Is Succinyldicholine the Substrate of Choice for the Measurement of Cholinesterase Activity?

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We have developed a succinyldicholine-based assay for serum cholinesterase (EC 3.1.1.8) to help establish whether patients with suspected sensitivity to drugs of this type have enzyme abnormalities that cannot be detected by conventional laboratory techniques. Although the method discriminates between cholinesterase activities of drug-sensitive and nonsensitive people as well as an assay involving propionylthiocholine does, it has not revealed cholinesterase abnormalities in patients who experienced prolonged apnea after succinyldicholine, in whom no enzyme defect could be demonstrated by other procedures. Of 50 individuals who were apneic for between 20 and 180 min, only one had a cholinesterase activity less than the mean for E₁₄ homozygotes by more than 2.5 SD. We conclude that the number of patients who experience clinical problems due to enzyme abnormalities that at present go unrecognized is small. Consequently, although succinyldicholine might eventually become the substrate of choice for cholinesterase, its advantages over propionylthiocholine are not yet sufficient to encourage its use.

Additional Keyphrases: heritable variants • choline oxidase • apnea • propionylthiocholine substrate compared • enzyme activity

Prolonged respiratory paralysis is a rare but serious and potentially life-threatening complication attending the use of succinyldicholine and related short-acting muscle relaxants. Because these drugs are inactivated in plasma by cholinesterase (acycholine acylylhydrolase, EC 3.1.1.8), their use in patients with decreased activity of the enzyme may give rise to serious problems in anesthesia. Such a situation may commonly arise in pregnancy (1), liver disease (2), poisoning from organophosphorus insecticides (3), and in patients with genetically determined enzyme abnormalities (4).

While clinical observation, personal and family history, and the use of peripheral nerve stimulators can lead to a strong suspicion of sensitivity, confirmation relies heavily on the measurement of cholinesterase activity and establishment of phenotype by use of selected enzyme inhibitors. The atypical, fluoride-resistant, J, Kalow, and silent variants have all been associated with increased sensitivity to succinyldicholine.

Because enzyme substrates may be chosen for their ease of analysis rather than any ability to maximize clinically useful information, the interpretation of cholinesterase activity in the absence of genetic abnormalities can be difficult. Few authors have been able to define activities at which sensitivity is substantially increased (5–7). Furthermore, because of the frequently poor correlation between enzyme activity and apparent sensitivity assessed clinically, a theory has developed that as-yet-unrecognized cholinesterase variants exist which, it is supposed, can metabolize in vitro the substrates used to measure them but do not metabolize succinyldicholine in vivo (8).

Testing such a hypothesis requires an assay for cholinesterase based on succinyldicholine as the substrate. Previously described techniques have incorporated changes in pH (9), the use of 14C labels (10), or choline oxidase (EC 1.1.3.17) from rat liver preparations (11), but none is easily applied to routine use. We have developed a simpler system involving commercially available choline oxidase and have assessed its usefulness for finding additional cholinesterase variants and in defining more clearly patients' risk of sensitivity. In this system, choline released by cholinesterase from succinylcholine is oxidized to betaine aldehyde by choline oxidase, with the formation of hydrogen peroxide. This, in the presence of peroxidase (EC 1.11.1.7), converts phenol and 4-aminoazophenazone to a red quinone dye in proportion to the rate of hydrolysis of succinyldicholine.

We compared the assay with a propionylthiocholine system, currently considered the best available for distinguishing between the cholinesterase activities of succinyldicholine-sensitive and nonsensitive patients (6).

Materials and Methods

Blood Samples

Cholinesterase reference ranges were established by assaying serum obtained from male laboratory staff (ages 23–45 years, n = 39) and from nonpregnant women of reproductive age who were not taking oral contraceptive medication (n = 52). No subjects were included who suffered from any condition likely to affect cholinesterase activity or who possessed genetic abnormalities identifiable by measurement of dibucaine and fluoride inhibition. All other blood samples were taken from the bank of material held by the Cholinesterase Investigation Unit of this hospital:

a) 76 patients and their relatives referred for investigation during a three-month period.

b) 87 individuals with proven genetic abnormalities of cholinesterase.

c) 50 patients who had suffered an apnea of 20 min or more after administration of succinyldicholine but for whom routine investigations of cholinesterase status gave normal results.

Samples were stored at −20 °C, which is suitable for long-term stability (12). Routine assays involved measurement of enzyme activity towards propionylthiocholine at 25 °C (6) and ascription of phenotype on the basis of inhibition of benzoylcholine hydrolysis by dibucaine (13), fluoride (14), and the dimethylcarbamate of (2-hydroxy-5-phenylbenzyl) trimethylammonium bromide (Ro 02-0883; Roche Pharma-
Reagents

Peroxidase was obtained from Hughes and Hughes, Romford, U.K.; choline oxidase (from Alcaligenes species) and succinylcholine chloride were from Sigma Chemical Co., Poole, U.K.

Calcium chloride: Dissolve 7.8 g of CaCl₂ · 2 H₂O in 100 mL of water.

4-Aminophenazone: Dissolve 6.0 g in 100 mL of water.

Buffer: Tris HCl, 50 mmol/L, pH 7.4, containing 2 g of Triton X-100 detergent per liter.

Color reagent: Dissolve 50 U of choline oxidase, 110 U of peroxidase, and 10 mg of phenol in 50 mL of buffer. Add 100 μL each of the calcium chloride and 4-aminophenazone solutions. Store at 4 °C, and make up freshly each week.

Substrate: Succinylcholine chloride, 25 mmol/L in water. This is stable for up to four weeks when stored at 4 °C.

Procedure

Mix 50 μL of substrate and 1 mL of color reagent in a cuvette and allow to equilibrate at 37 °C for 3 min. Add 50 μL of serum and measure the rate of change in absorbance (ΔA) at 500 nm against a blank solution in which the serum has been replaced by buffer. Enzyme activity, defined as micromoles of choline liberated per minute per litre of serum, is determined from the color change observed when known amounts of choline chloride are added to the color reagent: Enzyme activity (U/L) = (ΔA/min) × 1923.

More than 90% of the choline released is derived from the first step of succinylcholine hydrolysis; the next step, conversion of succinylmonocholine to succinic acid, proceeds very slowly (16).

Results

Details of the laboratory assessment of the method are summarized as follows:

The substrate concentration of 1.14 mmol/L is optimal for the usual cholinesterase variant. The Michaelis constant (Kₘ) of 37 mmol/L for the usual enzyme is in agreement with that reported by other workers (17). The within- and between-run coefficients of variation for the assay, determined for a serum specimen with cholinesterase activity in the upper part of the reference range, were 4.5% and 5.6%, respectively. The assay is not affected by slight sample turbidity, but gross lipemia causes problems because of high initial absorbance. Similarly, there is no interference from bilirubin until its concentration in serum exceeds 200 μmol/L; at 365 μmol/L, enzyme activity is inhibited by 24%. Reference ranges (mean ± 2 SD) for serum from E₁⁺E₁⁻ homozgyotes are 45–82 U/L for males and 34–72 U/L for females. These are significantly different (p < 0.005), in accordance with reports for assays using other substrates (18). The combined reference range for both sexes is 35–82 U/L.

Results for 76 patients referred for routine investigation are shown in Figure 1, where they are compared with those obtained with propionylthiocholine.

The optimum dividing line between the cholinesterase activities of sensitive and nonsensitive individuals has been recommended as 2.5 SD below the mean activity for E₁⁺E₁⁻ homozgyotes as measured with propionylthiocholine substrate at 37 °C (5). For succinylcholine the corresponding cutoff value is 29 U/L. Of 22 patients presenting either with clinical evidence of prolonged apnea or a susceptible cholinesterase genotype, 19 had cholinesterase activities of less than 29 U/L. Two of those remaining were of E₁⁺E₁⁻ genotype, in which our experience is not invariably associated with sensitivity. One was normal by all criteria and is included with the 50 normal patients considered later. One patient with a nonsensitive genotype also had an activity of less than 29 U/L but had not been exposed to succinylcholine, so that no conclusions can be drawn.

Assay of samples from 87 patients, selected because of either having a susceptible cholinesterase genotype or being heterozygous for the usual and another variant, gave the results shown in Figure 2. All individuals with genotypes invariably associated with succinylcholine sensitivity had activities of less than 29 U/L. However, only two of the four E₁⁺E₁⁻ heterozygotes fell into this category. In addition, one E₁⁺E₁⁻ patient with a prolonged reaction to the drug had an activity of 33 U/L. Two E₁⁻E₁⁻ heterozygotes had low cholinesterase activities; one had experienced an apnea but the other had not been exposed to succinylcholine and therefore the risk of sensitivity could not be ruled out. These findings compare favorably with those of Evans and Wroe, who used propionylthiocholine at 25 °C (6).

The results for 50 patients normal by current biochemical criteria but suspected of sensitivity on the basis of an apnea in excess of 20 minutes are included in Table 1. Only one had an activity of less than 29 U/L. This patient is to be investigated further.

Discussion

The choice of procedure for the analysis of any biochemical variable depends upon a number of criteria, including ease of assay, precision, and cost. However, the primary consideration, especially with enzyme measurements, should be the ability of the method to solve the clinical problem posed. This is of particular importance with cholinesterase because the attribution of succinylcholine sensitivity relies so heavily on laboratory investigations.

In theory, a method for measuring cholinesterase activity based on hydrolysis of succinylcholine will reflect the in vivo metabolism of the drug, and therefore the duration of the drug's action, more precisely than an assay based on a
Table 1. Cholinesterase Activities in Patients with Apnea after Treatment with Succinyldicholine

<table>
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<tr>
<th>Duration of apnea, min</th>
<th>Propionylthiocholine, in µM</th>
<th>Succinyldicholine, in µM</th>
<th>Duration of apnea, min</th>
<th>Propionylthiocholine, in µM</th>
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* Length of apnea unstated but greater than 20 min.

*Activity more than 2.5 SD below mean normal for E₁₁ homozgyotes.

All patients had cholinesterase inhibitor numbers characteristic of the usual phenotype as determined by inhibition of benzoylcholine hydrolysis by dibucaine, fluoride, and Ro O2-0083 (see text).

nonpharmacological substrate. This assumption was apparently substantiated by the work of Agarwal et al. (19), who examined serum from 21 patients with clinical sensitivity but with no cholinesterase abnormality. In six of these they observed reduced catalytic activity when cholinesterase was measured with a succinyldicholine substrate. This, they supposed, indicated the presence of cholinesterase variants that were not revealed by the techniques in routine use.

The system which we describe is at least as good as one making use of propionylthiocholine to identify drug sensitivity, whether from genetic abnormalities or pathological decreases in cholinesterase activity (Figure 1). However, we have not been able to provide any evidence for the existence of new cholinesterase variants. Only one of 30 patients, clinically suspected of sensitivity but biochemically normal, had an activity of less than 29 U/L. These observations contrast with those of Agarwal et al. Although there are significant differences between their analytical system and ours, we do not believe these are sufficient to explain the disagreement. Rather, we think that much of the explanation may lie in Agarwal's use of benzoylcholine as the routine substrate. Aliphatic and aromatic choline and thiocholine esters give different hydrolysis ratios when acted upon by usual and atypical enzymes (20), and cholinesterase activities measured by using benzoylcholine have only minimal interpretive value.

We recognize that it is probably impossible for laboratory conditions ever to simulate entirely those prevailing at the time the anesthetic is administered: substrate concentration, the patient's ventilatory state, and the presence of other anesthetic drugs that might influence cholinesterase will differ between the laboratory and the operating theater. Nonetheless, we believe that our work demonstrates that the number of patients who are sensitive to succinyldicho-
line due to cholinesterase abnormalities and who are failing to be recognized is small.

The recent commercial availability of choline oxidase has led to the development of succinylcholine-based cholinesterase assays other than our own that appear to be similar in their ability to identify succinylcholine-sensitive patients (17, 21). However, until the information provided by such methods significantly improves upon that available from nonpharmacological substrates—e.g., propionyl- or possibly butyrylthiocholine—a widespread change in cholinesterase methodology seems unlikely.

References