1969

Atypical cholinesterase

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A THESIS

Presented to the Faculty of

The College of Medicine in the University of Nebraska

In Partial Fulfillment of Requirements

For the Degree of Doctor of Medicine

Under the Supervision of Jerry Schenken, M.D.

Omaha, Nebraska

February 1, 1969

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ATYPICAL CHOLINESTERASE

At least two distinct forms of cholinesterase exist within the body. Cholinesterase I, also known as specific cholinesterase, true cholinesterase, E-type cholinesterase, and acetyl cholinesterase is present in red cells and nervous tissue. Its function is poorly understood but it apparently has both muscarinic and nicotinic functions in neurohumoral transmission.[1] Cholinesterase II, also known as atypical cholinesterase, nonspecific cholinesterase, pseudocholinesterase, S-type cholinesterase, and acyl-choline-acyl-hydrolase, is synthesized in liver and is present in plasma and many other tissues.[5]

The estimation of plasma cholinesterases may have clinical value in such conditions as liver and biliary tract diseases, malnutrition, fluorophosphate ester poisoning and in patients being treated with thio-TEPA for cancer. Plasma cholinesterase levels may also be reduced after ingestion of such drugs as chlorpromazine, morphine, estrogens, cortisone, quinine, prostigmin, physostigmine, and procaine.[2]

Serum cholinesterase hydrolyzes succinylcholine as it does procaine and other esters of benzoic acid. Initially, it is transformed to succinyl monocholine and choline. Succinyl monocholine, a weak neuromuscular blocker about 1/20 as potent as succinylcholine, is in turn hydrolyzed to succininc acid and choline. Under physiologic conditions the first reaction

occurs six times more rapidly than the second. Serum cholinesterase acts only while succinylcholine is present in the blood. Enzymatic activity increases with a rising concentration of the drugs. The rate of hydrolyses is rapid, about 90% of the injected dose is destroyed in the first minute; heme enzyme activity is of importance only briefly but serves to limit the amount of relaxant that reaches the neuromuscular end-plate. Termination of action is probably caused by diffusion from the end-plate into the interstitial fluid.

It has been noted that certain individuals given succinylcholine undergo profound protracted apnea responsive only to assisted ventilation. In these cases, known as "suxamethonium apnea", the plasma pseudocholinesterase levels were found to be lower than normal. It was noted that they also demonstrated invitro resistance to enzymatic inhibition by such compounds as dibucaine, fluoride and N-butanol. This inhibition was not present in patients lacking this susceptibility to succinylcholine.[3,4,6] The need for determination of this plasma enzyme prior to administration of such anesthetic agents is obvious.

Genetic studies in families exhibiting this peculiarity in the pseudocholinesterase system revealed that the plasma levels tended to cluster in at least three areas. It has subsequently been clarified that the genes for the first found loci of pseudocholinesterase (represented by E_1) are probably

alleles of the same locus. By describing the patients as E_1^u (usual), E_1^a (atypical), E_1^s (silent), and E_1^{fl} (fluoride) ten genotypes have been described. A patient who is homozygous for E_1^u is usual or normal and will show satisfactory inhibition of serum cholinesterase by dibucaine, fluoride and N-butanol. Those who are homozygous for E^a are atypical and do not show satisfactory invitro inhibition of their cholinesterase level. These patients are definitely at risk to develop suxamethonium apnea although the attack rate is not 100% and is somewhat dose related. The absence of plasma pseudocholinesterase (homozygous E_1^s) demonstrates the so-called silent gene in which no pseudocholinesterase activity is demonstrated in plasma. These cases are extremely rare.[3]

Heterozygotes, that is combinations of E_1^u and E_1^a show partial invitro inhibition but do not to the degree of the true atypicals. These patients are apparently not att risk to develop suxamethonium apnea.

Normally approximately 80% of the pseudocholinesterase activity in plasma can be inhibited invitro by positively charged quaternary nitrogen containing molecules such as dibucaine. The per cent inhibition is known as the dibucaine number. Surveys of the general population reveal that 94% of people tested show about 80% inhibition of their pseudocholinesterase level by dibucaine and can be classified as homozygous for E_1^u. Approximately 3% will show 40-60% inhi-

bition and are apparently heterozygotes showing a combination of E_1^u and E_1^a or E_1^{fl}. Only 1 in 4,000 patients will show only 20% invitro inhibition by dibucaine, are homozygous for E_1^a, and are definitely at risk for suxamethonium apnea. The negatively charged fluoride ion may also be used as an invitro inhibitor.[1] This inhibition is immediate, constant, and reversible, as studied by dialysis and dilution techniques. It is presumably a direct effect by the fluoride ion and not by complexing with such metals as magnesium and calcium or phospahte. The relationship of the fluoride and dibucaine inhibition in heterozygotes has not been clearly established. It is possible that they are biochemical facets representing the same gene. However, studies of inhibitions by these two compounds have shown that in heterozygotes ($E_1^a \cdot E_1^u$) the inhibition is relatively less by fluoride than by dibucaine.[1] The neutral compound N-butanol has also been used as an inhibitor.[7] In all cases the atypical homozygotes as well as the usual homozygotes are clearly defined. Unfortunately the definition of the heterozygotic phenotypes is not always clear nor separable depending on the inhibitor used.[6]

Laboratory methods for quantitating the amount of cholinesterase present in plasma measure enzyme activity and do not differentiate between the normal and atypical variety.[4] Thus low enzyme levels may mean reduced amount of normal

enzyme or the presence of the atypical enzyme in varying amounts.

Many substrates have been utilized for the determination of plasma true and pseudocholinesterases. Tribytyrin, acetyl-beta-methylcholine, benzoylcholine, phenylacetylcholine, and acetylcholine have all been used. At that the cholinesterases are relatively nonspecific and both also hydrolyze a variety of noncholine esters. Substrate specificity has been poorly defined but it is fairly well established that red cell true cholinesterase will hydrolyze acetyl-beta-methylcholine but not benzoylcholine, while the plasma pseudocholinesterase will react in just the opposite fashion.[6]

The cases which were studied in this paper underwent varying degrees of suxamethonium apnea after administration of succinylcholine. The pseudocholinesterase activity in plasma of these individuals as well as members of their families was determined by the method to be described. For controls, approximately 30 normal individuals were tested utilizing the same procedure.

Method

The method for determination of atypical forms of pseudocholinesterase used in this study was that of Kalow and Genest.[3] Cholinesterase activity was determined by ultraviolet spectrophotometry at 240 nanometers using $5X10^{-5}$ M benzoylcholine, buffered at pH 7.4, as substrate. The zero

order reaction was determined with and without the addition of dibucaine. The degree of inhibition expressed as a per centage was recorded as the "dibucaine number".

Benzoylcholine is a relatively stable compund showing an absorbence maximum at 240 nm. Enzymatic activity is determined kinetically (time-rate) by observing the loss of absorbance as the benzoylcholine is hydrolyzed to benzoate and choline. Relatively large concentrations of the product, benzoate, produce little absorbance at this wavelength. Benzoylcholine is a relatively resistant to spontaneous hydrolysis except by the action of bacterial or fungal growth and must be stored at 5°C. Excess substrate has an inhibitory effect just as it does when acetylcholine is used to estimate true cholinesterase. For this reason, very dilute substrate concentrations were used. The destruction of benzoylcholine by serum cholinesterase proceeds at least 35 times faster than that of acetylcholine when substrate concentrations in the order of 10^{-5}M are used.

Human serum or plasma was diluted 1:50 in M/15 phosphate buffer, pH 7.4, and added to benzoylcholine substrate with and without the addition of dibucaine as an inhibitor.[3] Constant time-rate spectrophotometric anaylsis was determined at 240 nm using a Gilford 2000 recording spectrophotometer with automatic cuvette changer, thermoregulator at 25°C., and 1 cm square quartz cuvettes. Reactions were observed over a

period of 3 minutes and a change in absorbanne per minute with and without the inhibitor was determined. The dibucaine number, that is, the percentage of inhibition, was calculated as 100 times /1-(decrease absorbance in presence of dibucaine divided by decrease in aabsorbance without dibucaine)/. Total esterase units were arbitrarily expressed as micromols of acetylcholine hydrolyzed by 1 cc of serum in one hour at 37° and were arbitrarily compared to the hydrolysis of benzolycholine. The total value was not a part of the study other than the observation that the homozygous atypicals tended to have lower total absorbance values than did normals.

Case Studies

Case 1. LR, 6-year-old white male, developed prolonged post anesthetic apnea while undergoing a hernia repair. His dibucaine number was 18. The father and mother had dibucaine numbers of 67 and 72 respectively. Five siblings had numbers of 71, 68, 71, 85, and 86. A 14-year-old sibling had a D.N. of 28. Interestingly enough this sibling had undergone a hernia repair some 3 years before without incident in which succinylcholine was administered.

Case 2. TL, 62-year-old female, experienced approximately 8 hours post-anesthetic apnea while having a hiatal hernia repaired. Her D.N. was 18. Normal siblings had dibucaine numbers of 68, 66, and 84, but one 61-year-old sister had a D.N. of 23.

Case 3. RW, 55-year-old female, experienced profound 12 hour apnea after receiving succinylcholine during the course of an intramedullary nail pinning of a fractured hip. Her D.N. was 25. A son and grandsons had numbers of 68, 67, and 86.

Case 4. WD, Sr., 34-year-old male, experienced severe apnea following succinylchoine administration while having a hernia repair. His dibucaine number was 14. His children had D.N.'s of 61 and 66.

Results

By this method normal patients (homozygotes ($E_1{}^u \cdot E_1{}^u$) tended to have dibucaine numbers between 70-85% as did normals whose total may have been somewhat low secondary to liver disease. Abnormal homozygotes ($E_1{}^a \cdot E_1{}^a$) clustered between 16 and 25. Heterozygotes by the dibucaine method were noted to be between 50 and 65.

It is interesting to note that the parents who were apparently homozygotes for the gene $E_1{}^a$ had total cholinesterase levels significantly lower than the 0.040-0.050 Absorbance units per minute -- of the normal range, running from 0.025A to 0.030A per minute. It is interesting to speculate on the apparent heterozygotic nature of the various ralatives studied.

Conclusion

The relationship of inhibitible pseudocholinesterase activity in serum and suxamethonium apnea appears well

defined although the normal function of plasma pseudocholinesterase is poorly understood. The methods available for the determination of dibucaine inhibitable pseudocholinesterase are reasonably simple and precise. This determination should be performed on all patients who have had prior post-anesthetic apneic reactions themselves or have a family history of a similar event.

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