Medical Intelligence

Succinylcholine Hydrolysis:

A Review

Roger W. Litwiller *

IN A RECENT PAPER, Pilz[1] stated that succinylcholine is not, under physiologic conditions, hydrolyzed in the plasma. He believes that an inhibitory serum protein he has isolated prevents the rapid hydrolysis of succinylcholine in blood. The tail amino acid of this protein is 5-ethoxytryptophan, a compound supposedly excreted in increased amounts in patients who show prolonged apnea following administration of succinylcholine. It is his contention that succinylcholine *in vivo* is rapidly hydrolyzed, not by a serum or plasma enzyme, but rather by a tissue enzyme that he has isolated from a homogenate of human lung. According to Pilz, the hydrolytic effect of horse serum on succinylcholine reported by Glick[2] was due to saponification, not enzymatic hydrolysis. He emphasizes that benzoylcholine and other substrates, and not succinylcholine, are being used in the study of plasma cholinesterases. He argues that this does not give a measure of the enzyme associated with the hydrolysis of succinylcholine. Pilz's stimulating contentions led me to re-examine the literature for evidence of the role of plasma cholinesterase in succinylcholine hydrolysis.

Succinylcholine has been known to medical science for more than 60 years. In 1906, Hunt and Traveau[3] studied the effects of various choline esters, including succinylcholine, on blood pressure. At that time use of curarized animals prevented the observation of its neuromuscular blocking action. Forty years later, workers in Great Britain, Italy and the United States independently described the neuromuscular blocking activity of succinylcholine. Its short duration of action was noted, and optimism arose about the controllability of muscle paralysis with this drug. Rapid hydrolytic degradation by plasma cholinesterase supposedly was responsible for the brevity of action.[2, 4]

In 1941 Glick,[2] while working with various esters of choline, found that the choline salts of succinic acid was hydrolyzed by horse serum. He used the Warburg manometric method in his analysis and observed that the choline esters of dicarboxylic acids, *e.g.*, succinic acid, hydrolyzed relatively slowly, with a rate 4 per cent of the maximum hydrolysis rate achieved with acetylcholine. Bovet-Nitti,[4] using the Warburg manometric technique, showed that succinylcholine was destroyed by rabbit cholinesterase. Castillo and de Beer[5] noted in 1950 that cat serum deactivated succinylcholine. They incubated succinylcholine in cat serum and found that after 18 hours it would no longer block neuromuscular transmission in the cat. However, if physostigmine, an anticholinesterase, were added to the serum–succinylcholine mixture, succinylcholine still would block neuromuscular transmission in the cat after 18 hours. This implied that physostigmine, by inhibiting the cholinesterase in serum, prevented the destruction of succinylcholine. Ginzel et al.[6] studied the hydrolysis of various choline esters of dibasic acids, including succinic acid. They found that all the substances investigated were split by horse serum, the speed of hydrolysis increasing with the length of the polymethylene chain of the dibasic acid. They noted that eserine increased the duration of action of the substances.

* Medical student, Class of 1970.

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In 1952, Whittaker and Wijesundra,[7] using chromatographic methods to separate and identify various constituents of a horse serum esterase–substrate (succinylcholine) mixture at different stages of hydrolysis, found succinylcholine hydrolysis taking place in two steps. Succinylmonocholine and choline were the first hydrolysis products. Succinylmonocholine in turn underwent hydrolysis, giving succinic acid and choline. At optimum conditions, the reaction proceeded at 3–4 per cent of the maximum rate achieved using acetylcholine as the substrate, agreeing with rate of hydrolysis reported by Glick.[2]

Evans *et al.*,[6] with the aid of a micromanometric technique, demonstrated *in vitro* that succinylcholine was hydrolized by the "pseudo" acetylcholinesterase of human serum, but not by the true acetylcholinesterase found in human red blood cells. Like Glick [2] and Whittaker and Wijesundra,[7] they observed that hydrolysis of succinylcholine by the serum enzyme proceeded much more slowly than the enzymatic hydrolysis of acetylcholine. An inverse relationship between the level of serum enzyme and the duration of apnea following succinylcholine administration was also reported at this time.

Using the Warburg apparatus to study human plasma, human red blood cell hemolysate and eserinized plasma, Fraser [9] found that neither red blood cell hemolysate nor eserinized plasma destroyed succinylcholine. The non-eserinized plasma, however, caused hydrolysis of succinylcholine. In *in vivo* experiments using the chick and the cat, he found that destruction of the red blood cells (true cholinesterase) did not potentiate the effect of succinylcholine; however, inhibition of plasma cholinesterase potentiated the paralytic effect of this drug.

Schroeder and Himes [10] compared the effects of succinylcholine only and succinylcholine previously incubated with various animal plasmas on the anterior tibialis–gastrocnemius muscle preparation of the intact dog. Depolarizing capacity of succinylcholine was markedly attenuated by prior incubation with plasma. Depolarizing capacity was intact for succinylcholine not incubated with plasma prior to administration.

Tsuji *et al.*[11] employed Warburg's manometric technique to assay the hydrolysis of succinylcholine by human plasma. Under the conditions of their study, the rate of hydrolysis of succinylcholine was proportional to the concentration of plasma. The enzymatic hydrolysis of succinylcholine in human plasma was a zero-order reaction within the substrate concentration studied. The rate of the reaction remained constant in the face of increasing substrate concentration. Therefore, at the lowest substrate concentration studied, 12.75 μM succinylcholine/ml plasma, all the sites of the enzyme capable of hydrolyzing succinylcholine were doing so at their maximum rates. In agreement with Whittaker and Wijesundra,[7] they found a two-step hydrolysis, with succinylmonocholine and choline as the first hydrolysis products. The succinylmonocholine then was hydrolyzed more slowly to succinic acid and choline. The enzymatic hydrolysis of succinylcholine proceeded at about 4 per cent of the hydrolysis rate of acetylcholine. Heat, as well as neostigmine, an anticholinesterase, reduced the hydrolysis rate of succinylcholine.

Using human plasma cholinesterase concentrate (Cholase), Foldes *et al.*[12] studied the kinetics of the succinylcholine plasma cholinesterase reaction with a substrate concentration of 2.2×10^{-2} M, the maximum observed velocity was 2.1×10^{-2} M/l × min. There was a definite relationship between the activity of plasma cholinesterase in various species and the dose of succinylcholine needed to produce muscle relaxation.[13]

Wang and Ross [14] reported prolonged action of succinylcholine in cancer patients receiving the experimental drug AB-132 (ethyl-N-(bis[2, 2 dimethylenimido] phosphoro) carbamate). Further studies revealed that this drug also acted as a cholinesterase inhibitor.

Further evidence for the role of plasma cholinesterase in the hydrolysis of succinylcholine arose from a study of one of the drug's uses. Reports of prolonged apnea [15, 16] following succinylcholine administration began to appear shortly after its introduction. Examination of these cases led to the recognition of a familial tendency toward prolonged apnea following succinylcholine administra-

tion. Numerous investigators, including Ernst and Smith [17] with the aid of dibucaine inhibition and acetylcholine hydrolysis, correlated the prolongation of apnea with the absence of typical plasma cholinesterase and the presence of an atypical plasma cholinesterase.

Goedde and Schmidinger,[18] using spectrophotometric tests and ^{14}C-labelled succinylcholine, studied the degradation of succinylcholine in serum at usual clinical levels, *i.e.*, less than 5×10^{-5} M, and demonstrated enzymatic hydrolysis under such conditions. Borders *et al.*[19] found that concentrated plasma cholinesterase, given parenterally in large quantities, shortened the period of apnea following succinylcholine administration in the normal patient. Altland and Goedde [20] further reported that plasma cholinesterase, when given to patients with abnormal plasma, shortened the period of apnea following administration of succinylcholine.

Kalow [21] has done much of the work that allows the classification of the varieties of plasma cholinesterase. It is now well established that the liver is the site of synthesis of plasma cholinesterase.[22] Hepatic disease represents one of the many conditions associated with plasma cholinesterase deficiency.[23] A pseudocholinesterase is known to exist in glial tissue, cardiac muscle, intestine and skin.[24, 25] Evidence now exists that the pseudocholinesterase found in the intestine and glial tissue has an amino-acid sequence identical to that of plasma cholinesterase.[26] This implies that the same structural gene controls the synthesis of these cholinesterases. At present, at least four different genes are known to occupy this locus.[27]

Plasma cholinesterase, also called pseudocholinesterase, serum cholinesterase, acylcholine acylhydrolase, or EC 3.1.1.8, has a molecular weight variously reported as between 168,000 [28] and 300,000.[29] The enzyme migrates with the α_2-globulins in conventional electrophoresis.[29] Svensmark [30] demonstrated that human serum cholinesterase is an acid glycoprotein containing several residues per molecule of sialic acid, a derivative of neuraminic acid. These may be removed without loss of enzymatic activity.

Harris *et al.*[31] showed the heterogeneity of plasma cholinesterase by two-dimensional electrophoresis (the first stage on paper and the second in starch gel). He found four zones, each with staining properties and responses to activators and inhibitors consistent with the presence of cholinesterase. In about 5 per cent of individuals a fifth zone of activity appeared.[32] This zone seems to be associated with increased plasma cholinestersase activity. Although he made no attempt to correlate his findings with the zone of increased activity on electrophoresis Neitlich [33] described an individual with increased plasma cholinesterase activity. Study of the family revealed that this trait probably was inherited as an autosomal dominant. Svensmark [22] has separated three fractions from human heptic tissue by chromatography and gel filtration. Fraction II is identical to human plasma cholinesterase with respect to electrophoretic mobility and enzyme properties. Fractions 1d and 1e differ from plasma cholinesterase in that they are not sialoproteins (glycoproteins with sialic acid as the carbohydrate component). However, they have enzymatic properties identical to those of plasma cholinesterase. Svensmark believes that fractions 1d and 1e represent precursors and fraction II the final product, *i.e.*, plasma cholinesterase.

At various times, investigators other than Pilz have raised the question of the role of plasma cholinesterase in succinylcholine hydrolysis. Fraser,[9] using a 0.2 mg/kg dose of eserine in cats, observed that maximum plasma cholinesterase inhibition occurred in less than ten minutes, while maximum potentiation of the paralytic effect of succinylcholine did not occur until one hour after the eserine had been given. This led him to suggest that factors other than plasma cholinesterase might influence the duration of succinycholine action; de Beer *et al.*[34] also suggested this. In spite of these questions, there is little in the literature to support Pilz's contention regarding the hydrolysis of succinylcholine. The tail amino acid, of the inhibitory protein prepared by Pilz, 5-ethoxytryptophan, is a compound excreted in increased amounts in the urine of patients who respond to succinylcholine with prolonged apnea.[1] Rubinstein *et al.*[35] found this substance in the patient with atypical serum cholinesterase.

Very recently, Doenicke *et al.*[36] re-examined Pilz's work. They found that, at the substrate concentrations used by Pilz, plasma cholinesterase action was almost totally inhibited. Using a method described by Pilz,[37] they also observed rapid enzymatic degradation of succinylcholine in serum. It should be pointed out that Pilz [1] did his experimental work at a *p*H of 8.6, which is far beyond the physiological *p*H. Fraser [9] states that succinylcholine is unstable in alkaline solution, with slow spontaneous hydrolysis occurring at *p*H 8.2.

If Pilz's [1] contention were true, *i.e.*, if hydrolysis of succinylcholine were to take place only in the parenchyma of the lungs, patients on cardiopulmonary bypass should show increased sensitivity to succinylcholine. They do not.

In conclusion, at the present time, much of the available evidence supports the concept that succinylcholine is hydrolyzed primarily in plasma by a cholinesterase synthesized in the liver. This evidence has been accumulated in numerous experiments with various substrates. With the recent use of succinylcholine as a substrate,[18] it has become even more convincing. Whether or not the provocative work of Pilz eventually will lead to establishment of other or additional mechanisms for the hydrolysis of succinylcholine *in vivo* remains to be seen.

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Drugs

BARBITURATE INTOXICATION Polyvinylpyrrolidone of molecular weight 10,000 (PVP-10) distributes rapidly throughout extracellular fluid. It is excreted rapidly and completely by glomerular filtration and is nontoxic after acute intravenous administration to rabbits, so its effects on rabbits intoxicated with phenobarbital and secobarbital were studied. Significant quantities of both barbiturates were found at serum concentrations of PVP attained by intravenous administration of 2 g/kg per hour of PVP-10. Renal clearance of the barbiturates was enhanced and the toxic state ameliorated more rapidly than by treatment with dextran of the same molecular weight. The effect was attributed to binding by PVP of a portion of the barbiturate in a non-reabsorbable form in tubular urine, as well as binding in a nondiffusible form in the extracellular space. *(Ruedy, J., and Chernecki, W.: Use of a Binding Agent, Polyvinylpyrrolidone, in the Treatment of Experimental Barbiturate Intoxication in Rabbits, Canad. J. Physiol. Pharmacol. 46: 829 (Nov.) 1968.)*