

GENETICALLY ENGINEERED HUMAN ACETYLCHOLINESTERASE

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This is a continuation of application Ser. No. 07/496,554, filed Mar. 20, 1990, now abandoned.

FIELD OF THE INVENTION

The invention relates to genetically engineered human acetylcholinesterase. The invention is also directed to the cloning and production of human acetylcholinesterase. The invention is further directed to the production of antibodies interacting with said protein. The invention also relates to pharmaceutical compositions comprising acetylcholinesterase for treatment and prophylaxis of organo-phosphorous compounds poisoning. The compositions of the present invention may also be used to relieve post-surgery apnea. Methods of treating or preventing organophosphorous poisoning or post-operative apnea by employing the pharmaceutical compositions of the invention are also within the scope of the application. The invention further relates to human cholinesterase probes which may be employed for diagnosing progressing ovarian carcinomas and hemocytopenic disorders. Methods of diagnosing such tumors or hemocytopenic disorders are also envisaged within this application. Furthermore, methods of treating hemocytopenic disorders are also considered.

Throughout this application, various publications are referenced by Arabic numerals within parentheses. Full citations for these references may be found at the end of the specification immediately preceding the claims. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art as known to those skilled therein as of the date of the invention described and claimed herein.

BACKGROUND OF THE INVENTION

Properties of Cholinesterases

Cholinesterases (ChEs) are highly polymorphic carboxylesterases of broad substrate specificity, involved in the termination of neurotransmission in cholinergic synapses and neuromuscular junctions. ChEs terminate the electrophysiological response to the neurotransmitter acetylcholine (ACh) by degrading it very rapidly (1). ChEs belong to the B type carboxylesterases on the basis of their sensitivity to inhibition by organophosphorous (OP) poisons (2) and are primarily classified according to their substrate specificity and sensitivity to selective inhibitors into acetylcholinesterase (ACHE, acetylcholine acetylhydrolase, EC 3.1.1.7) and butyrylcholinesterase (BuChE, acylcholine acylhydrolase, EC 3.1.1.8) (3). Further classifications of ChEs are based on their charge, hydrophobicity, interaction with membrane or extracellular structures and multisubunit association of catalytic and non-catalytic "tail" subunits (4,5).

The severe clinical symptoms resulting from OP intoxication (6) are generally attributed to their inhibitory interaction on AChE (7). OPs are substrate analogues to ChEs. The labeled OP diisopropylfluorophosphate (DFP) was shown to bind covalently to the serine residue at the active esteratic site region of ChEs, that is common to all of the carboxyl-esterases (8,9). However, the binding and inacti-

vation capacity of OPs on ChEs is considerably higher than their effect on other serine hydrolases. Furthermore, even within species the inhibition of ChEs by different OPs tends to be highly specific to particular ChE types (10). In order to improve the designing of therapeutic and/or prophylactic drugs to OP intoxication, it was therefore desirable to reveal the primary amino acid sequence and three dimensional structure of human ACHE, and to compare them to those of human BuChE, as well as to the homologous domains in other serine hydrolases.

AChE may be distinguished from the closely related enzyme BuChE by its high substrate specificity and sensitivity to selective inhibitors (11). Both enzymes exist in parallel arrays of multiple molecular forms, composed of different numbers of catalytic and non-catalytic subunits (12). However, in humans, as in other species, they display a tissue-specific mode of expression. BuChE, assumed to be produced in the liver, is the principal species in serum (13). In contrast, AChE is the major cholinesterase in various human brain regions (14), including the cholinceptive basal brain ganglia (15).

Extensive research efforts by several groups resulted in recent years in the isolation of cDNA clones encoding the electric fish AChE (16,17), *Drosophila* AChE (18,19) and human BuChE (20,21). However, the primary structure of mammalian, and more particularly, human AChE remained unknown.

Interaction of Cholinesterases with Organophosphorous Insecticides and War Gases

The use of organophosphorous (OP) anticholinesterase compounds in war (22) and as agricultural insecticides (23) resulted, over the last 40 years, in an interesting number of cases of acute and delayed intoxication. These included damage to the peripheral and central nervous system, myopathy, psychosis, general paralysis and death (24). Estimations are that 19,000 deaths occur out of the 500,000 to 1 million annual pesticide-associated poisonings (25). Previous animal studies demonstrated that methyl parathion administration suppressed growth and induced ossification in both mice and rats, as well as high mortality and cleft palate in the mouse (26). In humans, malformations of the extremities and fetal death were correlated with exposure to methyl parathion in 18 cases (27). In addition, a neonatal lethal syndrome of multiple malformations was reported in women exposed to unspecific insecticides during early pregnancy (28).

Complete inhibition of ChEs by the administration of OP poisons is lethal (6). This inhibition is achieved by formation of a stable stoichiometric (1:1) covalent conjugate with the active site serine (7), followed by a parallel competing reaction, termed "aging", which transforms the inhibited ChE into a form that cannot be generated by the commonly used reactivators (7) such as active-site directed nucleophiles (e.g., quaternary oximes) which detach the phosphoryl moiety from the hydroxyl group of the active site serine (70). The aging process is believed to involve dealkylation of the covalently bound OP group (7), and renders therapy of intoxication by certain organophosphates such as Sarin, DFP and Soman, exceedingly difficult (29).

Use of preparations comprising ChEs for therapeutical purposes has been demonstrated to be effective at laboratory level: purified AChE from fetal calf serum has been shown to protect rats from 2 lethal doses of Soman (a war OP poison) with half life of 5-6 days (37,38). Purified BuChE from human serum has been shown to improve the symptoms of OP-intoxicated patients (31).