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N-[^{18}F]fluoroethylpiperidin-4-ylmethyl butyrate: a novel radiotracer for quantifying brain butyrylcholinesterase activity by positron emission tomography

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Abstract—In Alzheimer's disease, cerebral cortical butyrylcholinesterase (BChE) activity is reported to be elevated. Our aim was to develop a novel ^{18}F-labeled tracer for quantifying cerebral BChE activity by positron emission tomography. With in vitro screening of *N*-[^{14}C]ethylpiperidin-3- and 4-ylmethyl esters, *N*-[^{14}C]ethylpiperidin-4-ylmethyl butyrate was selected as a lead for ^{18}F-labeling, affording *N*-[^{18}F]fluoroethylpiperidin-4-ylmethyl butyrate. The ^{18}F-labeled butyrate showed the required properties for in vivo BChE measurement, that is, the lipophilic nature of the authentic ester, high specificity to BChE, a moderate hydrolysis rate, and the hydrophilic nature of the metabolite.

There are two major forms of cholinesterases, acetylcholinesterase (AChE, EC 3.1.1.7) and butyrylcholinesterase (BChE, EC 3.1.1.8), in the mammalian brain. BChE is found in neurons and glial cells as well as in neuritic plaques and tangles in Alzheimers's disease (AD).[1] In AD, while AChE activity decreases progressively, BChE activity is higher compared to age-matched controls.[2] Two lipophilic acetylcholine analogues, *N*-[^{11}C]methylpiperidin-4-yl acetate ([^{11}C]MP4A: **1**) and propionate ([^{11}C]MP4P: **2**), have been developed[3] and clinically used to quantify regional cerebral AChE activity in AD patients[4,5] using positron emission tomography (PET). Based on the same principle as **1** and **2** (the lipophilic substrate analogue method), ^{11}C-labeled butyrylcholine analogues such as *N*-[^{11}C]methylpiperidin-4-yl butyrate ([^{11}C]MP4B: **3**) have been developed as potential tracers for the measurement of cerebral BChE activity by PET.[6,7]

In the lipophilic substrate analogue method, the ideal tracer must satisfy the following conditions: First, the unchanged tracer must be lipophilic enough to cross the blood–brain barrier. Second, the tracer must have high specificity for the target enzyme. Third, the hydrolysis rate of the tracer in the brain region of interest must be moderate.[8] Finally, the metabolite must be hydrophilic enough to be retained in the brain (metabolic trapping). Among the conditions, optimization of the tracer's metabolic rate is very important, because reliability of enzyme activity estimation is highly dependent on the rate.[8] Based on the computer-simulation study,[8] it is suggested that the metabolic rate of MP4A and MP4P are appropriate for the human cerebral cortex.

The aim of this study was to develop an ^{18}F-labeled tracer with high specificity to and a moderate hydrolysis rate with human cerebral BChE. Because of the longer half-life (^{18}F, 110 min; ^{11}C, 20 min) and shorter positron range (^{18}F, 650 keV; ^{11}C, 960 keV) of ^{18}F compared to ^{11}C, ^{18}F is an attractive label. Introduction of the [^{18}F]fluoromethyl group to the N-atom of piperidine is not possible, due to instability of the fluoromethyl group attached to the secondary amine.[9] Another method is *N*-[^{18}F]fluoroethylation of the piperidine. However, the *N*-fluoroethyl derivatives of **1** and **2** are reported to have low reactivity to BChE.[10] Furthermore, the concentration of BChE activity in the human cerebral cortex is much lower than that of AChE. There-

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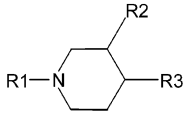
fore, to develop an ^{18}F-labeled tracer with moderate BChE-reactivity, the ester structure needs to be modified so as to have increased reactivity with BChE.

Primary alcohol esters are known to be hydrolyzed by cholinesterases more rapidly than secondary alcohol esters.[11] We have therefore designed a series of primary esters (butyrate and valerate) of *N*-[^{14}C]ethylpiperidinemethanols (Table 1), which are used to identify a structure most suitable for ^{18}F-labeling. The ^{14}C-labeled esters are modified from **3** in three respects: first, conversion from the secondary ester to the primary ester to increase the hydrolysis rate by BChE; second, change of the acyl group from the butyryl (**4**, **5*R***, **5*S***) to the valeryl group (**6**, **7*R***, **7*S***); and third, change of the position of an acyl group on the piperidine ring including optical isomers.

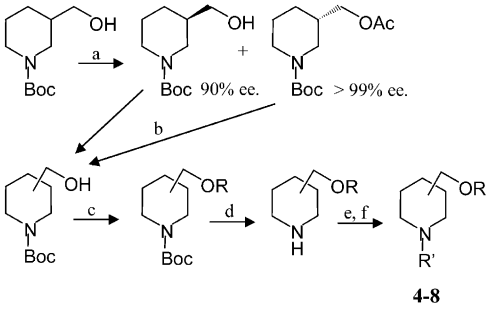
The esters[12] were prepared from corresponding *N*-Boc-piperidinol or piperidinemethanols as starting materials as shown in Scheme 1. The optical separation of the *R* and *S* isomers of *N*-Boc-3-piperidinemethanol was performed by the literature method with lipase PS.[13] By the calculation from literature optical rotations of *N*-Boc-3-piperidinemethanols, the optical purity was 90% ee for *R* isomer and more than 99% ee for *S* isomer. *N*-[^{14}C]ethyl and *N*-[^{18}F]fluoroethyl labeling was performed with [^{14}C]ethyl iodide (55 Ci/mol) and [^{18}F]fluoroethyl bromide (20 Ci/μmol), respectively. Since the radiolabeled compounds display no special absorption on UV spectrum (200–400 nm), the specific activity of them were estimated same specific activity as [^{14}C]ethyl iodide and [^{18}F]fluoroethyl bromide used for radiolabeling. The radiochemical purities of the labeled compounds were more than 98%. Though primary esters at the second position of the piperidine or pyrrolidine ring are known to migrate to the N atom,[6,14] all the primary esters synthesized in this study were quite stable (decomposition rate; <0.0001 min^{-1}) in a phosphate buffer (pH = 7.4, 0.1M).

To estimate the BChE specificity and hydrolysis rate of the designed esters in the human brain, we used purified

Table 1. Structure of compounds



Compd	R1	R2	R3
		Reference compounds	
1 (MP4A)	Me	H	$OCOCH_3$
2 (MP4P)	Me	H	$OCOC_2H_5$
3 (MP4B)	Me	H	$OCOC_3H_7$
		Evaluated compounds	
4	Et	H	$CH_2OCOC_3H_7$
5*R*	Et	(*R*)$CH_2OCOC_3H_7$	H
5*S*	Et	(*S*)$CH_2OCOC_3H_7$	H
6	Et	H	$CH_2OCOC_4H_9$
7*R*	Et	(*R*)$CH_2OCOC_4H_9$	H
7*S*	Et	(*S*)$CH_2OCOC_4H_9$	H
8	CH_2CH_2F	H	$CH_2OCOC_3H_7$



4-8

Scheme 1. Reagents and conditions: (a) lipase PS, vinyl acetate, $CHCl_3$; (b) NaOHaq, MeOH; (c) butyryl chloride or valeryl chloride, pyridine, CH_2Cl_2; (d) 4M-HCl/AcOEt; (e) 30% CH_3CHO, sodium triacetoxy-borohydride, CH_2Cl_2 for **4**–**7S**, and 1-fluoro-2-tosilethane, K_2CO_3, dimethylformamide, 80 °C for **8**; (f) [^{14}C]C_2H_5I, K_2CO_3, acetone, 70 °C or 1-bromo-2-[^{18}F]fluoroethane, DMF, 130 °C. R = butyryl or valeryl, R′ = ethyl of fluoroethyl.

human enzymes, AChE (human erythrocytes) and BChE (human serum).[6] In a pH 7.4 phosphate buffer containing AChE or BChE, the first-order hydrolysis rate constant of ^{14}C or ^{18}F-labeled ester was measured and normalized for unit enzyme concentration, affording k_{AChE} and k_{BChE}, the normalized rate constant (unit; min^{-1}Unit^{-1}mL^{-1}) of the ester for AChE and BChE. Concentrations of AChE and BChE activity in the human cerebral cortex were measured by Ellman's method ([AChE] = 0.6 Unit/g tissue, [BChE] = 0.1 Unit/g tissue). The AChE-rate and BChE-rate of each ester were calculated as: AChE-rate = k_{AChE} × [AChE] and BChE-rate = k_{BChE} × [BChE]. Total-rate was defined as: Total-rate = AChE-rate + BChE-rate. The BChE specificity (%) of each ester was defined by the ratio of BChE-rate to total-rate multiplied by 100 (%).

With BChE (Fig. 1A), the hydrolysis rate of each of the esters synthesized ranged from 0.31 (**4**) to 0.85 min^{-1} g^{-1} mL^{-1} (**5*S***). For the positional effect, the 3yl-esters (butyrate; **5*R*** and **5*S***, valerate; **7*R*** and **7*S***) were hydrolyzed more rapidly than corresponding 4yl-esters (butyrate; **4**, valerate; **6**). With AChE (Fig. 1B), each ester showed a much lower hydrolysis rate compared to with BChE, ranging from 0.0024 (**6**) to 0.017 min^{-1}g^{-1}mL^{-1} (**5*R***). The same positional effect was observed as BChE, i.e., the hydrolysis rate of 3-yl ester was larger than that of 4-yl ester. Each of the butyryl esters (**4**, **5*R***, **5*S***) showed a 2–4 times higher hydrolysis rate than that of the corresponding valeryl esters (**6**, **7*R***, **7*S***). Regarding the chiral effect, R isomers of butyryl and valeryl ester (**5*R***, **7*R***) showed higher hydrolysis compared to the corresponding S isomers (**5*S***, **7*S***). Ignoring other effects such as hydrolysis by other enzymes,[6] each of the esters examined showed more than 97% of BChE-specificity (Fig. 1C). The total-rate of each ester in the human cerebral cortex was almost same as BChE-rate (Fig. 1A), since AChE-rate was negligible. Compound **4** showed an estimated hydrolysis rate of 0.32 min^{-1} g^{-1} mL^{-1}, being the closest to the rate of **1** (0.16 min^{-1} g^{-1} mL^{-1}, Table 2), one of established esters, suggesting that **4** may be most suitable for ^{18}F-labeling.

Based on these results from in vitro screening of *N*-[^{14}C]ethyl labeled esters, *N*-[^{18}F]fluoroethylpiperidin-4-

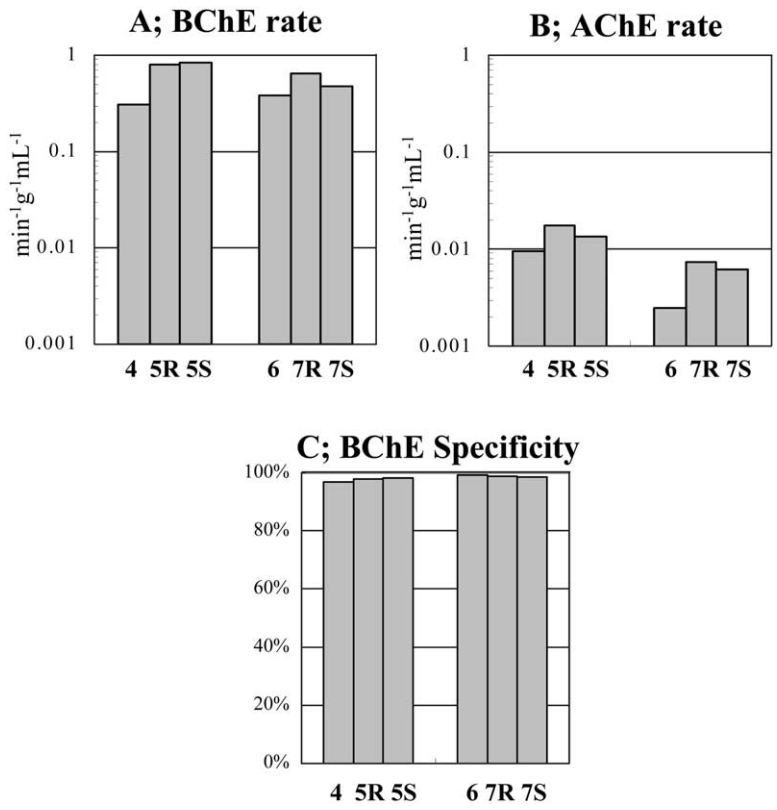


Figure 1. The hydrolysis rates (min^{-1}) of primary esters, butyryl esters (**4**, **5*R*** and **5*S***) and valeryl esters (**6**, **7*R*** and **7*S***) of *N*-[^{14}C]ethyl piperidinemethanols with pure human BChE (panel A; BChE-rate) and AChE (panel B; AChE-rate) at the unit tissue concentration (g/mL) and BChE specificity (%) (panel C) of each ester estimated for the human cerebral cortex.

ylmethyl butyrate (**8**) was finally synthesized and its biochemical and physicochemical properties were compared with those of a [^{11}C]MP4A, [^{11}C]MP4P and [^{11}C]MP4B (Table 2). The total-rate (0.20 min^{-1}) of **8** estimated for the human cerebral cortex was almost the same as that of MP4A (0.16 min^{-1}), suggesting that **8** would have an appropriate hydrolysis rate in the human brain. The BChE-specificity of **8** was estimated as 98%, indicating that **8** satisfies the required conditions for in vivo BChE measurement by PET, so far as biochemical properties are concerned. The partition coefficients (log P) of authentic ester and metabolite of **8** were 0.62 and −1.3, respectively, suggesting that the ester form is sufficiently lipophilic and its metabolite is moderately hydrophilic. Actually in our preliminary study, high initial uptake was observed in rat cerebral cortex after intravenous injection of **8** (1.2% dose/g tissue at 1 min), while its metabolite hardly crossed the blood–brain barrier (<0.1% dose/g tissue at 1–30 min) after intravenous injection.

Table 2. Comparison of *N*-[^{18}F]fluoroethylpiperidine-4-ylmethyl butyrate (**8**) with established PET tracers, [^{11}C]MP4A and [^{11}C]MP4P, and a previously-developed BChE-Tracer, [^{11}C]MP4B

Compd	Total-rate[a]	Specificity to BChE[b] (%)	Log P[c] (ester)	Log P[c] (metabolite)
8	0.200	98	0.62	−1.3
MP4B	0.033	98	0.21	−2.2
MP4P	0.035	—	−0.22	−2.2
MP4A	0.160	—	−0.30	−2.2

[a] The estimated hydrolysis rate in the human cerebral cortex (unit; $min^{-1}g^{-1}mL^{-1}$) calculated in the same manner as in Figure 1.
[b] Calculated by the ratio of BChE-rate to total-rate.
[c] Partition coefficients measured in a mixture of 1-octanol/phosphate buffer (0.1M, pH 7.4).

In conclusion, we have developed a novel [^{18}F]-labeled compound, *N*-[^{18}F]fluoroethylpiperidin-4-ylmethyl butyrate, which satisfies the required conditions for BChE measurement by PET.

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12. Compound **4**: *N*-Ethylpiperidin-4-ylmethyl butyrate; ^{1}H NMR (δ, $CDCl_3$): 0.89–0.95 (6H, *m*, 2×CH_3), 1.10–1.37 (4H, *m*, 2×CH_2), 1.58–1.69 (3H, *m*, 2×CH, CH), 1.81–1.89 (2H, *m*, 2×CH), 2.25 (2H, *t*, CH_2), 2.36 (2H, *q*, CH_2), 2.90–2.94 (2H, *m*, 2×CH), 3.89 (2H, *t*, CH_2). ^{13}C NMR ($CDCl_3$): 12.10, 13.67, 18.46, 28.92, 35.42, 36.19, 52.57, 52.91, 68.60, 173.72. Anal. calc for ($C_{12}H_{23}NO_2$): C 67.57, H 10.87, N 6.57. Found: C 67.55, H 10.86, N 6.55. IR (neat) cm^{-1} 1718.
Compound **5*R*** and **5*S***: (*R*)- and (*S*)-*N*-Ethylpiperidin-4-ylmethyl butyrate; *R* isomer; $[\alpha]_D^{20}$ +7.3° (c = 1.1, $CHCl_3$), *S* isomer; $[\alpha]_D^{20}$ −7.9° (c = 1.1, $CHCl_3$). ^{1}H NMR (δ, $CDCl_3$): 0.89–1.10 (6H, *m*, 2×CH_3) 1.27–1.37 (3H, *m*, CH, CH_2), 1.56–1.71 (4H, *m*, 4×CH), 1.78–1.92 (2H, *m*, 2×CH), 2.25 (2H, *t*, CH_2), 2.36 (2H, *q*, CH_2), 2.76–2.89 (2H, *m*, 2×CH), 3.82–3.99 (2H, *m*, 2×CH). ^{13}C NMR ($CDCl_3$): 11.99, 13.66, 18.45, 24.86, 27.42, 35.92, 36.18, 52.75, 53.67, 56.79, 67.09, 173.66. Anal. calcd for ($C_{12}H_{23}NO_2$): C 67.57, H 10.87, N 6.57. Found: C 67.56, H 10.86, N 6.58. IR (neat) cm^{-1} 1716.
Compound **6**: *N*-Ethylpiperidin-4-ylmethyl valerate; ^{1}H NMR (δ, $CDCl_3$): 0.85–0.94 (6H, *m*, 2×CH_3), 1.25–1.36 (6H, *m*, 3×CH_2), 1.54–1.69 (3H, *m*, 2×CH, CH), 1.81–1.89 (2H, *m*, 2×CH), 2.27 (2H, *t*, CH_2), 2.36 (2H, *q*, CH_2), 2.90–2.94 (2H, *m*, 2×CH), 3.89 (2H, *t*, CH_2). ^{13}C NMR ($CDCl_3$): 12.10, 13.66, 22.24, 27.40, 28.90, 34.02, 35.40, 52.56, 52.90, 68.61, 173.90. Anal. calcd for ($C_{13}H_{25}NO_2$): C 68.68, H 11.08, N 6.16. Found: C 68.70, H 11.07, N 6.15. IR (neat) cm^{-1} 1706.
Compound **7*R*** and **7*S***: (*R*)- and (*S*)-*N*-Ethylpiperidin-4-ylmethyl valerate; *R* isomer; $[\alpha]_D^{20}$ +7.0° (c = 1.1, $CHCl_3$), *S* isomer; $[\alpha]_D^{20}$ −7.7° (c = 1.1, $CHCl_3$). ^{1}H NMR (δ, $CDCl_3$): 0.86–0.94 (6H, *m*, 2×CH_3) 1.28–1.37 (3H, *m*, CH, CH_2), 1.55–1.67 (6H, *m*, 4×CH, CH_2), 1.82–1.90 (2H, *m*, 2×CH), 2.25–2.37 (4H, *m*, 2×CH_2), 2.70–2.81 (2H, *m*, 2×CH), 3.82–3.95 (2H, *m*, 2×CH). ^{13}C NMR ($CDCl_3$): 11.97, 13.67, 18.88, 22.23, 27.05, 27.40, 34.00, 35.90, 52.74, 53.65, 56.78, 67.09, 173.81. Anal. calcd for ($C_{13}H_{25}NO_2$): C 68.68, H 11.08, N 6.16. Found: C 68.69, H 11.08, N 6.15. IR (neat) cm^{-1} 1712.
Compound **8**: *N*-fluoroethylpiperidin-4-ylmethyl butyrate; ^{1}H NMR (δ, $CDCl_3$): 0.87 (3H, *t*, CH_3), 1.27–1.36 (2H, *m*, 2×CH), 1.51–1.66 (5H, *m*, 3×CH, CH_2), 1.96–2.03 (2H, *m*, 2×CH), 2.18–2.23 (2H, *m*, 2×CH), 2.56 (1H, *m*, CH), 2.66 (1H, *m*, CH), 2.88–2.92 (2H, *m*, 2×CH), 3.84–3.87 (2H, *d*, CH_2), 4.41 (1H, *m*, CH), 4.56 (1H, *m*, CH). ^{13}C NMR ($CDCl_3$): 13.53, 18.33, 28.67, 34.92, 36.03, 53.50, 58.21, 58.47, 68.33, 80.68, 82.89, 173.54. Anal. calcd for ($C_{12}H_{22}FNO_2$): C 62.31, H 9.59, N 6.06. Found: C 62.31, H 9.60, N 6.05. IR (neat) cm^{-1} 1735.
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