Journal of Labelled Compounds and Radiopharmaceuticals
J. Labelled Cpd. Radiopharm. **44**, 31-41 (2001)

Synthesis of Piperidinyl and Pyrrolidinyl Butyrates for Potential *In Vivo* Measurement of Cerebral Butyrylcholinesterase Activity

Tatsuya Kikuchi[1,3], Kiyoshi Fukushi[1], Nobuo Ikota[1], Takao Ueda[2], Shin-ichiro Nagatsuka[1,2], Yasushi Arano[3] and Toshiaki Irie[1*]

[1] Advanced Technology for Medical Imaging, National Institute of Radiological Sciences, Chiba
[2]Laboratories, Daiichi Pure Chemicals Co Ltd, Ibaraki
[3]Department of Radiopharmaceutical Chemistry, Chiba University, Chiba
Japan

SUMMARY

Biochemical changes in postmortem brains of Alzheimer's disease patients include decreased acetylcholinesterase and choline acetyl transferase activity, indicating reduced activity of the central cholinergic system, while butyrylcholinesterase (BChE) activity increases. A method that can measure regional BChE activity in the brain *in vivo* may be useful for investigating the relationship between BChE and Alzheimer's disease. Seven compounds, either piperidinyl or pyrrolidinyl butyrates, were synthesized as BChE substrate radiotracers to map central BChE activity *in vivo* by positron emission tomography (PET). ^{14}C-labeled compounds were assayed to determine their hydrolysis rates by BChE and the partition coefficient. The five esters of secondary alcohols had lipophilic properties sufficient to pass readily through the blood-brain barrier while the metabolites were sufficiently hydrophilic to be retained in the brain. The esters showed moderate hydrolysis rates by BChE and high specificity for BChE relative to acetylcholinesterase, while two esters of primary alcohols were hydrolyzed too rapidly to estimate reliably the local cerebral BChE activity. From these results, we conclude that one or more of these five esters, when labeled with ^{11}C, would be a useful tracer for quantification of BChE activity by PET.

Key Words: Butyrylcholinesterase, positron emission tomography, Alzheimer's disease, ^{14}C labeling, piperidinyl butyrates, pyrrolidinyl butyrates

INTRODUCTION

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) both hydrolyze acetylcholine, while BChE also hydrolyzes benzoylcholine and butyrylcholine (1).

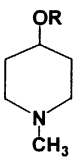
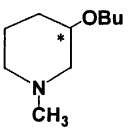
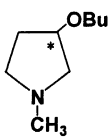
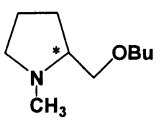
*Author for Correspondence: Toshiaki Irie, Ph.D., Advanced Technology for Medical Imaging, National Institute of Radiological Sciences 9-1, Anagawa 4-chome, Inage-ku, Chiba-shi, Chiba 263-8555, Japan, Tel: +81-43-206-3191, Fax: 81-43-251-7147, e-mail: t_irie@nirs.go.jp

Received 14 August 2000
Revised 18 September 2000
Accepted 25 September 2000

However the physiological role of BChE has not been established. In Alzheimer's disease (AD), central BChE activity increases with disease progression, while AChE activity decreases as determined by postmortem studies (2- 4). Although human AChE activity in the brain has been mapped *in vivo* by positron emission tomography (PET) using ^{11}C-labeled acetylcholine analogs (MP4A, PMP) (5) and these compounds have been used for diagnosis of AD (6, 7), similar studies of BChE activity have not yet been performed.

The aim of this study is to develop a tracer that can measure BChE activity of the brain *in vivo* by PET. Seven compounds, either piperidinyl or pyrrolidinyl butyrates, were designed as radiotracers with a principle of irreversible metabolic trapping type like PMP or MP4A, in other words they should have high specificity for BChE and the proper lipophilicity to cross the blood-brain barrier (BBB), and their hydrophilic metabolites should be trapped in the brain at the site of the hydrolytic enzyme. The synthesized esters are given below (Table 1). ^{14}C-labeled compounds were assayed to determine the partition coefficient with 1-octanol/ phosphate buffer, their hydrolysis rates by BChE and their specificity for BChE using purified human AChE and BChE.

Table 1. Structure of Designed Compounds

	R=Ac	MP4A	*N*-methylpiperidin-4-yl acetate
	R=Pr	PMP or MP4P	*N*-methylpiperidin-4-yl propionate
	R=Bu	MP4B	*N*-methylpiperidin-4-yl butyrate
		MP3B_R and MP3B_S	(*R*)-*N*-methylpiperidin-3-yl butyrate and (*S*)-*N*-methylpiperidin-3-yl butyrate
		5R3B_R and 5R3B_S	(*R*)-*N*-methylpyrrolidin-3-yl butyrate and (*S*)-*N*-methylpyrrolidin-3-yl butyrate
		5R2B_R and 5R2B_S	(*R*)-*N*-methylpyrrolidine-2-methyl butyrate and (*S*)-*N*-methylpyrrolidine-2-methyl butyrate

*; asymmetric carbon atom Ac; $COCH_3$ Pr; $COCH_2CH_3$ Bu; $COCH_2CH_2CH_3$

RESULTS AND DISCUSSION

Design and Chemistry

The compounds were designed with piperidinyl and pyrrolidinyl structures containing a tertiary-amine structure and a butyryl ester with different classes (primary and secondary) and positions, to have sufficient lipophilicity for high brain extraction, specificity for BChE and hydrolysis rate variations (1). The reaction steps are depicted in Fig. 1-3, including preparations of optical isomers with enzymatic (8, 9) and chemical (10, 11) methods before radiolabeling, and each authentic sample could be synthesized by direct butyrylation of the N-methyl alcohols. However, primary alcohol esters (5R2B_R, 5R2B_S and their demethyl precursors) were unstable because migration of the butyryl group to the N atom occurred in their unprotonated form (5). Furthermore, since they had too rapid a hydrolysis rate at pre-assay when using horse BChE, further studies were not performed with the two tracers. The esters were radiolabeled rapidly by a one-step reaction with [^{14}C]methyl iodide in acetone and with high yields and purities. Thus, it appears that these tracers are well suited for labeling with ^{11}C for PET studies, after slight modification (use of DMF instead of acetone and higher reaction temperature).

Table 2. Partition Coefficients (P) and Hydrolysis Rates (K)

	P*	K_{BChE}**	K_{AChE}**	(K_{BChE}/K_{AChE})
MP4B	1.6	0.33	0.0009	378
MP3B_R	5.9	2.97	0.0059	501
MP3B_S	6.2	0.17	0.0006	280
5R3B_R	1.3	0.84	0.0020	424
5R3B_S	1.1	0.65	0.0004	1750
MP4OH	0.006	-	-	-
MP3OH	0.015	-	-	-
5R3OH	0.005	-	-	-

MP4OH, MP3OH and 5R3OH are the hydrolyzed metabolites of MP4B (or MP4A), MP3B and 5R3B, respectively.

*Partition coefficients (P) in a mixture of 1-octanol / phosphate buffer (0.1M, pH7.4).

**K_{BChE} and K_{AChE} are the first-order rate constant for hydrolysis by corresponding enzymes (Human BChE or AChE).

The partition coefficient of each synthesized ester was higher than that of MP4A (P; 0.5) and the coefficients of each metabolite were lower and similar (Table 2). From our previous experience with [^{11}C]MP4A in human PET study (high brain extraction of [^{11}C]MP4A and good retention of its metabolite [^{11}C]MP4OH in the brain (6)), it is expected that each tracer will be able to pass through BBB easily and its metabolite will be retained in the brain.

Enzymatic Properties

The hydrolysis rates and specificity measurements (ratio of K_{BChE}/K_{AChE}) are shown in Table 2. Each ester exhibited high specificity for BChE. The distinct position of the ester and its fundamental structure (piperidinyl or pyrrolidinyl) resulted in a wide range of enzymatic reactivity (nearly 20-fold). For example, optical isomers MP3B_R and MP3B_S, piperidinyl esters, showed a greater difference in hydrolysis rates than the pyrrolidinyl esters, 5R3B_R and 5R3B_S. This result could be explained by their stereo structures. The pyrrolidinyl esters are flatter, so the isomers may have less three-dimensional difference.

The hydrolysis rates of five butyryl esters in human cortex were estimated from measured concentrations of BChE (about 0.1 Unit/tissue g) and AChE (0.6 Unit/tissue g), ignoring other effects such as hydrolysis by other enzymes; the values obtained show a moderate range of hydrolysis rates (Table 3). The values were comparable with those for MP4A (0.15 /min/g/mL) and PMP (0.04 /min/g/mL) that were successfully applied to human PET studies. Tracers with too high hydrolysis rates *in vivo* will not accurately reflect BChE activity. Furthermore, BChE activity is increased in AD in contrast to reduced AChE activity. The hydrolysis rates with real tissue data will differ less than expected. Actually in our study of MP4A, the calculated hydrolysis rate (0.15 /min/g/mL), using the reactivity with purified human AChE (0.25 /min/Unit/mL) and

Table 3. Estimated Hydrolysis Rates of Esters in Human Cortex

	MP4B	MP3B_R	MP3B_S	5R3B_R	5R3B_S
Hydrolysis Rate (/min/g/mL)	0.033	0.30	0.017	0.084	0.065

The values were calculated as: K_{BChE} [BChE] + K_{AChE} [AChE], where [BChE] and [AChE] are the concentrations (Unit/g) of corresponding enzymes in human cerebral cortex.

the AChE activity in human cortex (0.61 Unit/g), was near the experimentally measured value (0.16 /min/g/mL).

We conclude that the results indicate the feasibility of measuring central BChE activity *in vivo* by PET using one or more of these tracers.

EXPERIMENTAL

Synthesis

[^{14}C]Methyl iodide (2.04 GBq/mmol) was obtained from Amersham International Ltd. All starting materials for chemical synthesis were purchased from Aldrich Chemical Co. Other chemicals were of the highest grade available commercially. Authentic samples were analysed by ^{1}H and ^{13}C nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectroscopy using JNM FX-100 (100 MHz) and JNM AL-300 (300 MHz) spectrometers and also by elemental analysis. Optical rotations were measured on a JASCO DIP-360 polarimater.

(R)-N-Methylpyrrolidin-3-yl butyrate (5R3B_R)

(*R*)-*N-tert*-Butoxycarbonyl-3-hydroxypyrrolidine (1.0 g, 5.3 mmol) was dissolved in 10 mL methylene chloride and pyridine (790 mg, 10 mmol) was added. To this solution, butyryl chloride (850 mg, 8.0 mmol) was added and the reaction mixture was kept at room temperature for 14 hr. After adding 100 mL ethyl acetate, the organic layer of the mixture was washed with water, 5 %-HCl, water, saturated $NaHCO_3$ and finally water. The organic solution was then dried over anhydrous $MgSO_4$. The residue that was obtained by evaporating the solvent was purified by column chromatography (silica gel C-200, hexane : ethyl acetate = 4 : 1), yielding (*R*)-*N-tert*-butoxycarbonylpyrrolidin-3-yl butyrate as an oil (1.31 g, 83 %) (first step in Fig. 1).

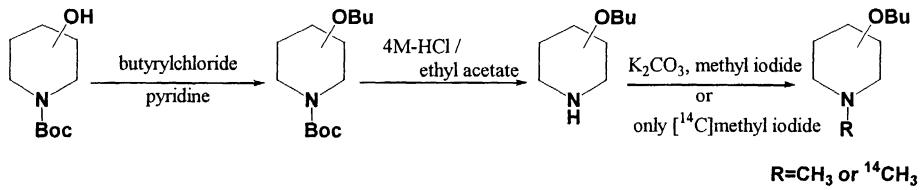


Figure 1. General synthesis of piperidyl esters. Pyrrolidyl esters were also synthesized in the same manner.

(*R*)-*N*-*tert*-Butoxycarbonylpyrrolidin-3-yl butyrate (500 mg, 1.95 mmol) was dissolved in 5 mL ethyl acetate, and 5mL 4 M-HCl/ethyl acetate was added under ice-cold conditions. After the solution was allowed to stand for 1 hr at room temperature, ethyl acetate was evaporated under vacuum below room temperature, yielding (*R*)-pyrrolidin-3-yl butyrate hydrochloride as an oil (380 mg). This compound was dissolved in 5 mL chloroform, and 5 mL saturated NH_3-chloroform solution was added under ice-cold conditions. After the mixture was reacted for 5 min at room temperature, the solution was filtered to remove the insoluble solid and the solvent was then evaporated under diminished pressure, yielding (*R*)-pyrrolidin-3-yl butyrate ($[\alpha]_D^{20}$ +11.5° (*c*=1, $CHCl_3$)) (second step in Fig. 1).

(*R*)-Pyrrolidin-3-yl butyrate (300 mg, 1.91 mmol) was dissolved in 5 mL acetone, and 200 mg K_2CO_3 and 0.24 mL methyl iodide were added. The solution was allowed to stand for 6 hr at room temperature. The residue, that was obtained by filtering to remove the insoluble solid and evaporated under diminished pressure to remove the solvent, was then purified by column chromatography (silica gel C-200, EtOH : ethyl acetate = 1 : 10) to yield (*R*)-*N*-methylpyrrolidin-3-yl butyrate (140 mg, 43 %) (final step in Fig. 1).

5R3B_R ; $[\alpha]_D^{20}$ +1.5° (*c*=2, MeOH). ^{1}H-NMR(δ , $CDCl_3$): 0.89(3H, *t*, CH_3), 1.57(2H, *m*, CH_2), 1.78(1H, *m*, CH), 2.26(4H, *m*, $2\times CH_2$), 2.31(3H, *s*, N- CH_3), 2.63(2H, *m*, CH_2), 2.77(1H, *m*, CH), 5.13(1H, *m*, CH). ^{13}C-NMR($CDCl_3$): 13.51(*q*), 18.28(*t*), 32.45(*t*), 36.14(*t*), 41.83(*q*), 54.74(*t*), 61.98(*t*), 74.15(*d*), 173.44(*s*). Anal calc for ($C_9H_{17}NO_2$): C 63.12, H 10.00, N 8.18. Found: C 62.99, H 10.26, N 7.95. IR(neat) cm^{-1} 1734.

(S)-N-Methylpyrrolidin-3-yl butyrate (5R3B_S)

(*R*)-*N*-*tert*-Butoxycarbonyl-3-hydroxypyrrolidine (1) (500 mg, 2.7 mmol, $[\alpha]_D^{20}$ –22.7° (*c*=1, $CHCl_3$)) was dissolved in 10 mL THF, and benzoic acid (685 mg, 5.6 mmol), triphenylphosphine (1.47 mg, 5.6 mmol) and azodicarboxylate (975 mg, 5.6 mmol) were added and stirred for 1 hr at room temperature. After adding 100 mL ethyl acetate, the reaction mixture was washed with water, saturated $NaHCO_3$ and water. The

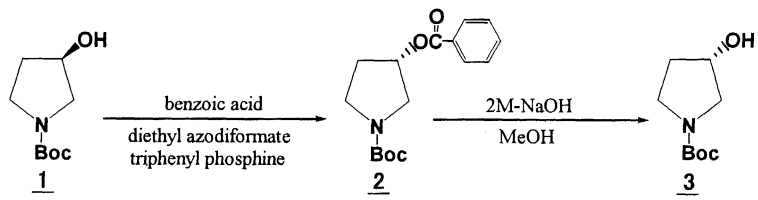


Figure 2. Preparation of (*S*)-*N*-*tert*-butoxycarbonyl-3-hydroxypyrrolidine with Mitsunobu reaction

organic layer was then dried over anhydrous $MgSO_4$ and the residue that was obtained by evaporating the solvent was purified by column chromatography (silica gel C-200, hexane : ethyl acetate = 4 : 1) to yield (*R*)-*N*-*tert*-butoxycarbonylpyrrolidin-3-yl benzoate (**2**) as crystals (640 mg, 82 % $[\alpha]_D^{20}$ +39.5° (*c*=1.1, $CHCl_3$), mp. 65℃). The benzoate (500 mg, 1.7 mmol) was dissolved in 5 mL MeOH and 2 mL 2 M-NaOH was added and reacted for 1 hr at room temperature. Then 100 mL ethyl acetate was added to the reaction mixture and washed with water. The organic layer was dried over anhydrous $MgSO_4$ and the residue that was obtained by evaporating the solvent was purified by column chromatography (silica gel C-200, hexane : ethyl acetate = 1 : 1), yielding (*S*)-*N*-*tert*-butoxycarbonyl-3-hydroxypyrrolidine (**3**) as an oil (290 mg, 90 %, $[\alpha]_D^{20}$ +22.9° (*c*=1, $CHCl_3$)). (*S*)-*N*-methylpyrrolidin-3-yl butyrate (5R3B_S) was synthesized, in the same manner as described for the preparation of 5R3B_R (Fig. 1).

(R)- and (S)-N-methylpyrrolidine-2-methyl butyrate (5R2B_R and 5R2B_S)

(*R*)-*N*-Methylpyrrolidine-2-methyl butyrate (5R2B_R) and (*S*)-*N*-methylpyrrolidine-2-methyl butyrate (5R2B_S) were synthesized in the same manner as described above for the preparation of 5R3B_R (Fig. 1) from the corresponding (*R*)- or (*S*)-*N*-*tert*-butoxycarbonyl-2-hydroxypyrrolidine.

5R2B_S; $[\alpha]_D^{20}$ –32.4° (*c*=1.1, $CHCl_3$). ^{1}H-NMR(δ , $CDCl_3$): 0.89(3H, *t*, CH_3), 1.25-1.80(5H, *m*, CH, 2×CH_2), 1.80-1.95(1H, *m*, CH), 2.20-2.40(4H, *m*, 2×CH_2), 2.37(3H, *s*, N- CH_3), 3.00(1H, *m*, CH), 4.04(2H, *m*, CH_2). ^{13}C-NMR($CDCl_3$): 13.56(*q*), 18.32(*t*), 22.70(*t*), 28.24(*t*), 36.07(*t*), 41.36(*q*), 57.56(*d*), 63.74(*t*), 66.34(*t*), 173.60(*s*). Anal calc for ($C_{10}H_{19}NO_2$): C 63.12, H 10.00, N 8.18. Found: C 62.99, H 10.26, N 7.95. IR(neat)

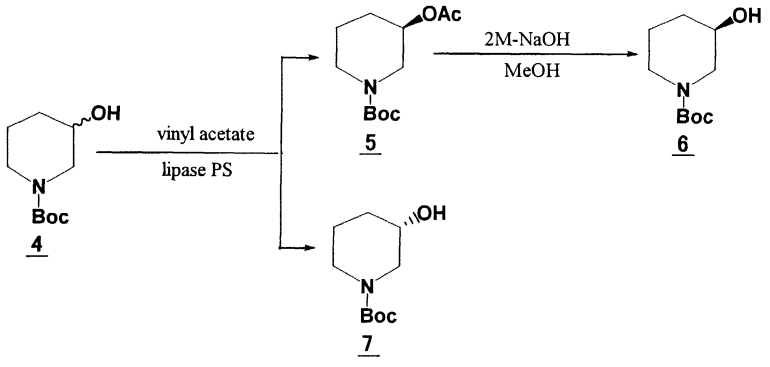


Figure 3. Preparation of (*R*)- and (*S*)-*N*-*tert*-butoxycarbonyl-3-hydroxypiperidine in an enzymatic reaction

cm^{-1} 1734.

(R)- and (S)-N-methylpiperidin-3-yl butyrates (MP3B_R and MP3B_S)

The racemic *N*-*tert*-butoxycarbonyl-3-hydroxypiperidine (**4**) (4.0 g, 20 mmol) was stirred in 100 mL pentane for 4 days at room temperature with 15 mL vinyl acetate and 2 g lipase PS (pseudomonas sp.; Amano pharmaceutical Co., Ltd. Japan). The residue, that was obtained by filtering to remove the insoluble solid and evaporated under diminished pressure to remove the solvent, was then purified by column chromatography (silica gel C-200, hexane : ethyl acetate = 1 : 3) to yield (*R*)-*N*-*tert*-butoxycarbonylpiperidin-3-yl acetate (**5**) (1.05 g, 22 %, $[\alpha]_D^{20}$ +9.9° (*c*=1.1, $CHCl_3$)) as an oil.

Additionally, about 3 g of recovered hydroxyl compounds enriched with non-reacted **7** was treated twice with lipase PS in the same manner as described above and purified by column chromatography to afford optically pure (*S*)-*N*-*tert*-butoxycarbonyl-3-hydroxypiperidine (**7**) as a crystals (0.9 g, 23 %, $[\alpha]_D^{20}$ +9.9° (*c*=1, $CHCl_3$), mp. 40-42℃) (Fig. 3).

The compound **5** (0.5 g, 2.1 mmol) was dissolved in 5 mL MeOH and 2.5 mL 2 M-NaOH was added and reacted for 2 hr at room temperature. Then 50 mL ethyl acetate was added and washed with water. The organic layer was dried over anhydrous $MgSO_4$ and the residue that was obtained by evaporating the solvent was purified by

column chromatography (silica gel C-200, hexane : ethyl acetate = 1 : 1), yielding (*R*)-*N*-*tert*-butoxycarbonyl-3-hydroxypiperidine (**6**) as crystals (390 mg, 95 %, $[\alpha]_D^{20}$ –9.6° (*c*=1, $CHCl_3$), mp. 40-42 ℃) (Fig. 3).

(*R*)- and (*S*)-*N*-methylpiperidin-3-yl butyrate (MP3B_R and MP3B_S) were synthesized in the same manner as described above (Fig. 1) from the corresponding (*R*)- or (*S*)-*N*-*tert*-butoxycarbonyl-3-hydroxypiperidine.

MP3B_R; $[\alpha]_D^{20}$ +15.5° (*c*=2, $CHCl_3$). ^{1}H-NMR(δ , $CDCl_3$): 0.88(3H, *t*, CH_3), 1.39(1H, *m*, CH), 1.55(3H, *m*, CH_2, CH), 1.65(2H, *m*, CH_2), 2.10-2.30(4H, *m*, 2 × CH_2), 2.19(3H, *s*, N- CH_3), 2.38(1H, *m*, CH), 2.57(1H, *m*, CH), 4.82(1H, *m*, CH). ^{13}C-NMR($CDCl_3$): 13.49(*q*), 18.38(*t*), 22.32(*t*), 28.59(*t*), 36.24(*t*), 46.19(*q*), 55.16(*t*), 58.98(t), 68.88(d), 173.02(s). Anal calc for ($C_{10}H_{19}NO_2$): C 64.83, H 10.34, N 7.56. Found: C 64.68, H 10.54, N 7.33.

N- Methylpiperidin-4-yl butyrate (MP4B)

N-Methylpiperidin-4-yl butyrate (MP4B) was synthesized from *N*-*tert*-butoxycarbonyl-4-hydroxypiperidine, in the same manner as described above for the preparation of 5R3B_R.

^{1}H-NMR(δ , $CDCl_3$): 0.90(3H, *t*, CH_3), 1.60-1.70(4H, *m*, 2 × CH_2), 1.87(2H, *m*, CH_2), 2.17-2.34(7H, *m*, N-CH_3, 2 × CH_2), 2.64(2H, *m*, CH_2), 4.76(1H, *m*, CH). ^{13}C-NMR($CDCl_3$): 13.56(*q*), 18.46(*t*), 30.34(*t*), 36.44(*t*), 45.59(*q*), 52.38(*t*), 68.85(*d*), 173.03(*s*). Anal calc for ($C_{10}H_{19}NO_2$): C 64.83, H 10.34, N 7.56. Found: C 65.02, H 10.22, N 7.78.

N-[^{14}C]Methylpiperidinyl and N-[^{14}C]methylpyrrolidinyl butyrates

A mixture of precursor ester (demethyl piperidinyl or pyrrolidinyl ester, 100 μ mol) and [^{14}C]methyl iodide (10 MBq) was heated in 2 mL acetone at 50 ℃ in a water bath for 20 min. An ethyl acetate solution containing anhydrous HCl (in excess of ester) was added to the reaction solution and the mixture was dried under N_2. The residue was dissolved in 200 μ L MeOH and was subjected to thin layer chromatography with a silica-gel plate and a mixture of ethyl acetate : iso-propanol : ammonia (15:5:1; vols.) as a developing solvent. The radioactive zone with Rf-value corresponding to the

authentic compound was collected and extracted with a 1:2 mixture of MeOH and chloroform. Radiochemical yields based on [^{14}C]methyl iodide were more than 80 % and purities were more than 99 % (final step in Fig. 1).

Hydrolysis Rate and Specificity

Purified BChE (horse serum and human serum) and AChE (human erythrocytes) were obtained from Sigma Chemical Co. BChE and AChE activities were measured with Ellman's method (12). In our study, 1 Unit of enzyme hydrolyzes 1.0 μ mol of acetylthiocholine iodide (AChE substrate) or butyrylthiocholine iodide (BChE substrate) per min at pH 7.4 at 21 ℃.

MP4B, 5R3B_R, 5R2B_R and 5R2B_S were pre-assayed with horse BChE, and because of too high a hydrolysis rate for 5R2B_R and 5R2B_S (70-fold higher than other two tracers), these compounds were not assayed as described below.

The human BChE was added to 1 %-triton X-100 phosphate buffer (0.1 M, pH 7.4), then the solution (100 μ L) was placed in tubes and pre-incubated at 37 ℃ for 30 min. Each ^{14}C labeled ester solution (15 kBq in 20 μ L buffer) was added to each tube to initiate the reaction. At designated intervals, 200 μ L EtOH was added to each tube to stop the reaction. 5 μ L of the solution was then applied to a silica-gel TLC plate and developed with a mixture of ethyl acetate : iso-propanol : ammonia (15:5:1;vols). The air-dried TLC plate was covered with a 5 μ m thick foil and placed in a cassette in contact with the imaging phosphor plate for 1 hr. The radioactivities corresponding to the Rf value of the unchanged esters and metabolites were quantitated using a bioimaging analyzer (BAS 2000 system, Fuji Photo Film Co.). The enzymatic hydrolysis rate (K_{BChE}) of each tracer was calculated as: $K_{BChE} = -Ln(A_2 / A_1) / (T_2 - T_1) / C$, where A_1 and A_2 represent the ^{14}C radioactivity of the unchanged ester remaining at times T_1 and T_2, respectively and C represents the enzyme concentration (Unit/mL) in the reaction solution. The hydrolysis rates by AChE (K_{AChE}) were measured in the same manner. Specificity between the enzymes was calculated as follows: $S = K_{BChE} / K_{AChE}$. Non-enzymatic hydrolysis rates of all five esters were very slow and therefore were neglected.

Partition Coefficient

The required ^{14}C labeled esters (five esters, MP4A (5) and their metabolites) were added to a 1:1 mixture of 1-octanol and 0.1 M phosphate buffer (pH 7.4), shaken vigorously and allowed to equilibrate for 1hr at room temperature. The ^{14}C concentrations in the organic and aqueous phases were measured with a liquid scintillation counter, and each partition coefficient was calculated as the ratio of the concentration in the organic phase to that in the aqueous phase.

ACKNOWLEDGMENTS

The authors are grateful to Prof. R. A. Koeppe, University of Michigan, School of Medicine, MI, USA for helpful discussions and for assistance in preparing the manuscript.

REFERENCES

1. Whittaker V.P. *Physiol. Rev.* **31:** 312-343 (1951)

2. Perry E. K., Perry R. H., Garry B. and Bernard E. T. *Lancet* **i:** 189 (1977)

3. Perry E. K., Bernard E. T., Garry B., Klaus B., Peter H. G. and Perry R. H. *Br. Med. J.* **25:** 1457-1459 (1978)

4. Davis P. and Maloney A. J. F. *Lancet* **ii:** 1403 (1976)

5. Irie T., Fukushi K., Akimoto M., Tamagami H. and Nozaki T. *Nucl. Med. Biol.* **21:** 801-808 (1994)

6. Iyo M., Namba H., Fukushi K., Shinotoh H., Nagatsuka S., Suhara T., Sudo Y., Suzuki K. and Irie T. *Lancet* **309:** 1805-1809 (1997)

7. Kuhl D. E., Koeppe R. A., Minoshima S., Sunyder S. E., Ficaro E. P., Foster N. L., Frey K. A. and Kilbourn M. R. *Neurology* **52:** 691-699 (1999)

8. Wong C. -H. *Science* **244:** 1145-1152 (1989)

9. Chen C. -S. and Sih C. J. *Angew. Chem. Int. Ed. Engl.* **28:** 695-707 (1989)

10. Grynkiewicz G. and Burzynska H. *Tetrahedron* **32:** 2109-2111 (1976)

11. Kurihara T., Nakajima Y. and Mitsunobu O. *Tetrahedron Letters* **28:** 2455-2458 (1976)

12. Ellman G. L., Courtey K. D., Andres V. Jr. and Featherstone R. M. *Biochem. Pharmac.* **7:** 88-95 (1961)

