymes.

Yoshida and Motulsky (13) found that the extra enzyme band detected in the patient reported by Neitlich disappeared on dilution, though the total enzyme activity remained proportional to the degree of dilution. Furthermore, because the extra enzyme band had a higher molecular mass than the normal C₄ enzyme, they concluded that the extra band resulted from an aggregation of components of enzyme molecules with different association properties. As
the two extra isoenzymes of serum ChE in our cases did not disappear on dilution, the mechanism of the emergence of the two isoenzymes in our cases seems to differ from that reported by Yoshida and Motulsky. Anyway, how the two additional isoenzymes emerged is not clear now, but they do not seem to be artifacts, because storage of serum for at least six months at -20 °C or freezing and thawing of the serum did not change the isoenzymic form.

It remains to be answered whether the presence of two additional enzyme components of the affected subjects is related to production of a structurally abnormal enzyme due to a mutation of either the E1 or E2 locus or results from some secondary modification of the protein.

We thank Ms. Mayumi Kamihira for her excellent assistance.

References