INHIBITION OF THE PLASMA CHOLINESTERASE VARIANTS BY PROPRANOLOL

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SUMMARY

The inhibitory effect of (±) propranolol 1.69 × 10⁻⁴ - 1.69 × 10⁻⁷ mol litre⁻¹ on normal and atypical plasma cholinesterase variants was investigated. The atypical enzyme is less sensitive to inhibition by (±) propranolol or either of its enantiomorphs than the usual enzyme. Propranolol 8.45 × 10⁻⁶ mol litre⁻¹ was used as differential inhibitor of 643 plasma samples from individuals of known genotype. Although the measurement of propranolol inhibition alone is not always unambiguous for assigning a definite genotype to a given individual, the correlation of propranolol inhibition with fluoride inhibition gives clear differentiation of the E,*,E,* and E,*,E,* phenotypes as well as other phenotypes.

Propranolol is one of the beta-blocking agents which has wide application therapeutically. It has been used in the medication of tremor (Marsden et al., 1967), but whilst there are some reports that this beta-blocker has a beneficial effect on essential tremor (Dupont, Hansen and Dalby, 1973; Morgan, Hewer and Cooper, 1973; Winkler and Young, 1974; Tolosa and Loewenson, 1975) others publish negative results (Balla, 1973; Foster, Longley and Steward-Wynne, 1973; Sweet et al., 1974).

We have observed that individuals receiving propranolol appear to be sensitive to suxamethonium and that this may be correlated with diminished plasma cholinesterase activity (Periss and Whittaker, unpublished observations). The decreased enzyme activity is restored to normal after therapy ceases. Propranolol is not metabolized by plasma cholinesterase (Schneck, Pritchard and Hayes, 1979). We investigated if propranolol could be used to differentiate the cholinesterase variants which segregate at the E, locus. Four allelic genes control biosynthesis of the enzyme at this locus. Thus usual E,*, atypical E,*, silent E,*, and fluoride resistant E,*,E,* genes give rise to 10 genotypes, all of which have been recognized.

The three heterozygotes with the silent gene E,*,E,*; E,*,E,* and E,*,E,* cannot be distinguished from the corresponding homozygotes E,*,E,*; E,*,E,* and E,*,E,* by inhibition measurements and the homozygote E,*,E,* has negligible enzyme activity even in the absence of added inhibitor.

Available screening tests have been criticized since it is sometimes difficult to assign a definite genotype to a given individual because of overlapping of some of the factors used to differentiate the variants. We therefore examined a large number of individuals with six known genotypes, using propranolol as differential inhibitor, and determined if plasma cholinesterase had stereo-specificity for either of the enantiomorphs, (+) or (−) propranolol.

MATERIALS AND METHODS

Plasma was obtained from venous blood samples and collected in heparinized tubes. The samples had been sent to Exeter for phenotyping of the cholinesterase variants. On arrival, the plasma was either phenotyped immediately or kept frozen until required.

Enzyme activity was assayed using benzoylcholinechloride 5 × 10⁻⁵ mol litre⁻¹ in phosphate buffer 0.067 mol litre⁻¹ pH 7.4 at 26°C by the method of Kalow and Lindsay (1955). The phenotype of each sample was determined by measurement of dibucaine number according to Kalow and Genest (1957) as well as the fluoride number by the method of Harris and Whittaker (1961). Propranolol was included in the reaction mixture at various concentrations. Enzyme activity was measured using plasma at a dilution of 1/200 as the source of enzyme in the absence of propranolol. The decrease in activity at a given concentration of propranolol was expressed as percentage of the activity in the absence of the drug. The concentrations of propranolol were 1.69 × 10⁻⁴ - 1.69 × 10⁻⁷ mol litre⁻¹. Plasma samples from at least nine individuals having the usual

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enzyme $E_1*E_1^*$ and at least five individuals having the atypical enzyme $E_1^*E_1^*$ were studied to obtain the inhibition profiles of the two enzymes. From these curves, the concentration of propranolol was selected which gave maximum differentiation of the variants. This optimal concentration of propranolol was used as a differential inhibitor to screen plasma from 643 individuals of known genotype. The samples contained six different phenotypes of the $E_1$ locus.

**RESULTS**

The average percentage inhibition of plasma cholinesterase activity using various concentrations of propranolol for several samples of the usual and atypical enzyme are given in figure 1. Propranolol $8.45 \times 10^{-6}$ mol litre$^{-1}$ provided optimal differentiation of the usual and atypical enzymes. This concentration was used to determine the propranolol numbers of 643 different individuals. The propranolol number is defined as the percentage inhibition of the rate of hydrolysis of benzoylcholine $5 \times 10^{-5}$ mol litre$^{-1}$ in phosphate buffer 0.067 mol litre$^{-1}$ at $26 ^\circ$C by propranolol $8.45 \times 10^{-6}$ mol litre$^{-1}$. The propranolol, dibucaine and fluoride numbers of the plasma samples investigated were separated into their various phenotypes, as defined by dibucaine and fluoride numbers, and are presented in table I.

![Figure 1](https://example.com/fig1.png)

**FIG. 1.** Inhibition of usual and atypical plasma cholinesterase with varying concentrations of (±) propranolol. $\times$ = mean percentage inhibition of $E_1^*E_1^*$ individuals; $\bigcirc$ = mean percentage inhibition of $E_1*E_1^*$ individuals; range of inhibition observed represented by vertical bars.

![Figure 2](https://example.com/fig2.png)

**FIG. 2.** Distribution of propranolol numbers in 643 individuals.
Table I. Distribution of propranolol numbers for plasma cholinesterase phenotypes

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Number investigated</th>
<th>Propranolol number</th>
<th>Dibucaine number</th>
<th>Fluoride number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>E₁⁻⁻</td>
<td>236</td>
<td>82.2</td>
<td>1.62</td>
<td>80.3</td>
</tr>
<tr>
<td>E₁⁺⁻</td>
<td>223</td>
<td>63.0</td>
<td>3.55</td>
<td>63.2</td>
</tr>
<tr>
<td>E₁⁺⁺</td>
<td>84</td>
<td>9.4</td>
<td>4.23</td>
<td>20.3</td>
</tr>
<tr>
<td>E₁⁻⁺</td>
<td>59</td>
<td>73.3</td>
<td>2.48</td>
<td>76.2</td>
</tr>
<tr>
<td>E₁⁺⁺⁺</td>
<td>35</td>
<td>39.0</td>
<td>7.52</td>
<td>48.6</td>
</tr>
<tr>
<td>E₁⁻⁺⁺</td>
<td>6</td>
<td>53.0</td>
<td>1.38</td>
<td>62.6</td>
</tr>
</tbody>
</table>

Discussion

Propranolol is a powerful inhibitor of plasma cholinesterase and the normal enzyme has a greater affinity for the drug than the atypical enzyme. The inhibition is reversible since the activity of the inhibited enzyme is restored after dialysis. For both enzymes, the inhibition is instantaneous and competitive.

The distribution of propranolol numbers among the 643 individuals is shown in figure 2. A continu-
ous distribution is found with no clear-cut segregation into different phenotypes except for $E^uE^f$. When the data are separated into the six phenotypes as defined by dibucaine and fluoride numbers, it is found from figure 3 that the continuous distribution of figure 2 can be regarded as being made up of six separate but, in some cases, overlapping distributions which is most obvious for the genotypes $E^uE^u$, $E^uE^f$ and $E^fE^f$. The histograms illustrate that the measurement of propranolol numbers alone is not sufficiently discriminating to assign unambiguously a definite genotype to a given individual. However, the resolution of the $E^uE^f$ phenotype from $E^uE^u$ is superior to that claimed by Dickson (1978) with the bromide number (BrN). The range of BrN for $E^uE^u$ and $E^uE^f$ reported by Dickson (1978) were 0-8 and 0-15 respectively. These are not very discriminating ranges of BrN for two different phenotypes and do not justify the claim that BrN give a better differentiation between $E^uE^u$ and $E^uE^f$ than previously used inhibitors.

The correlation diagram obtained when dibucaine numbers are plotted against propranolol numbers for each individual studied is shown in figure 4. A linear relationship is obtained and it is apparent that not only are the phenotypes $E^uE^u$, $E^uE^f$ and $E^uE^f$ resolved, but there is also a good resolution of $E^fE^f$ and $E^fE^u$. The phenotype $E^fE^u$, although segregating at the top of the $E^uE^f$ group is not clearly defined by this scatter diagram. When fluoride numbers are plotted against propranolol numbers for the 643 individuals, a linear relationship is again obtained (fig. 5). In this diagram, however, the phenotypes $E^uE^u$ and $E^uE^u$ do not segregate into distinct groups, and merging also occurs with the phenotypes $E^uE^f$ and $E^fE^f$. However, a few individuals have inhibition characteristics which appear to differ from those of the six phenotypes studied. Such discrepancies could be attributed to the existence of new phenotypes. Family studies are being undertaken in an attempt to recognize new phenotypes.

Rubinstein and others (1976) have described an $E^f$ gene segregating in a family and the same group have reported an $E^u$ gene segregating in two families (Rubinstein, Dietz and Lubrano, 1978). Although it was suggested that $E^u$ may be identical with the rare gene reported in a family by Lehmann and others (1960) and with the gene producing unusual alcohol numbers (Whittaker, 1968), this seems improbable. There are, however, several anomalies which could be resolved by the recognition of new variants of plasma cholinesterase. Many workers report that 30% of suxamethonium-sensitive individuals appear to have the usual phenotype (Bauld et al., 1974; Goedde et al., 1976; Whittaker, 1980) and although Viby-Mogensen and Hanel (1978) have been able to explain the sensitivity of some of these individuals, some with apparently $E^uE^u$ phenotypes are sensitive to suxamethonium. Whittaker, Spencer and Searle (1977) have reported a high frequency of the $E^f$ gene in individuals who have survived malignant hyperthermia and Ellis and others (1977) have confirmed that a high frequency of the $E^f$ gene has been found in relatives of known cases of malignant hyperthermia who, from muscle biopsy tests, would be expected to be prone to the syndrome. However, malignant hyperthermia has a dominant inheritance (Britt, Lecher and Kalow, 1969), whereas the $E^f$ gene is recessive and not all individuals with the $E^f$ gene would show malignant hyperthermia. A survey of Danish patients who have survived malignant hyperthermia does not show a high frequency of the $E^f$ gene (Viby-Mogensen and Hanel, private communication). A high frequency of the $E^u$ gene has been reported in Huntington's Disease (Whittaker and Berry, 1975), but Huntington's Disease is a dominant inheritance, in contrast to the $E^f$ gene. New genetic variants of plasma cholinesterase could explain some of these anomalies and, until an easy method for purifying the enzyme becomes available to enable identification of amino acid sequences in the polypeptide chains of the enzyme, it is worthwhile to explore new differential inhibitors which may expose new phenotypes.

The enantiomorph (+) propranolol has a greater affinity than (−) propranolol for both normal and atypical plasma cholinesterase variants, and these findings will be published in detail elsewhere.

ACKNOWLEDGEMENTS

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REFERENCES

Fig. 4. Distribution of dibucaine numbers and propranolol numbers of 643 individuals. x = single individual; • = more than one individual.

Fig. 5. Distribution of fluoride numbers and propranolol numbers of 643 individuals. x = single individual; • = more than one individual.


