A372

Title: IDENTIFICATION OF DNA MUTATIONS RESPONSIBLE FOR SERUM BUTYRYLCHOLINESTERASE VARIANTS WILL PERMIT A BETTER DIFFERENTIATION OF ABERRANT DRUG RESPONSES

Authors: BN La Du, MD, PhD, S Primo-Parmo, AFL van der Spek, MD
Institution: Department of Anesthesiology, University of Michigan, Ann Arbor, MI 48109-0572

Introduction: Serum butyrylcholinesterase (BChE) is essential for hydrolysis of succinylcholine, mivacurium, bambuterol, cocaine, heroin, aprophen and other drugs. Reduction of BChE's esteratic activity leads to prolonged relaxation after succinylcholine administration. Determination of the plasma's phenotype (by the level of esterase activity and its dibucaine and fluoride inhibition) allows identification of the 3 best known BChE variants (A, F, S). However, phenotyping is generally inadequate for identifying the more recently recognized quantitative variants; it does not distinguish sub-types of fluoride or silent variants. It leaves some abnormal responses unexplained, perhaps because benzoylcholine or butyrylthiocholine as the substrate may not mimic the behavior of succinylcholine. To gain further insight into the importance of BChE phenotypes in drug metabolism, our laboratory group has determined their molecular basis by nucleotide sequencing (1).

Methods: DNA was isolated from white blood cells harvested from EDTA plasma and the coding region of each BChE gene was amplified by the polymerase chain reaction (PCR). The nucleotide sequence in these regions was determined by the dideoxy sequencing method with a number of sequencing primers to identify the DNA abnormality in probands and their families carrying BChE variants.

Results: Twelve DNA defects were identified as either point (9) or frameshift (3) mutations. The atypical phenotype (A) was represented by a single nucleotide change within the 1722 bases coding for the BChE enzyme. The silent phenotype (S) differentiated into six sub-types – 3 point and 3 frameshift mutations. Two different point mutations can cause the same fluoride phenotype (F). Quantitative reductions in BChE activity (e.g. K, J, H, S), were as easily identified as the qualitative variants (e.g. A and F) by the specific DNA tests. To simplify and expedite the diagnosis of these genotypes, biotin labeled, allele-specific oligonucleotide probes and restriction fragment length polymorphism tests were developed to identify several of these DNA mutations.

Discussion: The 12 specific alleles identified can give rise to a large number of genotypes which may have importance with respect to differences in substrate hydrolyses. Combination of serum BChE phenotyping with these new DNA analytical methods increases the scope and accuracy of BChE typing (e.g. for succinylcholine-sensitive subjects with more than 1 mutation, i.e. the AS or AF types). These studies provide the basis for insight in the mechanism(s) of drug metabolism of succinylcholine, mivacurium, chloroprocaine and other drugs encountered in anesthetic practice (e.g. bambuterol, cocaine, heroin, aprophen, etc.) as well as the clinical importance of the BChE variants. These studies are extended by an improved spectrophotometric method permitting direct assessment of the pharmacokinetics of succinylcholine hydrolysis in vitro, in the serum of each of the above BChE genotypes. We propose to establish a referral center and national databank of BChE abnormalities, to which samples can be submitted. This will facilitate the study of genetic variants and their importance in the metabolism of drug substrate.

References: (1) Phenotypic and molecular biological analysis of human butyrylcholinesterase variants. La Du BN, Bartels CP, Nogueira CP, Hajra A, Lightstone H, van der Spek AFL, Lockridge O. Clin Biochem 1990; 23: 423-431

(Supported by NIH Grant GM 44887).

A373

TITLE: SATURABLE BINDING OF HALOTHANE TO SYNAPTOSOMAL MEMBRANES OF RAT BRAIN

AUTHORS: EA EL-Maghrabi, M.D., RG Eckenhoff, M.D. and H Shuman, Ph.D.
AFFILIATION: Dept of Anesthesia, and Inst. for Environmental Medicine, University of Pennsylvania, Phila, PA 19104

The hypothesis that inhalational anesthetics act directly on or bind specifically to membrane proteins remains controversial[1,2]. We have developed a photoaffinity technique which takes advantage of the photolabile nature of halothane allowing the use of classic pharmacological approaches to study the binding of inhalational anesthetics at a subcellular level. ^{14}C-halothane was incubated with synaptosomal membranes of whole rat brain in 1mM Na_2HPO_4, 0.32 M sucrose pH 7.4 and exposed to UV-light for 10 min. 10mM glutathione was added to quench free halothane radicals from binding artifactually to synaptosomes. Bound label was separated from free by vacuum filtration over Whatman GF/B filters pretreated with 0.05% polyethylenimine and washed with 10 ml of ice cold buffer. Retained radioactivity on filter was determined by liquid scintillation counting. Iterative computer analysis of competition and saturation curves showed that 63% ±4.6% of ^{14}C-halothane partitioning in brain synaptosomes was specific, with low affinity and high binding site concentration (K_D=0.68mM, B_{max}=79.1±4.8 umol/mg protein and Hill Coefficient=0.99±0.03). These data demonstrate saturable binding of ^{14}C-halothane to rat brain synaptosomes, and, because of the similarity of K_D and MAC (0.68, 0.31mM respectively), suggest relevance to anesthetic action. Supported in part by NIH GM07612 to E El-Maghrabi, and by an IARS Sankey Award to RG Eckenhoff.

References:
1. Topical Reviews in Anesthesia, Wright Bristol, Vol 1:1-84, 1980
2. Int Rev Neurobiol, 27:1-61, 1985

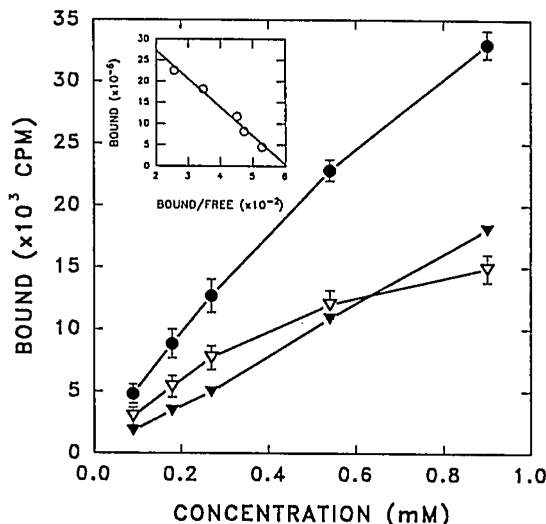

FIGURE LEGEND Scatchard analysis of ^{14}C-halothane photoaffinity labeling of rat whole brain synaptic membranes. Saturation isotherm before [•] and after [▽] removing the nonspecific binding [▼] as determined by adding 2.5mM unlabeled halothane. Results are means ± SD for three experiments. (Inset) Scatchard plot of data after removal of nonspecific binding.

Downloaded from http://pubs.asahq.org/anesthesiology/article-pdf/75/3/A373/642422/0000542-199109001-00373.pdf by guest on 05 December 2024