Effects of different pH on cholinesterases activity and bimolecular rate constant of dichlorvos in the tissues of food animals

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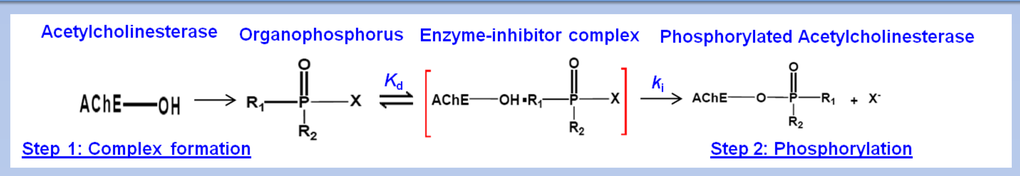
Background

- Cholinesterases (ChEs) are specialized carboxylic ester hydrolases that catalyse the hydrolysis of choline esters. Two types of ChE activity have been identified in mammalian tissues; these are distinguished according to their substrate specificity and sensitivity to the selective inhibitors. The first is acetylcholinesterase (AChE, EC 3.1.1.7). The second is butyrylcholinesterase (BChE, EC 3.1.1.8).
- Organophosphorus (OP) compounds are esters of phosphoric acid containing alkoxy, alkyl, amino or thioalkyl groups. These pesticides are widely used today in veterinary medicine to control parasites [1].
- The toxic effects of OP [dichlorvos (DDVP)] compounds are almost entirely due to covalent modification and hence inhibition of acetylcholinesterase (AChE) in the nervous system.
- An outline of the mechanism of inhibition of AChE by organophosphorus compounds is shown below.



Objectives

- To investigate the optimum pH for AChE and BChE from liver and muscle of sheep, cattle, and pig.
- A further aim was to study the most sensitive pH effect to the rate of constants (k_i) of DDVP-inhibited AChE and BChE from liver of food animals.

Methods

- Meat from food animals (5 sheep, 5 cattle and 5 pigs) was obtained from local abattoirs and transported in a cool box to the laboratory.
- To extract ChEs, samples of liver and muscle were cut into small pieces (3-5 mm^3), and homogenized using a mechanically-driven homogenizer (Model X520-D, T6 probe, Bennett and Company, Weston-super-Mare, UK) with phosphate buffer, pH 8.0 (ratio 1:9), and centrifuged at 9000 *g* for 5 min.
- AChE activity was determined using the Ellman (1961) method, adapted for a plate reader.
- For detection of optimum pHs, ChEs was measured for different pHs (6-9) at 20 °C. The increase and decrease in ChE activity over different pHs was then monitored (e.g. Figure 1A).
- For measurement of rate constants for inhibition (k_i), ChE was inhibited with 4, 6, and 8 µM DDVP in pHs 6.5, 7.5, and 8.5, respectively. The decrease in ChE activity over different times (0-60 min) was then plotted (e.g. Figure 1B).

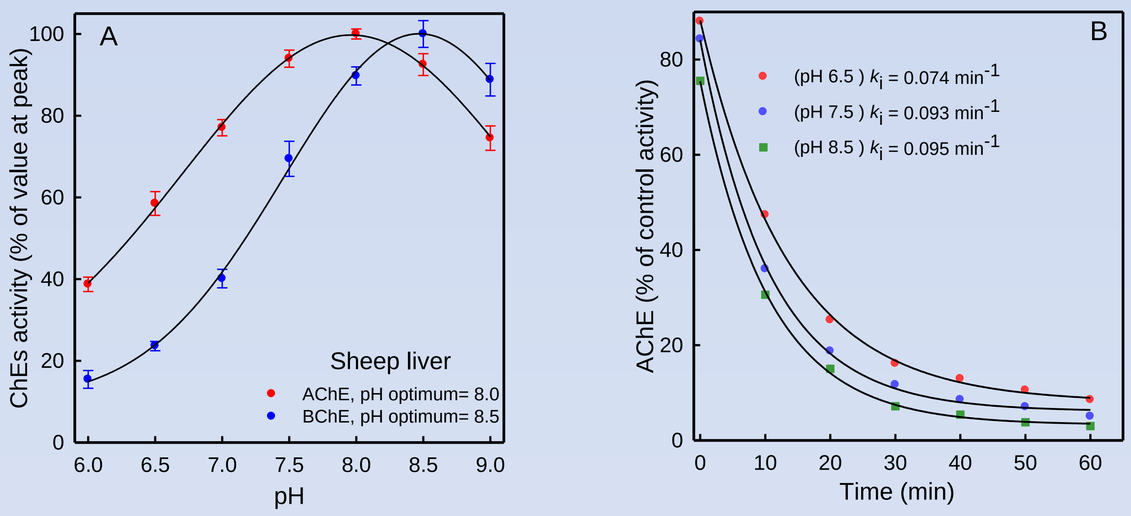


Figure 1: The figures above illustrate the data analysis used to obtain for optimum pH (A) and for rate constant for inhibition (B). The data are for sheep liver. Optimum pH data were fitted with a Gaussian peak using SigmaPlot 11 (Systat software, Inc.). For the rate constants (k_i) data the inhibition time courses at different times after inhibition were fitted with a single exponential decay.

Acknowledgements

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Results

Table 1: Optimum pH values for AChE and BChE from liver and muscle of food animals.

Tissues	Animals	AChE	BChE
Liver	Sheep	8.0	8.5
	Cattle	8.1	8.3
	Pig	7.9	8.5
Muscle	Sheep	8.1	7.8
	Cattle	8.2	7.8
	Pig	8.1	7.7

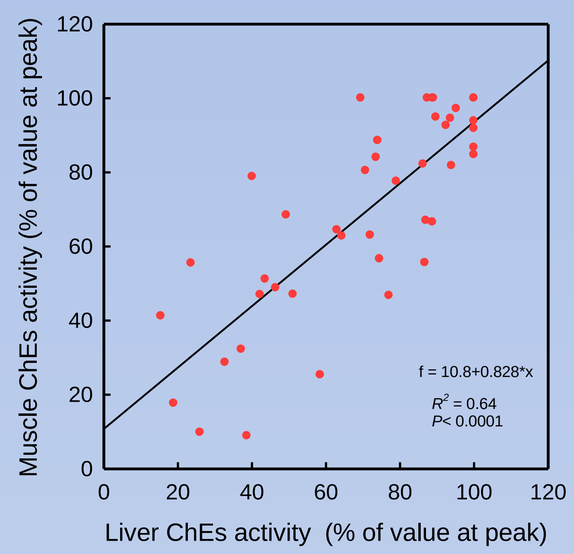

Figure 2: Comparison between liver and muscle ChEs activities (percent of value at peak) from food animals within a range of pHs (6-9).

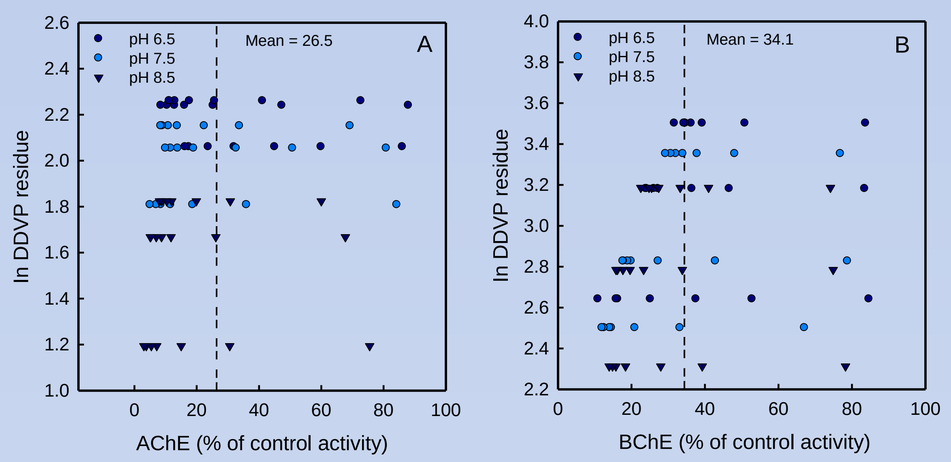

Figure 3: Residues of the DDVP (plotted on the y-axis) vs. the percent liver ChEs activities (x-axis) for food animal at different pHs. Vertical dashed lines are drawn at the mean percent control of ChEs activities.

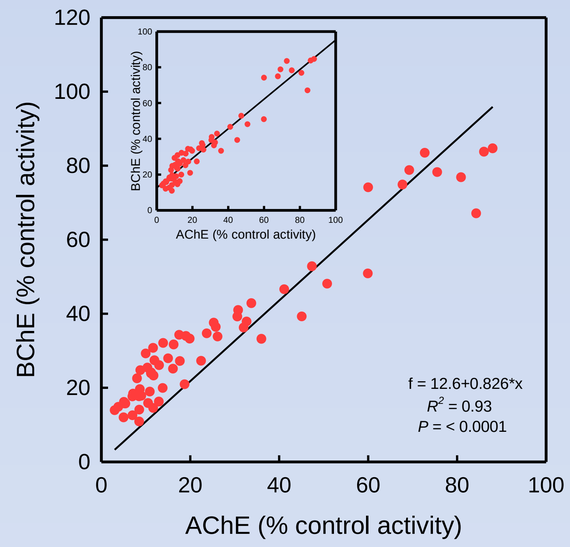

Figure 4: Comparison between AChE and BChE activities (percent control) in liver from sheep, cattle, and pigs inhibited with DDVP within three different pHs (6.5, 7.5, and 8.5).

Conclusions

- This is the first study that provided original data concerning an enzymological characterization of this inhibitor in food animals.
- In liver, the Optimum pH values in BChE was higher than AChE, while in muscle optimum pH values in AChE higher than BChE.
- In both AChE and BChE, k_i values for inhibition by DDVP compounds was higher in pH 6.5 than other pHs.
- In all animals residual ChEs activity after inhibition by DDVP compounds was lower in AChE than in BChE.