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SPECIES VARIATION IN THE SPECIFICITY OF CHOLINESTERASES IN HUMAN AND RAT BLOOD SAMPLES

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Abstract: Acetylthiocholine iodide (ATC) as a common substrate in the combined assay of red blood cell cholinesterase (RBC ChE) and butyrylcholinesterase (BuChE) do not provide the accurate individual enzyme activities. Hence, in the present study the two enzyme activities in the same sample were assayed with the help of two different substrate, ATC and butyrylthiocholine iodide (BTC). Specificity of BTC towards BuCHE was found in blood, plasma and serum, while ATC is nonspecifically hydrolysed by both RBC ChE and BuChE. ATC gives significantly higher enzyme activity ($P < 0.001$) in rat plasma/serum and significantly lower enzyme activity ($P < 0.0001$; $P < 0.001$) in human plasma/serum. The possible reasons are discussed for substrate specity in various species in the assay of ChEs.

Key words: species variation substrate specificity blood cholinesterases

INTRODUCTION

Simultaneous estimation of RBC ChE and BuChE activites in the same blood sample is desirable. But the use of a common substrate for the assay of the two enzymes may give inaccurate values, as these two enzymes differ in their substrate specificities. It is also of interest to note that their substrate specificities in different species are also different (1). The aim of the study was to interpret the characteristic of the species variation and substrate specificity of ChEs in fresh blood, plasma and serum samples from human and rat using ATC and BTC.

METHODS

Microsamples of blood (45 µl) were collected from orbital sinus in normal Wistar rats and the serum samples were obtained from the same rats after sacrifice with ether. In normal human volunteers microsamples of blood (45 µl) were drawn from finger tips cleaned with an alcohol wipe while serum samples were separately obtained from 2 ml of venous blood. Blood (45 µl) and serum (30 µl) were diluted respectively in 15 ml and 10 ml of buffer containing 5,5'-dithiobis-2- nitrobenzoic acid (2). Diluted blood was divided into 4 equal parts. For total ChE and BuChE determination, two parts of blood samples out of the four were separately treated with ATC and BTC respectively. After an incubation period of 10 min at 30°C, the reaction was arrested by addition of 50 µl of 10^{-3}M eserine and the samples were centrifuged at 4000 rpm for 15 min. The clear supernatants were read in a colorimeter at 415 mµ, using L-cysteine as a standard.

Other two blood sampels were centrifuged at 4000 rpm for 15 min and clear plasma samples were carefully separated from the sediments. Plasma as well as serum samples were individually treated with either ATC or BTC for the assay of enzyme activities. All the samples were incubated and the readings were taken as described earlier. The values were corrected with the respective blanks.

RESULTS

Both in human and rat, BuChE activity with BTC either in blood or plasma was not significantly different.

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However, in humans plasma, enzyme activity with ATC was significantly lower ($P < 0.0001$) compared to plasma enzyme activity with BTC. Serum samples also showed significantly lower enzyme activity ($P < 0.001$) with ATC compared to enzyme activity with BTC. However, in rat plasma or serum enzyme activity with ATC was significantly higher than those obtained with BTC ($P < 0.001$).

Human BuChE was shown to hydrolyse BTC preferably and at a faster rate than ATC (3). BuChE in serum samples of human, dog, cat, horse, and pig showed more preference to BTC compared to ATC (4). Both the ChEs were detected in human amniotic fluid and umbilical cord serum, but no AChE activity was detected in neonatal or adult serum *where the esterase activity was attributed only to BuChE (1). Thus the higher values obtained in our study with BuChE in serum and plasma samples can be explained on this basis.

TABLE I : Comparison of human and rat cholinesterase activities obtained with two different substrates.

Blood sample (n = 10)	*υM thiocholine/ml/min*			
	Acetylthiocholine Iodide (ATC)		*Butyrylthiocholine Iodide (BTC)*	
	Human	*Rat*	*Human*	*Rat*
Whole blood	23.24 ± 0.30	15.61 ± 0.64	18.94 ± 0.58[a]	5.14 ± 0.34[a]
Plasma	14.02 ± 0.38**	8.57 ± 0.54*	18.90 ± 0.43[a]	4.33 ± 0.41[a]
RBC	9.22 ± 0.51[b]	7.04 ± 0.63[b]		
Serum	11.26 ± 0.73**	8.02 ± 0.44*	16.10 ± 1.11	2.21 ± 0.05

[a]Represents the actual BuChE activity

[b]RBC ChE activity = Blood ATC value - Plasma ATC value

*Enzyme activities in rat plasma and serum with ATC were significantly higher ($P < 0.001$) while significantly lower ($P < 0.001$) in human serum.

**Enzyme activity with ATC in human plasma was significantly lower ($P < 0.0001$).

DISCUSSION

Rat plasma or serum BuChE activity exhibited a lower specificity with the substrate, BTC compared to ATC. This was true with the sera of chicken, mouse, hamster etc. where in addition to BuChE, some other hydrolysing enzyme was reported (3). Acetylcholinesterase (AChE) was detected along with BuChE in the plasma of these species and erythrocyte ghost cells were suggested to be the source for AChE which leaked into the plasma (4-7). This may account for the higher values with a non-selective substrate like ATC in the rat plasma/serum samples. AChE activity was found to be the predominant ChE activity in sheep and goat sera, while in teleosts, AChE was found in the place of BuChE (8-12).

As the BuChE activity was found to be almost same in the whole blood or separated plasma samples in our study, BTC can be added directly in the diluted blood samples and other estimation procedure may be carried out as described earlier. This simplifies the procedure where only BuChE activity is being required. For accuracy of RBC ChE activity whole blood and separated plasma should be individually treated with ATC and substraction of plasma enzyme activity from blood enzyme activity (total ChE activity) gives an accurate value of RBC ChE activity. This method will be useful in clinical and research conditions where accuracy in the estimation of both the ChE activities is required.

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