Shortened action of succinylcholine in individuals with cholinesterase C_5 isozyme

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To test the possibility that individuals with and without plasma cholinesterase C_5 isozyme have differences in neuromuscular sensitivity to succinylcholine chloride, we examined the effects of succinylcholine in these two groups of patients. Sera from 491 adult patients were examined for presence of the plasma cholinesterase C_5 isozyme by use of electrophoresis with polyacrylamide; 24 were positive for the C_5 isozyme. Plasma cholinesterase activity and duration of action of succinylcholine were measured in 12 C_5 positive patients and 18 C_5 negative patients, all of whom had the normal cholinesterase genotype. C_5 positive patients had 30.1 per cent higher mean plasma cholinesterase activity than C_5 negative patients. The duration of neuromuscular blockade, measured by the first twitch height evoked by train-of-four stimulation, was significantly shorter in C_5 positive patients than C_5 negative patients. The C_5 positive individuals had shorter duration of action of succinylcholine than C_5 negative individuals.

Key words
ENZYMES: cholinesterases, isoenzyme; NEUROMUSCULAR RELAXANTS: succinylcholine; TECHNIQUES: electrophoresis, polyacrylamide gel.

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Succinylcholine chloride (SCh) is hydrolysed by plasma cholinesterase (ChE, EC 3.1.1.8).[1] It is generally accepted that the short duration of action of succinylcholine is due to a rapid enzymatic hydrolysis by ChE. The duration of action of SCh has been shown to be dependent on the activity of plasma ChE.[2] ChE was electrophoretically separated to four isoenzymes C_1, C_2, C_3 and C_4. In addition to these four, a fifth slower moving component was found in some individuals, who were labelled C_5 positive (C_5^+) variants, while individuals who lack the component were labelled C_5 negative (C_5^-) variants.[3] Sera containing C_5 usually have relatively high ChE activity.[4] Though it is logical to speculate that C_5^+ individuals have a shorter duration of action of succinylcholine than those who are C_5^-, no investigation has been reported concerning the role of the ChE isozyme in determining responsiveness to succinylcholine.

The purpose of present study was to determine whether patients with the C_5 isozyme have a shortened duration of action of succinylcholine and to quantify the relationship between duration of succinylcholine neuromuscular blockade and the plasma ChE C_5 isozyme.

Methods

Four hundred and ninety one adult patients, 267 men and 224 women, undergoing elective surgery were studied. Informed consent was obtained from each patient and the study was approved by the hospital ethics committee. Blood samples for detection of the ChE C_5 component were withdrawn at the preanaesthetic examination. Presence or absence of the ChE C_5 component was established by electrophoresis. Electrophoresis of sera was performed using a miniaturized electrophoretic appara-

tus which was custom-made, according to specifications previously published.[5] Electrophoresis was carried out essentially as described by Davis.[6] (See Appendix for details.) Scanning profiles were obtained after applying the gels to a densitometer.

ChE activity was assayed spectrophotometrically in a Hitachi 200-20 recording spectrophotometer by the method of Kalow and Lindsay[7] which is based on the rate of hydrolysis of benzoylcholine in M/15 phosphate buffer pH 7.4 at 25° C which is followed by decrease in absorbance at 240 nm. Serum at a dilution of 1:50 was used as the source of enzyme, the final dilution in the reaction mixture being 1:100. Dibucaine and fluoride numbers were determined as described by Kalow and Genest[8] and Harris and Whittaker[9] respectively.

Eighteen C_5 negative patients who were randomly selected from the C_5 negative patients of ASA physical status I or II and all of 12 C_5 positive patients of ASA physical status I or II (all of whom had normal ChE genotypes (dibucaine number and fluoride number)), were entered in the study of neuromuscular blockade and measurement of ChE activity. For all tests of ChE activity, dibucaine and fluoride numbers, and monitoring of neuromuscular blockade, the observers were unaware of the C_5^- and C_5^+ identity of individual patients.

Premedication consisted of 0.4–0.5 mg atropine IM and 50 mg hydroxyzine IM 45–60 min before induction of anaesthesia. A blood sample to be used for measurement of ChE activity was withdrawn, and an intravenous infusion was started when the patient arrived in the operating room. Anaesthesia was induced with thiamylal 4–5 $mg \cdot kg^{-1}$ and maintained with 66 per cent nitrous oxide in oxygen and additional thiamylal. Respiration was assisted, if required, but hyperventilation was avoided by measuring arterial blood gases periodically, in addition to serum electrolytes, and serum pH. Body temperature was measured by rectal probe inserted before induction of anaesthesia.

After induction of anaesthesia, the ulnar nerve was stimulated at the wrist with a series of four supramaximal single stimuli (square wave pulses of 0.2 msec duration) delivered at 0.1 Hz (train-of-four) from a peripheral nerve stimulator (Myotest, Biometer Ltd., Denmark) using surface electrodes. The evoked force of contraction of the adductor pollicis was measured and recorded using a force transducer and a neuromuscular function analyzer

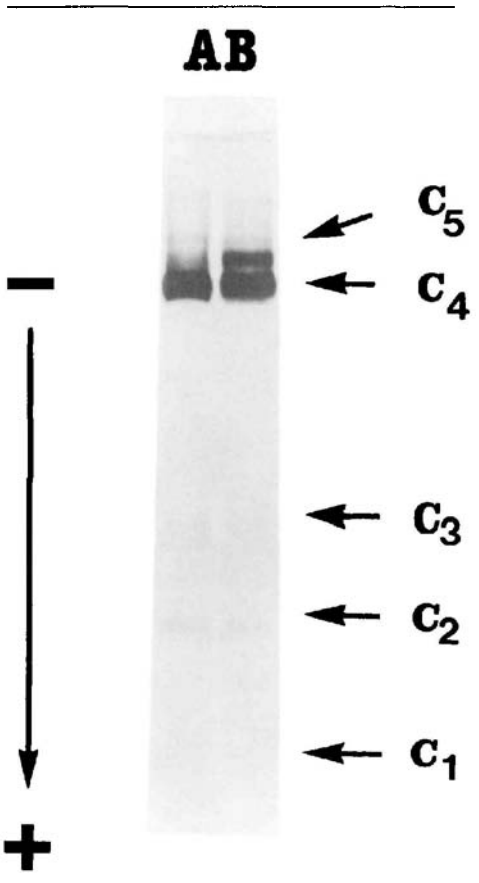

FIGURE 1 Polyacrylamide (seven per cent) gel electrophoresis of plasma cholinesterase of individuals without C_5 isozyme (A) and with C_5 isozyme (B). (A) demonstrates four isozymes of cholinesterase C_1, C_2, C_3, and C_4. A fifth slower-moving component, C_5, is seen in (B) in addition to four components.

(Myograph 2000, Biometer Ltd., Denmark) as described by Viby-Mogensen.[10]

When the response to the train-of-four stimulation was stable, the height of the first twitch of the train was taken as the standard control (control twitch height). Succinylcholine, 40 mg/m^2 body surface area, was given intravenously as quickly as possible. Monitoring of neuromuscular transmission was continued after succinylcholine administration, at least until the height of the first twitch of the train had returned to the control twitch height. The time from injection of succinylcholine to 20,

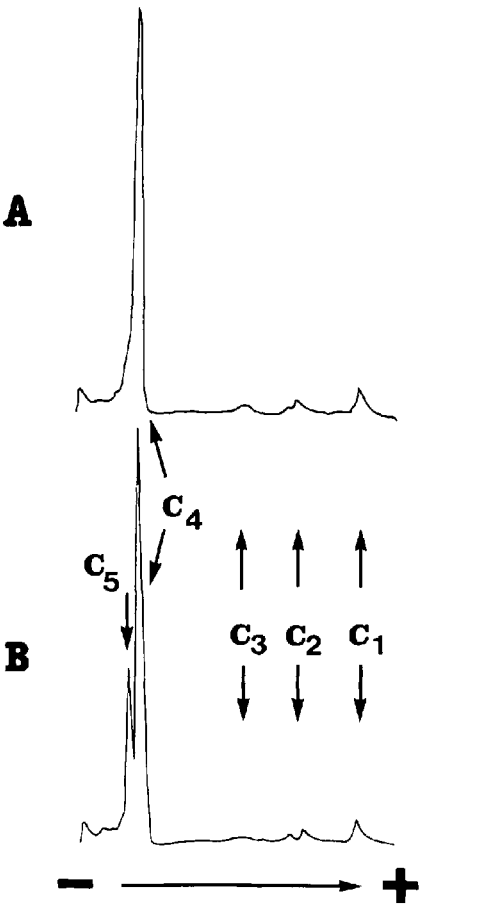

FIGURE 2 Gel scanning of electrophoretic profiles shown in Fig. 1 by densitometer. (A) C_1, C_2, C_3 and C_4; (B) C_1, C_2, C_3, C_4 and C_5. The cathode is to the right in both panels.

50, and 80 per cent recovery of control twitch height were determined in each case.

Statistical significance was estimated with an unpaired t-test.

Results

Electrophoresis of sera of patients demonstrated zymograms of ChE as in Figure 1. Four components of serum cholinesterase are shown in lane A. These isozymes have been designated C_1, C_2, C_3, and C_4 from cathode to anode. A fifth, slower-moving component can be seen clearly in lane B, in addition to the four components demonstrated in lane A. This component was named the C_5 component by Harris *et al.*[3] It has been shown that the C_4 band contributes the greatest portion of ChE activity, and the C_5 component – if present – contains the second largest portion of ChE activity in the C_5^+ enzyme as demonstrated by densitometry (Figure 2).

Both groups of patients were comparable with respect to sex, age, weight, height, and body surface area (Table I). Twenty-four of the 491 (4.9 per cent) patients studied were positive for the C_5 isozyme. Nine were positive for the ChE C_5 isozyme among the 267 males, and 15 of 224 females were ChE C_5 positive.

Plasma cholinesterase activity in the C_5^+ group was significantly higher than the C_5^- group. Patients whose sera contain the C_5 component had 30.1 per cent more ChE activity than those without this component. Dibucaine and fluoride numbers were within normal limits in all the patients who had ChE activity measured (Table II).

The pattern of neuromuscular blockade was characteristic of a depolarizing block in all cases. The duration of neuromuscular blockade by succinylcholine was significantly shorter in C_5^+ patients than C_5^- patients (Table III). Average times

TABLE I Patients' profile (Mean ± SEM)

Group	*Age (years)*	*Height (cm)*	*Body weight (kg)*	*Body surface area (m^2)*
C_5^- n = 18	44.0 ± 2.72	159.3 ± 1.06	55.3 ± 2.19	1.57 ± 0.031
C_5^+ n = 12	47.0 ± 3.38	157.4 ± 2.88	55.7 ± 2.28	1.57 ± 0.044

TABLE II Cholinesterase activity, dibucaine numbers and fluoride numbers (Mean ± SEM)

Group	*ChE activity (IU)*	*Dibucaine numbers*	*Fluoride numbers*
C_5^- n = 18	750.8 ± 26.9	78.5 ± 0.74	62.2 ± 1.02
C_5^+ n = 12	976.8 ± 58.2*	77.8 ± 0.94	63.3 ± 0.90

*Significantly different from C_5^- group ($p < 0.01$).

TABLE III Times to recovery of twitch height to 20%, 50% and 80% of control twitch height after intravenous administration of 40 mg/m² BSA succinylcholine (Mean ± SEM)

	20% Twitch height (sec)	*50% Twitch height (sec)*	*80% Twitch height (sec)*
C_5^- n = 18	886.7 ± 61.5	1105.3 ± 85.3	1409.7 ± 139.4
C_5^+ n = 12	670.8 ± 68.2*	787.5 ± 78.5*	916.3 ± 93.5†

*Significantly different from C_5^- group ($p < 0.05$).
†Significantly different from C_5^- group ($p < 0.02$).

to recovery of twitch height to 20, 50, and 80 per cent of control twitch height in C_5^+ individuals were 75.7, 71.2, 65.0 per cent of corresponding value in C_5^- individuals respectively.

Discussion

Harris *et al.*[3] described the appearance and enzymatic behaviour of serum ChE in normal serum as seen after two dimensional electrophoresis, first on paper and then on starch gel. Four components of serum ChE activity were found in the gels, corresponding to at least four isozymes which have been designated as C_1, C_2, C_3, and C_4, according to the decreasing order of mobility toward the anode. The slowest component (C_4) is the major enzyme, containing most of the serum ChE activity. Harris *et al.* found a fifth slower-moving component, in a few sera. This component was designated the C_5 component. Our experiments clearly demonstrate that the C_5 isozyme can be separated from other isozymes of ChE by electrophoresis with polyacrylamide. Harris *et al.*[11] provided evidence that the gene which determines the occurrence of C_5 is non-allelic to the genes on E_1 locus which control the bands C_1–C_4. Robson *et al.*[12] supported this hypothesis. This second gene has been designated the E_2^+ gene.[13]

The incidence of the C_5 variant in our patient population (all Japanese) was 4.9 per cent. The frequency of the C_5 variant in various populations was found to vary from 0.3 per cent to 10.1 per cent by Steegmüller.[14] She also reviewed the incidence of the C_5 variant in various populations as found by others, with values of 0 to 29 per cent reported. The calculated incidence of the C_5 variant was 9.4 per cent among Mongoloids (included 47 Koreans and 81 Thais). The number of samples tested was likely too few to give a reliable incidence. A possible explanation for the variations noted is that electrophoretic methods used by most authors are not sensitive enough to detect all patients with the C_5^+ phenotypes. Simpson[15] demonstrated that a polyacrylamide gel electrophoretic system (2.5 per cent acrylamide spacer gel, pH 6.7; seven per cent separating gel, pH 8.9) was able to detect 25 per cent more C_5^+ individuals than a 15 per cent starch gel system at pH 5.3. However Singh *et al.*[16] maintained that in their material the method described by Simpson did not help to detect any misclassification produced by the starch gel method.

The results of this study show that C_5 positive variants have 30 per cent more plasma ChE activity than those without this component. This is in accordance with the results of Harris *et al*,[4] who reported that subjects whose sera contained the C_5 component usually have 30 per cent more plasma cholinesterase activity than those without this component.

The duration of action of succinylcholine was about 30 per cent less in C_5^+ individuals than in C_5^- individuals, when it was administered on the basis of body surface area. Earlier studies have examined the correlation between the ChE level and duration of action of succinylcholine. Some found good correlations between the esterase level and duration of apnoea[17–24] while others found only partial correlation or no correlation.[25,26] In none of these studies was neuromuscular function monitored. In addition, few of the studies investigated the ChE phenotype and none determined the ChE C_5 isozyme.

Viby-Mogensen[2] reported that for patients with the geotypically normal enzyme the duration of succinylcholine action measured by the duration

of apnoea and the time to 100 per cent recovery of twitch height (train-of-four) increased with the decreasing plasma ChE activity. He did not investigate the ChE isozyme for detection of the C_5 variant, though he determined dibucaine, fluoride, chloride, scoline, and urea numbers. Neitlich[27] reported a genetic variant that increased the serum ChE two to three times the normal mean value with the appearance of a slow-moving band on zymograms obtained by disc-electrophoresis with acrylamide gels as the supporting medium. This variant was associated with resistance to succinylcholine in grip strength. Yoshida and Motulsky[28] showed the increased enzyme activity of the variant described by Neitlich could be attributed to an increase in the number of enzyme molecules. They concluded that the variant enzyme has approximately the same specific enzyme activity as normal and that the extra enzyme component of the variant plasma is structurally different from the C_5 enzyme component. They named the variant *E. cynthiana*.

Blitt *et al.*[29] showed no correlation between plasma ChE activity and duration of paralysis from succinylcholine using single-twitch nerve stimulation. This is probably because Blitt's patients showed only minor variations in enzymatic activity. High plasma ChE activity was detected in two German families by Delbrück *et al.*[30] They[31] showed that the ChE of the propositi exhibited isozyme separation patterns in polyacrylamide electrophoresis as well as in electrofocusing which were different from those of ChE from normal persons, but no differences could be seen with respect to the Km for substrates or the inhibition by dibucaine, fluoride or succinylcholine.

Scott *et al*[32] showed the properties of the C_5^+ enzyme to differ from the principal serum ChE in only minor ways. Muensch *et al.*[33] observed no difference at esteratic site of the enzyme with C_5 component from the normal enzyme without C_5. Therefore the difference in sensitivity to succinylcholine between C_5^- and C_5^+ variants probably results from the difference in quantity of ChE activity.

The results of our study indicate that the ChE C_5 isozyme, a genetic factor determined by the presence of the E_2^+ gene, contributes to decreased neuromuscular sensitivity to succinylcholine.

Appendix

Electrophoresis was carried out in seven per cent polyacrylamide gels for two hours at a constant current of 20 mA. The running gel buffer was 187.5 mM Tris-HCl (pH 8.8), the stacking gel buffer was 125 mM Tris-HCl (pH 6.8), and electrode buffer was 12.5 mM Tris-glycine (pH 8.3). After electrophoresis, the gels are washed in three changes of 125 mM phosphate buffer at pH 6.8 for 10 min each. Then the gels are incubated in a reaction mixture consisting of 50 ml of 125 mM phosphate buffer at pH 6.8, 1 ml of one per cent α-naphthyl acetate in acetone solution, and 100 mg of fast violet B salt for 30 min at 37° C. After incubation the stained gels are washed in running water.

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Résumé

Afin de vérifier la possibilité que les individus avec ou sans plasma cholinesterase C_5 isoenzyme présentent des différences de sensibilité à la succinylcholine, on a examiné les effets de succinylcholine chez ces deux groupes de patients. Les sérums de 491 patients adultes ont été examinés pour la présence de plasma cholinesterase C_5 isoenzyme par électrophorèse avec le polyacrylamide; 24 étaient positifs pour le C_5 isoenzyme. L'activité du plasma cholinesterase et la durée d'action de la succinylcholine ont été mesurée chez 12 patients ayant un C_5 positif et 18 patients ayant un C_5 négatif tous ayant un génotype normal de cholinesterase. Les patients avec C_5 positif ont présenté une activité moyenne de la plasma cholinesterase 30.1 pour cent supérieur à celles qui ont présenté un C_5 négatif. La durée du bloc neuromusculaire mesurée par la hauteur du premier twitch évoqué par une stimulation d'une ondée de quatre était significativement plus courte chez les patients ayant un C_5 positif que ceux qui ont un C_5 négatif. Les individus ayant un C_5 positif ont présenté une durée d'action de la succinylcholine plus courte que ceux qui ont un C_5 négatif.