

17. ACETYLCHOLINESTERASE AND BUTYRYLCHOLINESTERASE ACTIVITY MEASUREMENTS IN HUMAN BLOOD BY THE ELLMAN METHOD. I. EVALUATION OF PROCEDURE PROTOCOL

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INTRODUCTION

Absorption of organophosphorus compounds or carbamates is commonly assessed by measuring the decrease in acetylcholinesterase (AChE; EC 3.1.1.7) and butyrylcholinesterase (BChE; EC 3.1.1.8) activities in human blood. The procedure for plasma BChE activities is well established while this is not the case for the erythrocyte AChE. We suggest a procedure based upon separation of plasma from erythrocytes followed by separate measurements of BChE in plasma and AChE in unwashed erythrocytes. The enzyme activities are measured by the spectrophotometric method of Ellman (1).

EXPERIMENTAL PROCEDURE AND CONDITIONS

Venous blood is collected into dried heparinized tubes and subjected to centrifugation. Plasma is then withdrawn, and it is essential to withdraw all plasma without removing any erythrocytes. The erythrocytes are then suspended in water in a volume corresponding to the initial volume of whole blood, followed by 60-fold dilution with buffer (0.1 M phosphate buffer pH=7.4) and then frozen in order to haemolyse the erythrocytes. After thawing, the suspension is further diluted with buffer and the thiol reagent DTNB added (final DTNB conc. during enzyme assay 0.33 mM). Ten minutes after DTNB, acetylthiocholine (ATCh) is added (final ATCh conc. during enzyme assay 1.0 mM) and the increase in absorbance read at 412 nm against a blank containing haemolysed erythrocytes suspended in buffer. The BChE activity in plasma is also measured with 1.0 mM ATCh using the same buffer and DTNB reagent. The final assay volume is 3.0 mL. During enzyme assay, the final dilution of erythrocytes is 600-fold (in relation to whole blood) and of plasma 150-fold. The enzyme activities are measured at 25 or 37 °C.

The concentration of haemoglobin in the erythrocyte suspension is determined spectrophotometrically with a modified Zijlstra reagent containing potassium ferricyanide, sodium bicarbonate and Triton X-100 (2, 3). The final reaction volume is 1.5 mL, and the final erythrocyte dilution 120-fold. The absorbance is read against buffer at 546 nm at room temperature.

The activities of AChE are expressed as micromoles hydrolysed ATCh per minute and per milliliter whole blood, or per milligram haemoglobin. The activities of BChE are expressed per milliliter of plasma.

DISCUSSION

The described experimental conditions were evaluated in a previous study concerning routine measurements of cholinesterase activities in human whole blood and plasma (4). Phosphate buffer was chosen because the effect of temperature on the pH of that buffer is small, and the buffer can therefore be prepared and used over a broad temperature range (10 to 40 °C). ATCh is a suitable substrate for AChE and BChE. For routine purposes it is convenient to use the same substrate concentration (1.0 mM) for both enzymes. At pH=7.4 the rate of spontaneous hydrolysis of 1.0 mM ATCh is slow and corrections for the non-enzymic substrate hydrolysis are required only at 37 °C.

The absorbance of haemolysed unwashed erythrocytes is not stable. The absorbance measured against buffer decreases about 0.006 absorbance units over 10 min at 25 or 37 °C. That decrease is very small and need not be taken into account particularly because it is suggested that activities are measured against suspended haemolysed unwashed erythrocytes as a blank.

DTNB reacts not only with thiocholine, but also with thiol groups in the haemolysed erythrocytes. Under the above experimental conditions the reaction of DTNB with thiol groups in the haemolysed erythrocytes is completed within 10 min, and for that reason it is suggested to add the substrate 10 min after addition of DTNB. The reaction of DTNB with thiol groups in the erythrocytes reduces the DTNB conc. by only about 0.5%.

The suggested procedure is well reproducible. The within-run and between-run imprecision for the activity measurements of AChE in unwashed haemolysed erythrocytes are 2% each, and for the haemoglobin determination 1% each. The corresponding imprecision for plasma BChE activity measurements are 1-3 and 6-7% respectively (this paper and ref. 5).

SUMMARY

For routine assay of human blood cholinesterase activities it is suggested to separate erythrocytes from plasma by centrifugation and measure the acetylcholinesterase activity in unwashed erythrocytes and butyrylcholinesterase activity in plasma. The suggested substrate for both enzyme activity measurements is 1.0 mM acetylthiocholine.

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KEYWORDS

Acetylcholinesterase, butyrylcholinesterase, activity measurement, human plasma, human erythrocytes