Hydrolysis of Suxamethonium by Plasma from Subjects Responding to the Drug with Prolonged Apnoea, and by Plasma from their Relatives

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Summary. The hydrolysis of benzoylcholine, 0.05 mM, butyrylcholine, 10 mM, and suxamethonium, 0.025 mM, by the plasma of 10 subjects who developed prolonged apnoea following suxamethonium administered during anaesthesia, were measured. Plasmas from all available relatives in four families were similarly studied. The plasma of 3 subjects contained only atypical cholinesterase and hydrolysed suxamethonium, relative to butyrylcholine, at a rate of only 1.6% of that seen with usual human cholinesterase. One subject appeared to have only atypical cholinesterase on the basis of dibucaine and fluoride numbers. Suxamethonium hydrolysis, however, was sixteen times greater than that for the 3 homozygous atypical subjects. Family studies and inhibition of butyrylcholine hydrolysis by decamethonium established that this subject was heterozygous with about 20% of the total cholinesterase in the usual form. Two other subjects were also heterozygous for usual and either atypical or fluoride resistant genes. One of them hydrolysed suxamethonium at 25% of the usual rate, but the other had a normal rate of suxamethonium hydrolysis. Four subjects had no detectable anomaly of plasma cholinesterase, and hydrolysed suxamethonium normally. The apparent affinity of suxamethonium for usual and atypical cholinesterase was also determined and the significance of measurements of the hydrolysis of suxamethonium in relation to prolonged apnoea produced by the drug is discussed.

Suxamethonium is hydrolysed by plasma cholinesterase (acycholine acyl-hydrolase, E.C. 3.1.1.8.). In order to attempt to correlate the duration of action of suxamethonium with the rate of hydrolysis of suxamethonium by different forms of human plasma cholinesterase, it is desirable to employ substrate concentrations likely to occur in vivo. Few such studies have been reported so far. SCHMIDINGHER, HELD, and GORDDE (1966) used succinyldi (choline 14C-methyl) iodide to study hydrolysis between concentrations of 0.001 mM and 0.05 mM by a plasma containing only usual cholinesterase, and a plasma containing only atypical cholinesterase. KALOW (1959) used a titration technique to study hydrolysis of suxamethonium, 0.05 mM, and higher concentrations. In order to compare the rates of hydrolysis of suxamethonium, 0.025 mM, by plasma cholinesterase from different sources, HOBERGER and PECK (1969) developed a bioassay technique using the frog rectus abdominis muscle. This communication reports the rates of hydrolysis of suxamethonium, 0.025 mM, benzoylcholine and butyrylcholine by ten subjects who presented with prolonged apnoea after suxamethonium, together with studies on the plasma of relatives in four families which were studied in detail. Dibucaine (DN) and fluoride (FN) numbers were measured and on the basis of these, subjects were placed into one of three groups: the usual group with DN values around 80; the atypical group with DN values around 20; and the intermediate group with DN values between 27 and 69 (KALOW, 1962).

Methods

The enzyme source was serum or plasma without further purification. Comparison of the enzymic activity of serum and plasma from the same blood sample showed that their enzymic activities were identical within the limits of experimental error. Where possible fresh samples were used but if this was not possible, the plasma was stored at -15°C.

Hydrolysis of butyrylcholine, benzoylcholine and suxamethonium were measured as described by HOBBERGER and PECK (1969). The enzymic hydrolysis of butyrylcholine, 10 mM, was determined in a medium of 0.025M NaHCO₃, gassed with a mixture of 96% nitrogen and 5% carbon dioxide giving a pH of 7.45, using a Warburg apparatus which was thermostatically controlled at 37°C. Corrections were made for nonenzymic hydrolysis of substrate. In experiments designed to study the competitive inhibition of butyrylcholine hydrolysis by suxamethonium, corrections also were made for the hydrolysis of suxamethonium.

Enzymic hydrolysis of benzoylcholine, 0.05 mM, was determined spectrophotometrically by the method of KALOW and LINDSAY (1955), using a Hilger Watts u.v. spectrophotometer, Type 700. Enzyme samples were diluted with M/15 phosphate buffer, pH 7.4, and enzymic activity was measured at 26°C. The final concentration of human plasma (or serum) was usually 0.5%, but 1% when enzymic activity was low. The percentage inhibition of benzoylcholine hydrolysis by dibucaine (einecholine) 0.01 mM, (dibucaine number, DN), was determined from the enzymic hydrolysis obtained with aliquots of the same sample in the absence and presence of the inhibitor (KALOW and GENEST, 1957). The percentage inhibition of benzoylcholine hydrolysis by sodium fluoride 0.05 mM, (fluoride number, FN, HARRIS and WHITTAKER, 1961) was studied in the same way.

The rate of hydrolysis of suxamethonium 0.025 mM,
was studied by the method of HOBBS and PECK (1969). Plasma and substrate were incubated in a stoppered glass tube containing 6.7 mM Sorensen’s phosphate buffer, pH 7.4, placed in a thermostatically controlled water bath at 37°C, and shaken at a rate of 80/min. The final concentration of plasma used depended on its enzymic activity. With plasma containing usual cholinesterase the final concentration was 1%, while with plasma containing only atypical cholinesterase the final concentration was 10%. Aliquots of 0.3 ml were removed from the reaction mixture at 3 to 5 min intervals during the incubation and added immediately to 0.3 ml of frog Ringer solution containing the organophosphate anticholinesterase tetraethyl pyrophosphate, 0.01 mM, in order to arrest hydrolysis. The aliquots were diluted to 3 ml with frog Ringer solution a few minutes later, and then tested at once on the isolated frog rectus abdominis muscle suspended in frog Ringer solution at room temperature. The concentration of suxamethonium in test solutions was calculated by interpolation from a dose-response curve obtained with standard solutions of suxamethonium on the same rectus abdominis muscle. The time-course of hydrolysis of suxamethonium by plasma cholinesterase under these conditions, approached zero order kinetics.

For quantitative assessment of enzymic hydrolysis the time required for 50% hydrolysis (t$_{50}$) was used to calculate the umoles suxamethonium hydrolysed/ml plasma/h. Correction for nonenzymic hydrolysis was small and only required when the incubation period was 25 min or longer. Duplicate values obtained on one plasma were usually within a 15% variation or less.

Benzoylcholine chloride and butyrylcholine iodide were supplied by Sigma London Chemical Co. Ltd., suxamethonium chloride and decamethonium iodide by Koch-Light Laboratories Ltd., sodium fluoride by British Drug Houses Ltd., and dibucaine (cinchocaine hydrochloride) by Ciba Laboratories Ltd.

Results

Plasma of subjects presenting with prolonged apnoea after suxamethonium

Ten subjects presented with varying periods of apnoea after suxamethonium. Clinical details of these cases are given.

Subject I.A. Female. Age 29 years. Suxamethonium, 100 mg, produced apnoea lasting 50 min. during a submucous resection of the nasal septum. Recovery was complete.

Subject H. Br. Female. Age 61 years. Suxamethonium, 100 mg, produced apnoea lasting 25 min. The drug was given during bronchoscopy which confirmed the presence of a bronchial carcinoma. There was no evidence of hepatic involvement.

Subject A.D. Female. Age 28 years. During a lumbo-sacral fusion for spondylothesis of the 5th lumbar vertebra, suxamethonium, 60 mg, produced apnoea lasting 1 h 30 min. Recovery was complete and no other disease was present.

Subject N.S. Female. Age 24 years. No physical illness was present but episodes of depression required treatment by electroconvulsive therapy. An initial dose of suxamethonium, 40 mg, produced prolonged apnoea (duration not recorded). Subsequently very low doses of suxamethonium (5 to 15 mg) were found to be adequate.

Subject P.B. Male. Age 6 years. Two hours apnoea followed the administration of 20 mg suxamethonium given to facilitate endotracheal intubation prior to tonsillectomy. The subject was otherwise healthy and recovery was complete.

Subject N.J.G. Female. Age 20 years. Approximately 1 h of apnoea followed suxamethonium given during a dental extraction. Recovery was complete.

Subject P.M. Female. Age 25 years. During a submucous resection of the nasal septum, suxamethonium, 100 mg, produced apnoea lasting 30 min. Recovery was complete.

Subject L.M. Female. Age 19 years. Suxamethonium, 80 mg, given during an appendicectomy for acute appendicitis, produced apnoea lasting 25 min. Recovery was complete.

Subject J.G. Female. Age 41 years. Suxamethonium, 80 mg, produced apnoea lasting 45 min. The drug was given to allow endotracheal intubation prior to carotid arteriography performed after subarachnoid haemorrhage. No aneurysm was found, but mild hypertension was present. Recovery was complete.

Subject H.A. Male. Reported to have had prolonged apnoea following a usual dose of suxamethonium, but owing to loss of hospital records further details were not available.

Table 1 shows the rates of hydrolysis of suxamethonium, butyrylcholine and benzoylcholine by the different plasmas. In addition, the dibucaine and fluoride numbers are given together with the proposed genotype. For comparison mean values obtained with plasmas from 13 healthy control subjects with only usual human cholinesterase in their plasma, previously reported by HOBBS and PECK (1969), are included. The 10 subjects fell into three groups depending on the dibucaine number.

Group a) Four subjects with DNs between 80 and 87. The mean rate of hydrolysis of suxamethonium was 4.48 umoles/ml plasma/h, and the mean turnover of suxamethonium relative to butyrylcholine was 1.25%. The concentration of suxamethonium which inhibited the hydrolysis of butyrylcholine by 50% (I$_{50}$ value for suxamethonium), ranged between 2.0 and 2.3 mM. All these features indicated that only usual cholinesterase was present in these plasmas.

Group b) Three subjects with DNs of 17, 21 and 25. The mean rate of hydrolysis of suxamethonium was 0.028 umoles/ml plasma/h, and the mean turnover of suxamethonium relative to butyrylcholine was 0.018%. The I$_{50}$ values for suxamethonium and butyrylcholine ranged between 25 and 57 mM, and it was concluded that the plasma of these subjects contained only atypical cholinesterase. Detailed enzyme kinetic studies on these three plasmas have been reported by HOBBS and PECK (1969).

Group c) Three subjects with DNs of 27, 61 and 64. The plasma of subject N.S. hydrolysed suxamethonium at only 25% of the mean rate obtained with plasmas of the control subjects. Her DN, FN and I$_{50}$ values