DIFFERENTIATION OF SERUM CHOLINESTERASE VARIANTS BY SUCCINYLDICHLORINE INHIBITION

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SUMMARY

The inhibition by succinyldicholine (suxamethonium, Scoline) of the hydrolysis of benzoylcholine by serum cholinesterases has been studied. An analytical scheme for the differentiation of the genetically-determined cholinesterase variants is described and the ranges of percentage inhibition (Scoline number) encountered for each phenotype are presented. Correlation between succinyldicholine, dibucaine, fluoride and chloride inhibitions in 221 individuals of 6 recognized phenotypes are illustrated and it is suggested that these groupings are not homogeneous.

METHODS AND MATERIALS

Cholinesterase activity was estimated by the method of Kalow and Lindsay (1955) and phenotype determined simultaneously by dibucaine number (Kalow and Genest, 1957), fluoride number (Harris and Whittaker, 1961) and chloride number (Whittaker, 1968b) following the analytical scheme of King (1965) at 25°C. A Unicam SP8000 recording spectrophotometer was used, temperature control given by a Tecam water-bath.

Sera from six phenotypes, 100 normal homozygotes, E, E, 61 atypical heterozygotes, E, E, 25 fluoride-resistant heterozygotes, E, E, 20 atypical homozygotes, E, E, 3 fluoride-resistant homozygotes, E, E, and 12 atypical/fluoride-resistant heterozygotes, E, E, were studied.

RESULTS

At a benzoylcholine concentration of 50 µM, which is optimal for the usual enzyme at 25°C, the succinyldicholine concentration in the reaction mixture varied from 5 µM to 100 mM. The inhibition curves obtained for the six commonly-recognized phenotypes are shown in figure 1 and these follow the typical sigmoid pattern. The curves for the usual and the atypical homozygotes are almost identical with those obtained by Goedde, Held and Altland (1968). From these studies maximal differentiation of the phenotypes was obtained at a 1 mM concentration of succinyldicholine in the reaction mixture.

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By employing a 4 mM aqueous solution of succinyldicholine (397 mg succinyldicholine chloride dihydrate/litre) in the assay scheme of King (1965) the percentage inhibition (Scoline number) was determined in sera obtained from patients who had exhibited an abnormal response to the relaxant, from their relatives, and from normal individuals. The results obtained from this survey over a period of two years are illustrated in figure 2 and demonstrate that a differentiation of phenotypes is obtained at least as good as that obtained by the use of dibucaine inhibition. In figure 2, the closed circles for the usual phenotype each represent three sera, the open circles, one specimen. Three anomalous values obtained from serum samples of two sisters were assigned to the atypical homozygote group where they are shown as open circles.

Figures 3, 4 and 5 illustrate the phenotype differentiation obtained by succinyldicholine inhibition in conjunction with dibucaine, fluoride and chloride inhibition respectively. The best segregation is obtained with a combination of Scoline and fluoride numbers. The segregation obtained with dibucaine and fluoride inhibitions on the same population is shown in figure 6. A point in the centre of a symbol in these diagrams indicates two sera with identical numbers.

Preincubation of dilute serum in the presence of 1 mM succinyldicholine for periods up to 60 minutes did not alter the percentage inhibition significantly. This is not surprising as Kalow (1959a) showed that the normal and atypical enzymes at this concentration of succinyldicholine hydrolysed about 80 μmol and less than 5 μmol/min/l. respectively at 30°C. This is equivalent to hydrolysis of 96 nmol and less than 6 nmol per hour by 20 μl of serum which from figure 1 would not result in any significant change in inhibition. The figures given by Goedde,
Fig. 3. Distribution of Scoline and dibucaine numbers.

Fig. 4. Distribution of Scoline and fluoride numbers.

Fig. 5. Distribution of Scoline and chloride numbers.

Fig. 6. Distribution of dibucaine and fluoride numbers.
Held and Altland (1968) for spontaneous hydrolysis of succinylcholine are of the same order as that for the atypical enzyme.

**DISCUSSION**

The technical advantages of the use of succinylcholine in differentiating the serum cholinesterase variants are limited. Less skill is required in preparing the reagent. For a litre of solution an error of a few milligrams in the required 397 mg of succinylcholine chloride dihydrate will not alter the results, whereas with dibucaine and sodium fluoride 13.7 mg and 8.4 mg respectively must be accurately weighed.

There is doubtless an advantage in interpreting results with succinylcholine inhibition since the difference between the extremes of the usual phenotype and atypical homozygote are greater than that obtained with dibucaine. Figure 3 indicates, however, that there is a close correlation between the two inhibitor numbers.

It is only a comparison of figures 4 and 6 which shows any divergence of interpretation. Three of the specimens classified as $E^u E^f$ by Scoline and fluoride number combination (figure 4) would probably be classified as $E^u E^a$ by dibucaine and fluoride numbers (fig. 6). It is not possible by family studies to decide this matter although in one case involved in an apnoeic episode it may be possible at a later date, if there are any children. The point may appear trivial but it does introduce a wider concept.

In our hands the assay of cholinesterase activity has an error of $\pm 2.4\%$ and replicate estimates of dibucaine numbers around 20 and 80 have maximum variations of $\pm 3$ and $\pm 1$ respectively. The scatter of inhibitor constants for any phenotype cannot therefore be explained by a lack of precision in the assay procedure and the ranges of inhibitor numbers illustrated are real and not a result of experimental error. It should be emphasized that for the values reported here in which a result was doubtful or of a controversial nature it was confirmed by repeated estimations.

Kalow (1959b) considered two possible causes for the wide range of dibucaine inhibitions encountered in atypical homozygotes: that is, either there were numerous subtypes of cholinesterase with differing kinetic properties, or the amount of enzyme due to each gene was separately controlled. He concluded that the latter was the case and that homozygous sera contained a mixture of the two enzymes in various proportions, and further that these proportions were genetically determined.

Certainly among the “silent gene” homozygotes heterogeneity has been demonstrated (Goedde and Altland, 1968; Rubinstein et al., 1970) and either explicitly or implicitly the presence of subtypes has been suggested by chloride inhibition (Whittaker, 1968b; King and Dixon, 1970), by the effect of $n$-butyl alcohol (Whittaker, 1968a) or by substrate affinities (Irwin and Hein, 1966).

The three sera of controversial phenotype can readily be explained on either premise but the concept that there may be numerous subtypes of cholinesterase gains support from the three anomalous results from two sisters who were classified as atypical homozygotes despite the unusually high inhibition values. This, in turn, could suggest that there is not a single entity at present classified as the “usual”, “atypical” or “fluoride-resistant” enzyme but that each of these designations embraces a heterogenous population. There may therefore well be a continuous range of cholinesterases within that which is termed a phenotype, and depending not only upon the enzymic properties of the molecular species, mainly turnover number, but also on the relative stabilities of the different variants in the circulation.

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**REFERENCES**


Differenciation des variantes de la cholinesterase sérique à l’aide d’une inhibition par la succinyl-dicholine

**SOMMAIRE**

L’inhibition par la succinyl-dicholine (suxaméthonium, Scoline) de l’hydrolyse de la benzoylcholine par les cholinesterases sériques, a été étudiée. Un schéma analytique en vue d’une différenciation des variantes de la cholinesterase, génétiquement déterminées, est décrit et il est fait état des marges d’inhibition en pourcentage (Nombre de Scoline) reconnues pour chaque phénotype. Une illustration des corrélations existant entre les inhibitions exercées par la succinyl-dicholine, la dibucaine, les fluorures et les chlorures a été donnée à partir de deux-cent-vingt et un individus appartenant à six phénomètes reconnus et il est suggéré que ces groupements ne sont pas homogènes.

Differenciacion de las variantes de colinesterasa en el suero por inhibicion de la succinicolina

**RESUMEN**

Ha sido estudiada la inhibición por la succinicolina (suxametoniom, Scoline) de la hidrólisis de benzoilcolina por las colinesterasas séricas. Se describe un esquema analítico para la diferenciación de las variantes de colinesterasa de determinación genética y se presentan las escalas de inhibición porcentual (Número de Scoline) encontradas para cada tipo. Se ilustra sobre la correlación entre la inhibición de la succinicolina, dibucaina, fluoruros y cloruros, en doscientos veintiuno individuos de seis fenotipos reconocidos, sugiriéndose que estos grupos no son homogéneos.