# In Vivo Butyrylcholinesterase Activity Is Not Increased in Alzheimer's Disease Synapses

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***Objective:*** **We tested the premise that cholinesterase inhibitor therapy should target butyrylcholinesterase (BuChE) in Alzheimer's disease (AD), not acetylcholinesterase (AChE) alone, because both enzymes hydrolyze acetylcholine, and BuChE is increased in AD cerebral cortex.** ***Methods:*** **To examine this issue in vivo, we quantified human cerebral cortical BuChE activity using tracer kinetic estimates ($k_3$) of 1-[$^{11}$C]methyl-4-piperidinyl n-butyrate ([$^{11}$C]BMP) hydrolysis determined by positron emission tomography. Validation of the putative positron emission tomography method included regional distribution, positive correlation with age, and attenuation by the nonselective cholinesterase inhibitor physostigmine, but no attenuation by the AChE-selective inhibitor donepezil. Positron emission tomography scans in AD patients (n = 15) and control subjects (n = 12) measured both BuChE (using [$^{11}$C]BMP) and AChE activity (using N-[$^{11}$C] methylpiperidin-4-yl propionate, an established method).** ***Results:*** **As expected, AChE activity in AD cerebral cortex was decreased to 75 ± 13% of normal ($p = 0.00001$). Contrary to prediction, accompanying BuChE activity also was decreased to 82 ± 14% of normal ($p = 0.001$).** ***Interpretation:*** **Failure to observe increased [$^{11}$C]BMP hydrolysis in vivo makes it less likely that incremental BuChE contributes importantly to acetylcholine hydrolysis in AD. The findings do not support the premise that inhibitor therapy should target BuChE so as to prevent increased levels of BuChE from hydrolyzing acetylcholine in AD cerebral cortex.**

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There are two principal cholinesterases in the human brain, acetylcholinesterase (AChE; EC 3.1.1.7) and butyrylcholinesterase (BuChE; EC 3.1.1.8). AChE is membrane bound predominantly on presynaptic cholinergic neurons, but is also on postsynaptic cholinoceptive neurons.[1] BuChE has a neuroglial distribution.[2,3] The physiological role of AChE is to terminate the synaptic action of acetylcholine (ACh) through catalytic hydrolysis. ACh is hydrolyzed by both AChE and BuChE, but AChE catalyzes the hydrolysis of ACh much more efficiently than BuChE.[4] The physiological role of BuChE is poorly understood, particularly in the central nervous system. Recent attention has focused on this enigmatic enzyme and its possible relevance in the treatment of Alzheimer's disease (AD).[5–11]

Cholinesterase inhibitors have shown greater efficacy than placebo in clinical trials and are widely prescribed as symptomatic treatment to improve cognition and behavior in AD patients with mild or moderate dementia. The basis is the presynaptic cholinergic deficit that is found consistently in AD cerebral cortex.[12–14] The rationale is to increase the availability of ACh within cholinergic synapses by reducing its hydrolysis. Opinion differs on the issue whether these drugs should be selective inhibitors of AChE alone (eg, donepezil) or nonselective inhibitors of both AChE and BuChE (eg, rivastigmine). In the absence of cholinesterase inhibitors that are selective for BuChE, the potential efficacy of BuChE inhibition has been difficult to establish from clinical trials.[7,15] Proponents of AChE-selective inhibitors claim less peripheral adverse effects,[16,17] whereas some reject these results.[18] Proponents of nonselective inhibitors argue that if only AChE is inhibited, ACh will continue to be hydrolyzed by uninhibited BuChE that is known to be increased in AD cerebral cortex.[5–11] However, the magnitude and clinical significance of this process has not yet been established in vivo.[16]

To explore this issue directly, we tested the hypothesis that in vivo measures in cerebral cortex of AD patients with mild or moderate dementia would demonstrate the same discordant relation between AChE (decrease) and BuChE activity (increase) as has been documented from in vitro studies of postmortem AD

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brain.[2,9,19–23] First, we identified 1-[$^{11}$C]methyl-4-piperidinyl n-butyrate ([$^{11}$C]BMP) as a ligand superior for the in vivo measurement of cerebral BuChE activity based on specificity determined by in vitro assay and pharmacokinetics determined by in vivo measurements in primate brain.[24] Next, we validated the quantitative measure of in vivo human cerebral cortical BuChE activity using tracer kinetic estimates of [$^{11}$C]BMP hydrolysis determined by positron emission tomography (PET). Finally, we performed PET scans in AD patients and in healthy control subjects to measure both BuChE activity, using [$^{11}$C]BMP, and accompanying AChE activity, using *N*-[$^{11}$C]methylpiperidin-4-yl propionate ([$^{11}$C]PMP), a previously established method.[25–30] Recently, Roivainen and colleagues[31] reported using [$^{11}$C]BMP with PET to map BuChE activity in human brain, but no comparisons were made of AD patients versus control subjects.

## Subjects and Methods

### *Subjects*

A young control group consisted of 13 healthy subjects across an age range from 20 to 47 years (mean age, 30 ± 9 years; 4 female and 9 male subjects) and an elderly control group consisted of 12 healthy subjects across an age range from 55 to 68 years (mean age, 62 ± 7 years; 8 female and 4 male subjects). Healthy subjects had no history of significant general medical, neurological, or psychiatric illness, head injury with loss of consciousness, or drug or alcohol dependence, and they were not taking any medications with central nervous system actions. An AD group consisted of 15 subjects across an age range from 59 to 82 years (mean age, 73 ± 8 years; 9 female and 6 male subjects). AD subjects were not taking any medications with actions known to affect the central cholinergic system. All were required to be mildly or moderately demented and to have a clinical diagnosis of probable AD.[32] Mean Clinical Dementia Rating[33] was 1.1 ± 0.4. Mean Mini-Mental State Examination[34] score was 19 ± 4. This study was approved by an institutional review board. Written, informed consent was obtained from all subjects or their caregivers.

### *Radiotracers*

Details of preparation of [$^{11}$C]BMP[24] and [$^{11}$C]PMP[27] have been reported. In vitro assays have characterized piperidinyl esters as substrates for AChE and BuChE.[24–26,35] [$^{11}$C]PMP is a selective substrate for AChE, with a selectivity in ex vivo cholinesterase assays of 97% for AChE.[25] [$^{11}$C]BMP is not a substrate for AChE.

### *Scan Protocols and Analysis*

Data were acquired using a PET scanner (Siemens ECAT EXACT-47; CTI, Knoxville, TN) in three-dimensional mode. Final image resolution was 10.0mm (full-width at half-maximum) in-plane and axially. Each radiotracer was injected intravenously as a 444MBq (12mCi) dose. We limited a [$^{11}$C]BMP dose arbitrarily to a total injected mass less than 25μm/70 kg person. When used sequentially on the same day, [$^{11}$C]BMP was injected first, and then [$^{11}$C]PMP was injected after an interval of 2 hours. Tracer input functions were determined from serial blood samples withdrawn through a radial artery catheter. The fraction of unmetabolized tracer in plasma samples was determined. Data acquisition and analysis were performed as reported previously.[30] A three-compartment model[28,29] was used to yield pixel-by-pixel estimates of the rate constant $k_3$, which represented the hydrolysis rate (activity) of either BuChE or AChE. Regional values of $k_3$ were extracted using the three-dimensional stereotactic surface projection technique.[36] Data were not corrected for partial-volume effects. Any effect of cerebral cortical atrophy should account for less than an 8% reduction in the kinetically estimated values of [$^{11}$C]BMP$k_3$ or [$^{11}$C]PMP$k_3$.[28] Statistical methods included two-sample *t* tests assuming equal variances and different sample sizes for minimum detectable differences, two-tailed paired and unpaired *t* tests of significance, and two-factor repeated-measures analysis of variance. Reproducibility was estimated for test-retest variability, that is, the absolute difference in measured activity between the test and the retest divided by the average of the two studies and expressed as a percentage.

### *Inhibition Protocols*

Validation included measuring cerebral cortical responses of [$^{11}$C]BMP $k_3$ and [$^{11}$C]PMP $k_3$ after treatment with well-characterized drugs[37,38] known to be selective (donepezil) and nonselective (physostigmine) cholinesterase inhibitors. Young control subjects were scanned with [$^{11}$C]BMP (n = 4) or [$^{11}$C]PMP (n = 5)[28] before and after a constant rate infusion of physostigmine salicylate (Antilirium TM; Forest Pharmaceuticals, St. Louis, MO) in a dose of 1.5mg in 100ml saline over a period of 60 minutes.[28] Four AD patients (CDR1) were scanned with both [$^{11}$C]BMP and [$^{11}$C]PMP before and after treatment with donepezil hydrochloride (5mg/day; Aricept TM; Pfizer, New York, NY) for a 2- to 6-month period. After treatment, $k_3$ data were expressed as percentage decrease from pretreatment values (inhibition).

### *Alzheimer's Disease Protocol*

Both a [$^{11}$C]BMP and a [$^{11}$C]PMP PET scan were performed on each elderly control subject (n = 12) and AD subject (n = 15) to quantify in vivo BuChE activity and accompanying AChE activity in normal and AD cerebral cortex.

## Results

### *Validity of the 1-[$^{11}$C]Methyl-4-piperidinyl n-Butyrate Method*

REPRODUCIBILITY. In cerebral cortex of elderly control subjects, the coefficients of variation for intersubject (n = 12) measurements of [$^{11}$C]BMP $k_3$ and [$^{11}$C]PMP $k_3$ were 11 and 12%, respectively. Intrasubject test-retest measures made at 2-month intervals using [$^{11}$C]BMP (n = 5) and [$^{11}$C]PMP (n = 6) differed by 7 ± 5 and 6 ± 3%, respectively.

DISTRIBUTION. Our in vivo measures of [$^{11}$C]BMP $k_3$ and [$^{11}$C]PMP $k_3$ (Table) in healthy control cases agreed well with relative regional distributions of BuChE and AChE activity based on biochemical assay of postmortem human brain.[39] BuChE activity ranged from low in cerebral cortex to 2.5 times higher in cerebellum (Fig 1), and AChE activity ranged from low in cerebral cortex to 21 times higher in caudate nucleus.

AGE AND SEX. Cerebral cortical [$^{11}$C]BMP $k_3$ increased with age, whereas [$^{11}$C]PMP $k_3$ did not,[28] a finding in agreement with biochemical assays of postmortem brain.[19,23] [$^{11}$C]BMP $k_3$ in total cerebral cortex averaged 25% higher in elderly (age, 62 ± 7 years; n = 12) than in young (age, 30 ± 9 years; n = 13) subjects. Between either the age groups or the sex groups, the minimum detectable difference was 14% (80% power at $p < 0.05$). Using a two-factor analysis of variance (age and sex as factors), we noted a significant age effect ($p = 0.009$), with [$^{11}$C]BMP $k_3$ increasing with age. Neither sex effect ($p = 0.15$) nor age by sex interaction ($p = 0.17$) were significant. Relative to parietal cortex distribution, regional [$^{11}$C]BMP $k_3$ cerebral cortical distributions (frontal, temporal, occipital, visual, posterior cingulate) did not differ significantly ($p > 0.1$) between young and elderly subjects.

RESPONSE TO INHIBITORS. As predicted, acute administration of the nonselective inhibitor physostigmine (1.5mg infusion) inhibited both BuChE and AChE significantly in normal cerebral cortex (Fig 2). BuChE inhibition measured with [$^{11}$C]BMP (n = 4) was 49 ± 6% ($p = 0.001$), and AChE inhibition measured with [$^{11}$C]PMP (n = 5) was 52 ± 9% ($p = 0.0002$).[28] The inhibition levels of the two cholinesterases did not differ significantly ($p = 0.6$). As predicted, treatment with the selective AChE inhibitor donepezil (5mg/day for 2–6 months; n = 4) inhibited only AChE in AD cerebral cortex (see Fig 2). BuChE inhibition measured with [$^{11}$C]BMP was 4 ± 12% and was not significant ($p = 0.5$). Accompanying AChE inhibition measured with [$^{11}$C]PMP was 27 ± 5% and was significant ($p = 0.003$).

*Alzheimer's Disease*

When [$^{11}$C]BMP and [$^{11}$C]PMP PET scans were compared (Fig 3) between elderly control subjects (n = 12) and AD patients (n = 15), in vivo AChE activity in overall AD cerebral cortex was found to be decreased to 75 ± 13% of normal ($p = 0.00001$). Associated in vivo BuChE activity was not found increased, but instead was decreased to 82 ± 14% of normal ($p = 0.001$). The minimum detectable difference in cerebral cortex between elderly control subjects and AD patients was 13% with [$^{11}$C]BMP $k_3$ and 12% with [$^{11}$C]PMP $k_3$ (80% power at $p < 0.05$). The difference between [$^{11}$C]BMP $k_3$ decrease (mean, 18%) and [$^{11}$C]PMP $k_3$ decrease (mean, 25%) was significant ($p = 0.05$). The difference between the ratio [$^{11}$C]BMP$k_3$/[$^{11}$C]PMP$k_3$ measured in normal (0.94 ± 0.13) and in AD cerebral cortex (1.04 ± 0.17) was not significant ($p = 0.13$). As noted earlier, no compensatory increase in BuChE activity accompa-

*Table. Distribution of Butyrylcholinesterase and Acetylcholinesterase Activities: In Vivo versus Postmortem Biochemical Assay*

| Regions | Relative BuChE Activity (mean ± SD) | | Relative AChE Activity (mean ± SD) | |
|---|---|---|---|---|
| | In Vivo [$^{11}$C]BMP $k_3^{*a}$ | Postmortem Biochemical | In Vivo [$^{11}$C]PMP $k_3^{*a}$ | Postmortem Biochemical |
| Cerebellum | 2.50 ± 0.39 | 2.90 ± 0.04 | 8.97 ± 2.00 | 12.0 ± 2.2 |
| Putamen | 1.74 ± 0.11 | | 20.7 ± 3.6 | |
| Caudate nucleus | 1.58 ± 0.10 | 1.97 ± 0.23 | 21.4 ± 4.6 | 54.7 ± 6.7 |
| Thalamus | 1.28 ± 0.12 | | 2.60 ± 0.31 | |
| Pons | 1.14 ± 0.14 | | 5.44 ± 1.24 | |
| Cerebral cortex | | | | |
| Frontal cortex | 0.93 ± 0.06 | | 1.05 ± 0.07 | |
| Temporal cortex | 0.91 ± 0.04 | 1.47 ± 0.35 | 1.06 ± 0.08 | 1.5 ± 0.3 |
| Occipital cortex | 0.99 ± 0.05 | | 0.96 ± 0.05 | |
| Visual cortex | 0.96 ± 0.08 | | 0.94 ± 0.06 | |
| Posterior cingulate cortex | 0.99 ± 0.07 | | 1.10 ± 0.08 | |
| Parietal cortex | 1.00 ± 0.00 | 1.00 ± 0.25 | 1.00 ± 0.00 | 1.0 ± 0.1 |

Relative activity indices are relative to the corresponding parietal cortex measure. In vivo butyrylcholinesterase (BuChE) and acetylcholinesterase (AChE) activities were determined and were normalized to the parietal cortex measure in each elderly healthy control subject (n = 12). Postmortem indices were calculated from the published data of Atack and colleagues.[39] Activities in each elderly control brain (n = 3-5) were normalized to the mean parietal cortex measure.

[a]The rate constant $k_3^*$ represents cholinesterase activity, where the asterisk means relative to the parietal cortex.

SD = standard deviation; [$^{11}$C]BMP = 1-[$^{11}$C]methyl-4-piperidinyl n-butyrate; [$^{11}$C]PMP = *N*-[$^{11}$C]methylpiperidin-4-yl propionate.

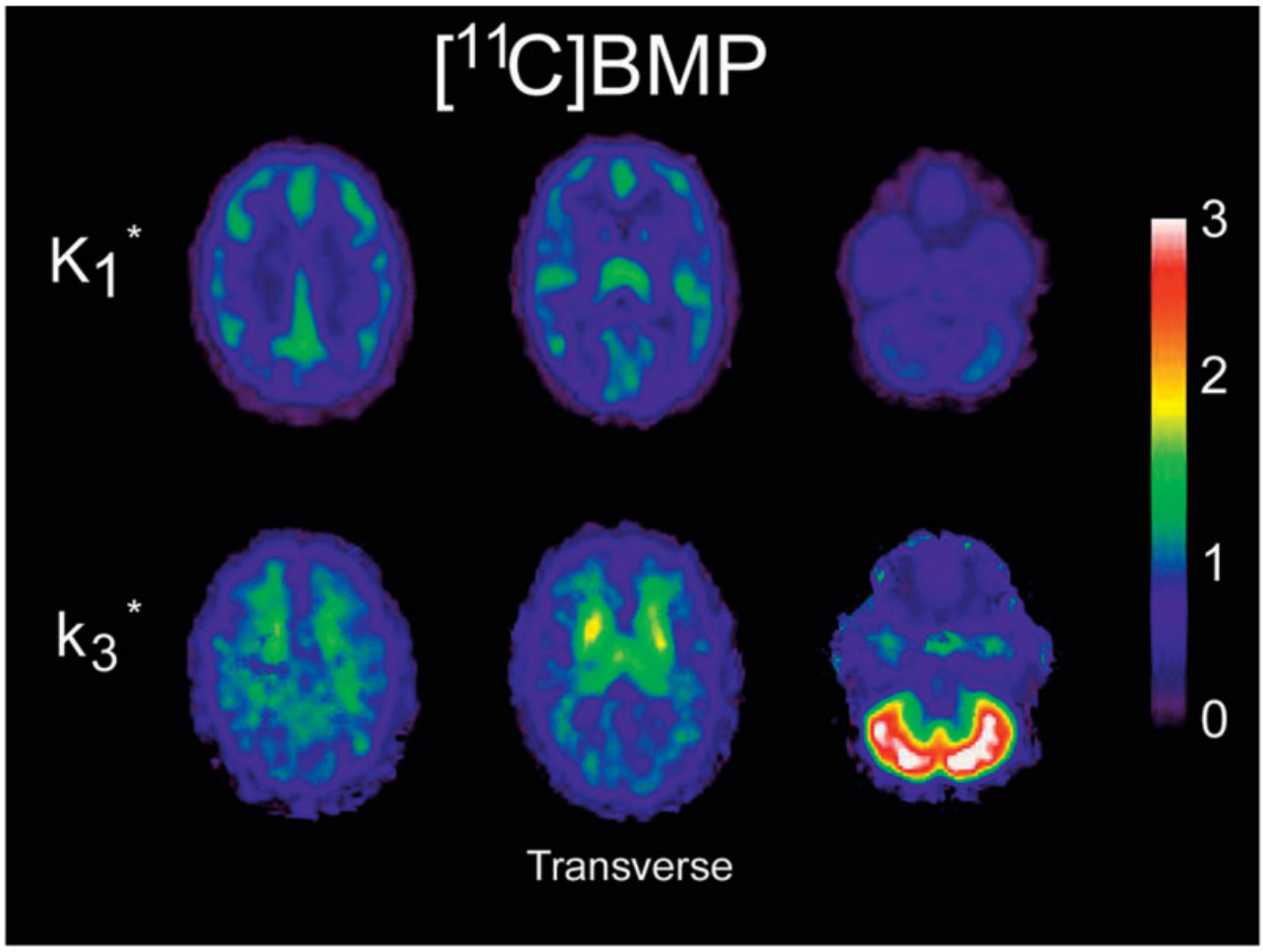


*Fig 1. Parametric images of 1-[[11]C]methyl-4-piperidinyl n-butyrate ([[11]C]BMP) kinetics within three brain levels of a healthy control subject, all normalized to mean values in parietal cortex. The rate constant $K_3^*$ represents delivery, and the rate constant $k_3^*$ represents butyrylcholinesterase activity, where the* asterisk *means relative to the parietal cortex. Compared with cerebral cortex, butyrylcholinesterase activity is higher in cerebellum, striatum, thalamus, and subcortical white matter, where it is localized in glial cells.[39]*

nied selective inhibition of AChE activity in AD cortex.

## Discussion

In vivo AD brain did not demonstrate the substantial increase in BuChE activity predicted from in vitro studies of postmortem AD brain.[2,9,19–23] Because we found that the activities of both cholinesterases were decreased in AD cerebral cortex, there was no in vivo evidence of the high ratios of BuChE/AChE that have been proposed as regional markers of AD by in vitro studies.[2,6] In addition, BuChE activity did not increase in human brain after months of selective AChE inhibition, an observation that was consistent with the reported absence of compensatory increase in BuChE activity in AChE-devoid brains of knock-out mice.[40]

We interpreted our results to be evidence that the incremental buildup of BuChE that is identified in postmortem AD brain does not contribute substantially to neuropil-associated BuChE activity in the living AD brain. We considered and rejected some alternative explanations. First, were the in vitro reports incorrect? We consider this unlikely. In vitro evidence is convincing from studies performed in multiple laboratories and with differing approaches that BuChE is increased in postmortem AD cerebral cortex. The in vivo neurochemical role and consequence of this incremental BuChE, however, has been speculative. An early study based on quantitative biochemical measures in autopsied brain found that total AChE was reduced 33% in AD temporal cortex, but total BuChE was increased 40%.[19] Histochemistry demonstrates both AChE and BuChE are bound to pathological AD substructures.[20] For example, BuChE emerges from neuroglia[2] and accumulates in pathological plaques, neurofibrillary tangles, and vessels affected by congophilic angiopa-

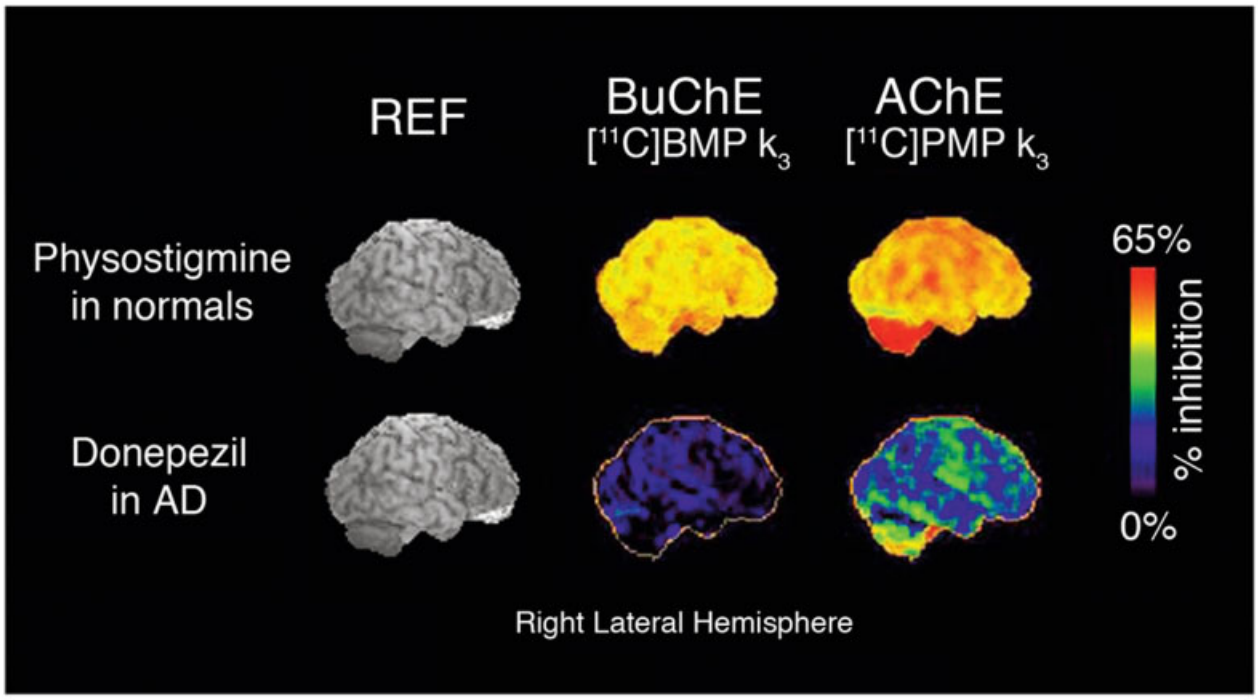


*Fig 2. Positron emission tomography (PET) scans using 1-[$^{11}$C]methyl-4-piperidinyl n-butyrate ([$^{11}$C]BMP) and N-[$^{11}$C]methylpiperidin-4-yl propionate ([$^{11}$C]PMP) quantified correctly the in vivo inhibition of butyrylcholinesterase (BuChE) and acetylcholinesterase (AChE) activities expected after treatment with nonselective and selective cholinesterase inhibitor drugs. After acute administration of the nonselective inhibitor physostigmine, mean inhibition in cerebrocortical, striatal, thalamic, and pontocerebellar zones was 49, 50, 54, and 51%, respectively, for BuChE (n = 4) and 52, 44, 61, and 64%, respectively, for AChE (n = 5). After treatment for several months with the selective AChE inhibitor donepezil (n = 4), AChE inhibition in the same zones was 27, 31, 36, and 46%, respectively, but BuChE was not inhibited in any zone. Image summations are right-left averages of percentage decrease from pretreatment values (percentage inhibition) plotted as three-dimensional stereotactic surface projections (reference anatomic map [REF]). Red color represents greater inhibition of BuChE or AChE by physostigmine or donepezil. AD = Alzheimer's disease.*

thy.[2,20,22,23] This accumulation, especially that enzyme bound to mature compact neuritic plaques,[41] is considered to account for the incremental BuChE observed in AD cerebral cortex tissue hemogenates.[23] In support of this distinction, no increase was found in BuChE immunochemistry associated with histologically normal AD neuropil, where cholinergic neurotransmission occurs.[9,21]

Second, did we scan our AD patients with mild or moderate dementia too early in the course of their disease for incremental plaque-bound BuChE to be expected? This is unlikely. The mature compact neuritic plaques that are reported to express increased BuChE activity[41] and the presynaptic cholinergic deficit are established already even earlier in the course of AD, before the onset of clinical symptoms.[42]

Third, did a mismatched control group obscure BuChE activity gain? The AD group (mean age, 73 years) was older than the elderly control group (mean age, 62 years), and BuChE activity increased with age in the control subjects (25% over 32 years). Such a bias would favor underestimating reduction, not gain, in BuChE activity. Accounting for this would equalize the BuChE and AChE declines in AD cerebral cortex to 75 ± 13% of normal.

Finally, was the [$^{11}$C]BMP PET measure flawed? Its rationale is based on the acetylcholine-analog PET method of Irie and colleagues.[25] This method has been accepted widely as a valid direct measure to quantify in vivo AChE.[28,30,43–45] Data reported here and previously[24,31] support the validity of the new [$^{11}$C]BMP PET measure as a method appropriate to the in vivo assessment of BuChE activity associated with cholinergic terminals. However, tissue heterogeneity on an anatomical scale below PET imaging resolution can complicate quantification of enzyme activity in foci distant from these terminals. For example, even though tissue homogenate assays might measure scarce, but highly concentrated, foci of incremental plaque BuChE that were sparsely perfused, this PET method would underestimate their activity, because delivery of [$^{11}$C]BMP tracer to the plaque enzyme would be limited. Nevertheless, the [$^{11}$C]BMP PET method serves well to measure BuChE activity that is associated with

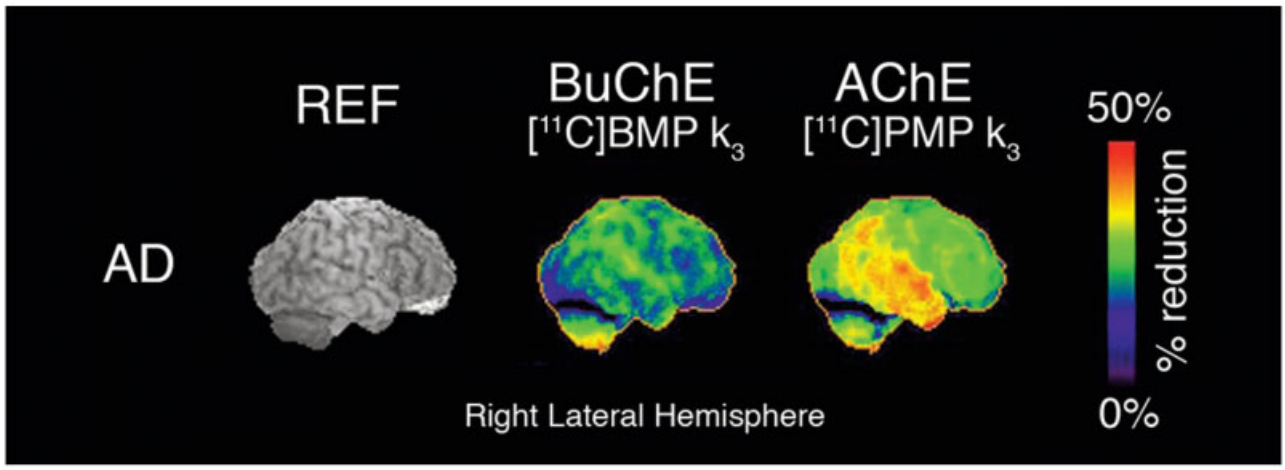


*Fig 3. Contrary to in vitro evidence that butyrylcholinesterase (BuChE) is increased substantially in Alzheimer's disease (AD) cerebral cortex, in vivo BuChE activity was not found to be increased. Positron emission tomography scans were performed in elderly control subjects (n = 12) and in AD patients (n = 15) using 1-[$^{11}$C]methyl-4-piperidinyl n-butyrate ([$^{11}$C]BMP) and N-[$^{11}$C]methylpiperidin-4-yl propionate ([$^{11}$C]PMP) to measure BuChE and acetylcholinesterase (AChE) activities, respectively. In cerebral cortical, striatal, thalamic, and pontocerebellar zones of in vivo AD brain, activity reductions averaged 28% for AChE and 18% for BuChE. Image summations are right-left averages of percentage reductions from normal plotted as three-dimensional stereotactic surface projections (reference anatomic map [REF]). Red color represents greater reductions of BuChE or AChE in AD in comparison with elderly control subjects.*

cholinergic terminals, and these are the critical sites where ACh is concentrated and where cholinesterase inhibition affects cholinergic neurotransmission.

ACh hydrolysis occurs predominantly within cholinergic synapses by outwardly facing, membrane-anchored, tetrameric (G4) molecular forms of AChE and BuChE.[46] Evidence for hydrolysis by BuChE is indirect. First, BuChE can hydrolyze ACh, but is less efficient than AChE.[4] Second, inhibition of BuChE in rat brain leads to a dose-dependent increase in ACh concentration in microdialysate.[6] Third, complete inhibition of both AChE and BuChE is fatal, but AChE-null knock-out mice survive, presumably because BuChE hydrolyzes ACh and prevents cholinergic overactivity.[40] Fourth, when AChE is inhibited in tissue sections, BuChE hydrolyzes the ACh surrogate acetylthiocholine iodide.[9] The actual contribution of terminal-associated BuChE to overall molar hydrolysis rate of ACh in living human cerebral cortex has yet to be quantified. But the incremental BuChE discovered in vitro in AD cerebral cortex is plaque-bound enzyme in the embryonic G1 molecular form,[21] not the membrane-anchored G4 BuChE that is considered to play a role in synaptic hydrolysis of ACh.[46]

There is no good evidence that plaque-bound cholinesterases, whether BuChE or AChE, hydrolyze endogenous ACh in the in vivo human brain. In quantitative comparisons, regional losses of cholinergic terminals and AChE activity did not differ throughout the in vivo AD cerebral cortex.[28] If in vivo AChE activity was bolstered strongly by incremental plaque-bound AChE, terminal loss should have been disproportionate to AChE activity loss, but it was not.

Our results suggest that, in AD, the hydrolysis of synaptic ACh by membrane-anchored G4 BuChE declines and is little affected by the buildup of the G1 BuChE that is found bound to neuritic plaques, neurofibrillary tangles, and vessels affected by congophilic angiopathy. In postmortem AD cerebral cortex, G4 forms of both AChE and BuChE are selectively lost,[21,47] and G4 AChE is decreased more than is G4 BuChE.[21] Our in vivo PET data are consistent with those in vitro data.

Consequently, our results did not support the premise that inhibitor therapy should target BuChE to prevent increased levels of BuChE from hydrolyzing ACh in AD cerebral cortex.[6,7,9] In normal brain, only a small amount of ACh is hydrolyzed by BuChE. Most ACh hydrolysis is catalyzed by AChE, which hydrolyzes ACh much more efficiently than does BuChE. In AD brain, we found that terminal-associated BuChE activity was not increased in vivo, rather it was decreased. In addition, we found that no compensatory increase occurred in terminal-associated BuChE activity after months of AChE inhibition therapy. The primacy of AChE inhibition as cholinomimetic therapy in AD is further supported by recent comparative clinical trials that show no difference in efficacy for cognitive improvement between AChE-selective (donepezil) and nonselective (rivastigmine) cholinesterase inhibitors.[48,49] Yet, current therapeutic drugs provide only about 30% inhibition of in vivo AChE activity in ce-

rebral cortex.[30,43–45] For more efficient reduction of ACh hydrolysis, drug development would be better targeted to increasing AChE inhibition without serious adverse effects, rather than to increasing BuChE inhibition.

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