ABNORMAL ENZYME PHENOTYPE ($E_1^a E_1^f$): NORMAL RESPONSE TO SUCCINYLCHOLINE

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SUCCINYLCHOLINE (Anectine, Scoline) apnoea is usually the result of a delay in the hydrolysis of this muscle relaxant due to the presence of an inherited defect in the serum cholinesterase. Many variants of serum cholinesterase have been identified by using substances which inhibit their activity to different degrees. This has been reviewed in various publications.¹⁻³

The heterozygote for the atypical and fluoride resistant genes ($E_1^a E_1^f$) has been reported to be sensitive to succinylcholine.⁴ Other phenotypes reported as invariably sensitive to succinylcholine are $E_1^a E_1^{a5.6}$, $E_1^f E_1^{f4.7-10}$, $E_1^s E_1^s$.¹¹⁻¹³ A search of the literature has revealed no reports disagreeing with these observations.

CASE REPORT

In August of 1973, a 16-year-old white male was admitted to St. Joseph's Hospital, Hamilton, through the Emergency Room for an appendectomy. His weight was recorded at 60 kg and he had the usual clinical findings of appendicitis. He was pre-medicated with meperidine 75 mg and hyoscine 0.4 mg.

Induction of anaesthesia was with thiopentone 300 mg and succinylcholine 60 mg and maintenance with Innovar[®] and nitrous oxide five litres per minute with oxygen two litres per minute. Relaxation was provided with a succinylcholine drip (0.1 per cent). About 300 mg of succinylcholine was used in total.

He arrived in the recovery room one hour later at 2250 hours and remained there until 2400 hours. The recovery room records note that he was awake, colour good, respiratory rate 18/min, pulse 80/min, and blood pressure 120/70 mmHg (15.96/9.31 kPa). He made an uneventful recovery and was discharged a few days later.

In September of 1976, three years later, the

same patient was admitted again to St. Joseph's Hospital for an elective tonsillectomy. On this occasion his weight was recorded as 70 kg and the anaesthetic management was as follows: pre-medication with meperidine 100 mg, hyoscine 0.4 mg; induction with thiopentone 250 mg, succinylcholine 60 mg. A nasotracheal tube was inserted. Maintenance: nitrous oxide 5 L/min, oxygen 2 L/min and Innovar[®], and again a succinylcholine drip (0.1 per cent) and controlled ventilation. The total succinylcholine administered was 160 mg. At the end of the one-hour procedure, the anaesthetic record states that, "the patient did not begin to breathe vigorously enough" and therefore ventilation was assisted manually for five to ten minutes. He was then transferred to the post-anaesthetic room. Because of the unusual pattern of ventilation at the end of the operation, a blood sample was drawn and sent for serum cholinesterase investigation.

METHODS AND MATERIALS

Serum cholinesterase activity was measured by the method of Kalow and Lindsay¹⁴ using a 50 μ mol/L benzoylcholine substrate. The percentage inhibitions of the enzyme by 10 μ mol/L dibucaine, 50 μ mol/L fluoride, 500 mmol/L chloride and 1 mmol/L succinylcholine were measured at 25° C according to the analytical scheme of King¹⁵ and King and Griffin.¹⁶ The ranges of inhibition demonstrated by the different phenotypes in the presence of these inhibitors has previously been published.³ The percentage inhibition under these conditions is expressed as the respective number - dibucaine number (D.N.), fluoride number (F.N.), chloride number (C.N.) and scoline number (S.N.).

In addition, urea 5 mol/L¹⁷ and bromide 250 mmol/L¹⁸ were also employed as inhibitors for serum cholinesterase. These are also expressed as the respective number - urea number (U.N.), and bromide number (B.N.). The ranges of inhibition for several phenotypes using these inhibitors are detailed in Table 1.

The reference range of serum cholinesterase activity under these conditions is 620-1370 U/L at 25° C.

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TABLE I

Phenotype	Inhibitor numbers	
	Bromide numbers BN	Urea numbers UN
E ₁ ^a E ₁ ^u	0-8	41-52
E ₁ ^a E ₁ ^r	0-12	63-68
E ₁ ^a E ₁ ^a	8-20	53-70
E ₁ ^a E ₁ ^r	18-22	84-95
E ₁ ^a E ₁ ^a	37-45	84-100

All enzyme activities were measured on a Beckman Acta III recording spectrophotometer with temperature control achieved by a circulating water bath (Model TE9-100, P.M. Tamson, Holland) and a dip cooler (Model PBC-4, Neslab Instruments Inc.). The reaction temperature in the cuvette was measured using a Yellow Springs Instrument Model 46 tele-thermometer. The interpretation of the effect of reaction temperature on the behaviour of the serum cholinesterase of different phenotypes is based on previously reported work.^{2,9,19-21}

RESULTS

The initial measurements of the patient's serum and subsequent repeat analyses are detailed in Table II. A sample was sent to the University Department of Pathological Bio-

chemistry, Royal Infirmary, Glasgow (Dr. J. King). All of the results indicated that this patient has the phenotype E₁^aE₁^r.

A further sample was obtained and the above measurements were repeated but with the addition of urea and bromide as inhibitors. Several other patients whose phenotypes had previously been established by the usual procedures were run as controls (Table III).

Temperature activities for the sera from the first four patients were determined at 5° C intervals from 20° C to 45° C. The results are presented in Figure 1.

DISCUSSION

This patient received succinylcholine 360 mg without any adverse affects. A sample was sent for cholinesterase phenotyping after his second operation, when he received 160 mg of succinylcholine, not because he was apnoeic but because he did not "... breathe vigorously enough". Repeat assays on different samples in two laboratories showed him to be consistently E₁^aE₁^r, a phenotype which has been reported as being invariably sensitive to succinylcholine. The usual response in a patient with this phenotype would have been total apnoea for several hours.

The use of bromide as an additional inhibitor also indicated that the patient's phenotype was E₁^aE₁^r. The other phenotypes listed in Table III

TABLE II

Patient sample	Cholinesterase activity (U/L)	Inhibitor numbers				Phenotype
		DN	FN	CN	SN	
8/9/76	515	45	41	31	51	E ₁ ^a E ₁ ^r
x/12/76*	502	54	36	34	50	E ₁ ^a E ₁ ^r
26/1/77	552	44	38	36	44	E ₁ ^a E ₁ ^r

*This specimen phenotyped by Dr. J. King.

TABLE III

Patient number	Cholinesterase activity (U/L)	Inhibitor numbers						Phenotype
		DN	FN	CN	SN	BN	UN	
1*	448	47	36	36	37	22	48	E ₁ ^a E ₁ ^r
2	964	82	53	12	89	4	41	E ₁ ^a E ₁ ^u
3	558	49	31	36	58	21	79	E ₁ ^a E ₁ ^r
4	830	84	58	17	91	3	45	E ₁ ^a E ₁ ^u
5	393	24	32	55	22	35	81	E ₁ ^a E ₁ ^a
6	539	79	47	11	85	4	54	E ₁ ^a E ₁ ^u OR E ₁ ^u E ₁ ^r

*Patient No. 1 is the subject of this case report.

FIGURE 1

also gave good correlation between the bromide number (B.N.) and the previously assigned phenotypes. Two of the controls (patients 4, 6, Table III) did not have family studies available and from their inhibitor numbers it was not clear whether they should be labeled $E_1^u E_1^u$ or $E_1^u E_1^f$. The bromide numbers obtained would support that $E_1^u E_1^u$ was probably the appropriate phenotype for these patients.

The temperature studies showed patterns for the $E_1^u E_1^u$ phenotypes (patients 2, 4) consistent with the previously published findings. The $E_1^a E_1^f$ control (patient 3) also exhibited a cholinesterase activity curve consistent with previous work, which indicated that progressive inactivation of the enzyme took place after 35° C was reached. However, the subject of this study (patient 1) did not exhibit the normal peak activity of $E_1^a E_1^f$ phenotypes at 35° C but continued to rise and probably reached a peak between 40 and 45° C. This continuing increase in activity at physiological temperature when compared to the enzyme activity obtained at the assay temperature of 25° C may partly explain why the patient was able to hydrolyse the succinylcholine which had been given. This finding again emphasizes that while it is necessary to measure activity at 25° C to phenotype with reasonable accuracy, a true index of serum cholinesterase activity can only be obtained if the estimation is performed at the

temperature at which the enzyme functions, that is 37° C.^{2,21}

The urea inhibition study had been modified slightly from the original report¹⁷ and from our usual analytical technique to avoid prior incubation of the serum with urea and also to avoid the problems of solubility of such high concentrations of urea. The original authors showed that inhibition increased with incubation time when serum is in the presence of 5 mol/L urea. In our study 2 mL of buffer containing 10 mol/L urea, 1 mL of substrate and 980 μ L of water were mixed, incubated at 25° C and 20 μ L of serum added and mixed immediately prior to measuring the change in absorbance in the spectrophotometer. Readings for the activity determinations were taken within the first minute. The uninhibited activity determinations necessary for calculation of the urea numbers were made under the same conditions but with the absence of urea from the buffer.

There was good agreement for the two $E_1^u E_1^u$ phenotypes between the published urea numbers (Table I) and those we obtained. The $E_1^a E_1^f$ control was not exactly in the published range but was certainly in the appropriate area for that phenotype. Patient 1 had a urea number of 48 which did not relate to the range of inhibition for the $E_1^a E_1^f$ phenotype. This urea number is more closely related to the values obtained with individuals homozygous for the usual enzyme. However, we have reservations about all of the urea inhibitor numbers. It is difficult in the course of the analysis to decide on the slope of the line to be taken from the spectrophotometer results for determining activity during urea inhibitions. The greater the inhibition the more difficult this can be. The problem is compounded by the fact that inhibition would appear to be increasing during the first minute of the test.

Our patient by at least five methods of inhibition has the phenotype $E_1^a E_1^f$ and represents the first reported example of such an individual not being sensitive to succinylcholine. There is an interesting variation with the temperature response and with urea inhibition which would suggest that the enzyme molecule is capable of changing configuration and presenting additional active sites. Sensitive phenotypes are usually discovered through apnoeas and subsequent family studies. It seems probable that many patients do have apnoeic episodes due to succinylcholine and other unknown causes but they are masked by the anaesthetic regime. On the other hand we presume that since the majority of apnoeas occur in the phenotypes $E_1^a E_1^a$, $E_1^a E_1^f$ or

$E_1^f E_1^f$ and the kinetics of these enzymes gives an explanation for the phenomenon, that all such phenotypes are necessarily sensitive. It is an assumption that appears to be invalid in the patient reported here but it is also of interest that a study of the enzyme kinetics as represented by the temperature activity curve appears to offer at least a limited explanation.

SUMMARY

The enzyme serum cholinesterase responsible for the hydrolysis of the muscle relaxant succinylcholine exists in the form of several variants. These may be identified in serum by using substances which inhibit their activity to different degrees. The heterozygote for the atypical and fluoride resistant enzymes ($E_1^a E_1^f$) is one of the phenotypes which has been reported to be sensitive to succinylcholine.

A case is described where succinylcholine given on two separate occasions did not induce apnoea in an individual phenotyped as $E_1^a E_1^f$ by at least five methods of inhibition. This is the first reported example of such insensitivity to the drug in this phenotype.

Temperature activities for the patient's serum over the range of 20° C to 45° C differed from that of an established $E_1^a E_1^f$ phenotype used as a control. There was a progressive inactivation of the control serum at temperatures higher than 35° C, as previously reported for this phenotype. Activity in the serum of the subject of this study did not exhibit the peak activity at 35° C but continued to rise and probably reached a peak between 40° C and 45° C.

The significance of these results in the context of current methods of phenotyping is discussed.

RÉSUMÉ

L'enzyme pseudo-cholinestérase qui sert à l'hydrolyse de la succinylcholine peut exister sous la forme de plusieurs variantes qu'on peut identifier grâce à des substances qui en inhibent l'activité à différents degrés. La forme hétérozygote de l'enzyme atypique et résistante au fluor ($E_1^a E_1^f$) est considérée comme sensible à la succinylcholine.

Les auteurs présentent le cas d'un patient dont le phénotype $E_1^a E_1^f$ avait été déterminé par cinq méthodes d'inhibition et qui n'a pas présenté d'apnée prolongée. C'est la première fois qu'on rapporte une insensibilité de ce phénotype à la succinylcholine.

L'activité sérique du malade entre 20° C et 45° C différait de celle du sérum d'un autre porteur du phénotype $E_1^a E_1^f$ déjà déterminé et servant de contrôle. Il y a eu inactivation progressive lorsque le sérum de contrôle a dépassé 35° C tel qu'observé précédemment pour ce phénotype. Par contre, cette pointe d'activité à 35° C n'a pas été démontrée dans le sérum du patient faisant l'objet de l'étude. Au contraire, l'activité s'est accentuée et un sommet a pu être atteint entre 40° C et 45° C.

Les auteurs discutent des résultats de cette étude à la lumière des connaissances actuelles sur les méthodes de phénotypage.

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