EFFECT OF STEREOCHEMISTRY ON ESTER HYDROLYSIS BY CHOLINESTERASES: IMPLICATIONS FOR RADIOTRACER DESIGN

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Keywords: acetylcholinesterase, butyrylcholinesterase, carbon-11

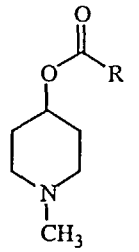
There is currently great interest in developing radiotracers to estimate cortical acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) enzymatic activity in Alzheimer's disease patients using *in vivo* imaging. As part of an ongoing effort to define structure-activity relationships (SAR) for cholinesterase substrates, we have recently published *in vitro* and *in vivo* kinetic results for a series of 1-[^{11}C]methyl-4-piperidinyl esters (1). These data exhibited the expected preference of AChE for short-chain esters (acetate and propionate) and the specificity of longer-chain esters (butyrate and pentanoate) for BuChE. We have also shown that this general pattern of selectivity extends to the 1-methyl-3-pyrrolidinyl esters, **1a,b** (2). As these latter compounds are chiral, it was of interest to extend the SAR to include the effects of stereochemistry on enzyme selectivity and cleavage rates.

Irie *et al.* previously reported that the AChE-mediated cleavage rates of both the acetate and propionate esters of (R)-1-methyl-3-piperidinol were much more rapid than those of the corresponding (S)-enantiomers (3). Beckett *et al.* have reported a similar relationship for the stereoisomers of α- and β-methylcholine esters (4,5). In our initial study, racemic mixtures of **1a** and **1b** exhibited *in vitro* AChE-mediated cleavage rates similar to the corresponding 4-piperidinyl esters, but the individual enantiomers were not tested (2).

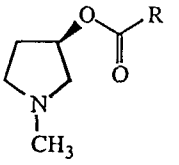
To more clearly define the structure-activity relationships for AChE and BuChE with respect to stereochemistry α to the ester functionality, we have prepared enantiomerically pure (R)-1-methyl-3-pyrrolidinyl propionate, **1a** and butyrate, **1b**, using the methods previously described for 1-methyl-4-piperidinyl propionate, PMP (6). Relative rates of substrate cleavage by purified enzymes (AChE or BuChE) were determined using a simple *in vitro* spectrophotometric assay (1). Esters **1a** and **1b** were also labeled with carbon-11, using methods similar to those reported for [^{11}C]PMP (6), and the *in vivo* biodistribution for each in CD-1 mice was determined.

Based on the work of Beckett *et al.* (4,5), it would be expected that a chiral center β to the ester functionality would also influence enzyme-mediated cleavage rates. To address this question we have also prepared

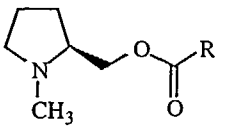
chiral esters of (S)-1-methyl-2-pyrrolidinemethanol, **2a-c**, and (±)-1-methyl-2-piperidinemethanol, **3a-c**. *In vitro* and *in vivo* investigations similar to those described above are currently underway.



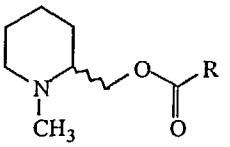
AMP, MP4A R = CH_3
PMP R = CH_2CH_3
nBMP R = $CH_2CH_2CH_3$



1a R = CH_2CH_3
1b R = $CH_2CH_2CH_3$



2a R = CH_3
2b R = CH_2CH_3
2c R = $CH_2CH_2CH_3$



3a R = CH_3
3b R = CH_2CH_3
3c R = $CH_2CH_2CH_3$

The preliminary *in vitro* spectrophotometric data for these investigations is given in Table 1. (R)-1-methyl-3-pyrrolidinyl propionate, (R)**1a**, is cleaved by AChE nearly three times faster than both racemic (±)**1a** and PMP (Table 1), implying that the (S)-enantiomer of **1a** may act as a weak AChE inhibitor. A much less dramatic stereochemical preference was observed for the BuChE-mediated cleavage of **1a**. This is also consistent with the *in vivo* distribution in mouse brain shown in Table 2. The retention fraction for (R)**1a** was higher than that for both PMP and (±)**1a** in all brain areas except striatum. Similarly, the retention fractions for (R)**1b** were higher than those of nBMP in all brain areas.

The racemic primary ester **3b** is also cleaved by AChE more rapidly than is PMP. This preference for the primary ester likely arises from the shorter amine-to-carbonyl distance in **3b**, which better approximates that of acetylcholine. In contrast, (S)-1-methyl-2-pyrrolidinemethyl acetate, **2a**, is cleaved by AChE at a rate only 1.5 times faster than PMP and about one-third the rate of 1-methyl-4-piperidinyl acetate (AMP, MP4A). These results indicate that AChE has a preference for the (R)-configuration at both the α- and β-positions whereas BuChE has little or no stereochemical preference.

Table 1. Relative *in vitro* ChE-mediated cleavage rates. Rates are determined as a change in absorbance (420 nm) of m-nitrophenol per minute per unit of enzyme using the indicated substrates and purified AChE or BuChE. Data are expressed relative to PMP=100 for AChE and nBMP=100 for BuChE.

Substrate	AChE (rate relative to PMP)	BuChE (rate relative to nBMP)
AMP,MP4A	100±0.5	33±1
PMP	20±0.3	115±2
(±)**1a**	17.2±0.3	76±13
(R)**1a**	52±1	115±8
(S)**2a**	32±1.5	n.d.
(±)**3b**	81±1.5	n.d.
*n*BMP	0.00	100±3
(±)**1b**	0.00	144±22

Table 2. Retention fractions calculated from *in vivo* regional distribution in mouse brain at 30 min. post-injection relative to initial uptake at 1 min. post-injection. Values for PMP and(*R*)**1a** are an average of 3 experiments (n = 12 total animals per time point), *n*BMP and **1b** are an average of 2 experiments (n = 8) and (±)**1a** values are from a single study (n = 4).

Substrate	striatum	cortex	cerebellum	hippocampus	thalamus
PMP	0.71±0.05	0.41±0.03	0.26±0.01	0.41±0.03	0.36±0.10
(±)**1a**	0.54±0.07	0.40±0.05	0.31±0.05	0.41±0.05	0.39±0.03
(*R*)**1a**	0.55±0.10	0.51±0.09	0.38±0.08	0.53±0.08	0.44±0.09
*n*BMP	0.11±0.03	0.08±0.02	0.10±0.02	0.10±0.02	0.12±0.04
(*R*)**1b**	0.20±0.04	0.15±0.02	0.18±0.03	0.18±0.03	0.23±0.03

References

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Acknowledgements. The authors wish to acknowledge the financial support of NIH grant T32-CA09015 (to X.S.) and DOE grant DE-FG02-87ER60561. Special thanks to Phil Sherman, Kyle Kuszpit and Leslie Doherty for assistance in the animal experiments.