PHENOTYPING OF INDIVIDUALS SENSITIVE TO SUXAMETHONIUM

The Cholinesterase Research Unit at the Royal Postgraduate Medical School

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It is 2½ years since the routine phenotyping for the cholinesterase variants occurring in individuals believed to be sensitive to suxamethonium was transferred from Exeter to the Royal Postgraduate Medical School, Hammersmith Hospital. An analysis of the distribution of variants occurring in these sensitive individuals is desirable, not only for comparison with earlier surveys, but also to ascertain the impact that the recognition of the E\textsubscript{k} and E\textsubscript{J} genes has made. These rare genes could be especially important in pregnant patients who are sensitive to suxamethonium. An unexpectedly high proportion of patients with the heterozygote, E\textsubscript{u}E\textsubscript{a}, appear to have a period of apnoea during Caesarean section (Whittaker, 1980). In this Unit, screening for the E\textsubscript{k} and E\textsubscript{J} genes has become routine practice, in spite of the fact that these genes can be recognized with confidence only when occurring as the heterozygotes with an E\textsubscript{a} gene or when confirmed by family studies.

MATERIALS AND METHODS

All blood samples were phenotyped by determining dibucaine (DN) and fluoride (FN) numbers using benzoylcholine as substrate (Whittaker, 1977). The RO2-0683 (dimethylcarbamate of (2-hydroxy-5-phenyl-benzyl)trimethylammonium bromide) inhibition characteristics were assayed at 25°C, in duplicate, using benzoylcholine 5 x 10^{-5} mol litre\(^{-1}\) as substrate and RO2-0683 10^{-6} mol litre\(^{-1}\) as differential inhibitor. The method was a modification of that described by Liddell, Newman and Brown (1963), but identical to that used by Evans and Wardell (1984).

SUMMARY

Four hundred and thirty blood samples from suxamethonium-sensitive individuals have been phenotyped by the Cholinesterase Research Unit following its transfer from Exeter to the Hammersmith Hospital. The distribution of genotypes has been shown to be similar to that found in Exeter. Screening for the E\textsubscript{k} and E\textsubscript{J} genes has not yielded any major differences in the gene frequencies of sensitive individuals, even during pregnancy. The uneven sex distribution of the patients, as well as other unusual points that have arisen, are discussed. A new gene for the biosynthesis of cholinesterase has probably been identified.

RESULTS

The distribution of genotypes found in patients referred to the Unit during the 2½ years at the Hammersmith Hospital is given in table I. This table includes an analysis of samples sent to Exeter during this period as well as the distribution of genotypes during the year before the transfer of the Unit. An estimate of the gene frequencies of the patients phenotyped during the 2½-year period is given in table II.

Table III records the genotypes found in pregnant patients reported in the two later surveys recorded in table I.

DISCUSSION

It is apparent from the data given in table I that there was no significant change in the distribution of genotypes found in the three surveys. Some differences did occur, but these can readily be explained by the low frequencies of the rare genotypes.

There was, however, an uneven sex distribution
in all the studies and, in fact, more than twice as many females as males occurred in the sensitive individuals. One must query whether the population for routine surgery is similarly weighted. In addition, one must ascertain whether the effect of pregnancy or the more widespread use of oral contraceptives, both of which are known to reduce plasma cholinesterase activity (Whittaker, 1986), are plausible explanations for this sexual imbalance. Leighton and colleagues (1986) have studied suxamethonium pharmacodynamics in peripartum patients. They used a peripheral nerve stimulator to determine the time from the injection of suxamethonium to 25% twitch height recovery among control patients, non-pregnant patients taking oral contraceptives, term-pregnant patients and postpartum patients. A similar duration of action of suxamethonium was found in the first three groups, but a slower recovery was observed in the postpartum patients. These results, obtained from women having the usual phenotype, E,\,U, imply that neither pregnancy nor oral contraceptives are the causes of our observed sexual imbalance in the E,\,U sensitive
The genotypes $E_i^aE_i^a$ and $E_i^aE_i^*$ are universally acknowledged as the principal categories of individuals sensitive to suxamethonium. The sensitivity of individuals having these atypical enzymes will be influenced neither by pregnancy nor by intake of oral contraceptives, since these enzymes do not hydrolyse the drug (Kalow, 1959). Analysis of the sex distribution of these genotypes in our three surveys should, therefore, give a better index of the surgical populations. Some sexual imbalance persists—albeit considerably smaller than found in our total surveys (F:M = 1.3). It is gratifying to find that, in one group of hospitals, the number of anaesthetics by sex classification during 1 year was 13695 female and 10329 male. We have not contacted other groups for their comparable statistics.

The frequency of the atypical phenotype ($E_i^aE_i^a$ and $E_i^aE_i^*$) is about 1 in 1800 and this can be used to give an approximate estimate of the number of anaesthetics which our service has covered during the past 3½ years. The number of atypical phenotypes recorded in table I is 278, so that an estimate of the number of anaesthetics covered by our surveys is 500400 or about 143000 per annum. It is apparent from the data in table II that pregnancy produces no major distortion in the gene frequencies found in suxamethonium-sensitive individuals. This is somewhat surprising since the $E_i^aE_i^h$ heterozygotes have a mean enzymic activity lower than that of the $E_i^aE_i^a$ heterozygotes (Rubinstein, Dietz and Lubrano, 1978). It was assumed, therefore, that the risk of susceptibility to suxamethonium was greater in the $E_i^aE_i^h$ genotype than in $E_i^aE_i^a$ individuals and especially in the pregnant patient (Evans and Wroe, 1980). Our results do not at present uphold this hypothesis. However, it is difficult to produce a rational explanation for the high proportion of usual phenotypes referred as sensitive to suxamethonium during pregnancy. All have reduced enzymic activity, but one must query whether the universal use of a nerve stimulator would reduce this high proportion of usual phenotype in our surveys. In many cases the patient was being over-ventilated and the resulting apnoea was, presumably, the result of hypocapnoea. Central ventilatory depression will also produce a period of apnoea which may be mistaken for sensitivity to suxamethonium. Also, the steroid hormones associated with pregnancy may become attached to the cholinesterase molecule so that the resulting steric hindrance effect could retard the hydrolysis of suxamethonium by partial blockade of the active site of the enzyme to suxamethonium in some pregnant patients.

We are often asked whether it is worthwhile for a busy anaesthetist to screen the relatives of a suxamethonium-sensitive individual. At Hammersmith, 154/430 individuals have been followed up with family studies. At present 739 relatives have been screened and 333 individuals have been issued with sensitivity cards. This is a considerable improvement on the response of our early days with the Unit (Whittaker and Vickers, 1970). It is desirable to screen the siblings, parents and children of a sensitive individual. Few anaesthetists screen the siblings, who are often domiciled in a different area, but we have found that general practitioners will frequently assist by taking blood samples from relatives. The purpose of a Reference Unit such as the one at Hammersmith is not only to provide reliable methodology for the phenotyping of the cholinesterase variants but to quote, on demand, the biochemical findings of any patient referred to us for phenotyping. In support of this service to anaesthetists we issue sensitivity cards only to individuals phenotyped in our laboratories. Each laboratory must establish its own range of variables for each genotype. We have observed a wide scatter of variables in different laboratories, even when the same method is used with basically similar conditions, and an interlaboratory study of cholinesterase phenotyping has shown many deficiencies in techniques (Evans, Wardell and Rapier, 1983). Greater difficulties are apparent when the experimental procedures are changed.

It is occasionally difficult or impossible to assign a genotype to an individual from the biochemical measurements. We have found two such individuals and confirmed our findings by repeat samples. The unusual phenotype found at the Hammersmith Hospital has had considerable family backup and our results are indicative of a new gene $E_i^h$, the Hammersmith gene, controlling the biosynthesis of plasma cholinesterase. Several members of the family had DN and FN values characteristics of $E_i^aE_i^a$, but very unusual RO2 numbers were invariably obtained. A detailed analysis of this family will be published elsewhere. It is only with the assistance of practising anaesthetists that we are able to show the existence of additional genes for cholinesterase.
The enzyme is stable and withstands the rigours of the British postal service at normal temperatures. Ten-millilitre samples of heparinized (or whole) blood from individuals showing an unusual reaction to suxamethonium should be sent, by first class post, to The Cholinesterase Research Unit in the Department of Anaesthetics at the Postgraduate Medical School, Hammersmith Hospital, London W12 0HS. Separated plasma or serum is much appreciated. It is desirable to have a record of the duration of apnoea as well as the patient's diagnosis to aid future analysis. In these days of computing patients records, it is mandatory to record a patient's sensitivity to suxamethonium. That this is not universal practice, is indicated by repeat samples from the same patient requested by the same hospital during successive pregnancies.

REFERENCES