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MEETING ABSTRACTS

Expi 293 CELLS EXPRESSING AN ENDOGENOUS WILD-TYPE BUTYRYLCHOLINESTERASE, AND A VARIETY OF ESTERASES THAT SELF-REACTIVATES AFTER PHOSPHYLATION BY ALL TYPES OF ORGANOPHOSPHORUS AGENTS

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A human embryonic kidney cell line (Expi293), adapted for suspension growth in serum-free medium, secretes a tetrameric butyrylcholinesterase (BChE). Expression levels are very low, but are increased 10-fold upon treatment with polyethylenimine. DNA sequencing shows that this enzyme is wild-type BCHE.

This endogenous BChE displays catalytic properties very close to that of natural huBChE with butyrylthiocholine and N-methylindoxyl acetate as substrates [1]. Several endogenous co-secreted esterases self-reactivate after inhibition by echothiophate, paraoxon, cresyl saligenin phosphate (CBDP), racemic coumarin(CM)-soman, CM-tabun and CM-VX. Overall reactivation rate constants, k_r, of diethylphosphorylated enzymes after inhibition by echothiophate and paraoxon are 0.171 min^{-1} and 0.059 min^{-1}, respectively, suggesting multiple OP-hydrolyzing enzymes. After phosphonylation by CM-soman, CM-tabun and CM-VX, k_r values range from 0.0375 min^{-1} to 0.0078 min^{-1}. kr of CBDP-inhibited enzyme is 0.028 min-1. Interestingly, an apparent aging rate is observed after phosphylation. The aging rate of the soman-phosphonylated enzyme(s) is approximately 2-fold slower than for wtBChE (half-time =16 min against 9 min for wtBChE [2]). The half-time for aging after inhibition by CBDP is 31 min whereas aging of wtBChE-CBDP is almost instantaneous [3]. Diethylphosphorylated enzyme(s) inhibited by paraoxon and echiothiophate age(s) with apparent k_a =0.162 min^{-1} and 0.057 min^{-1}, respectively. This difference also supports the multiple enzyme hypothesis. Further studies are in progress to indentify the different OP-reacting enzymes produced by this Expi293 cell line.

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