Correlation of brain amyloid with "aerobic glycolysis": A question of assumptions?

Michael E. Phelps[1] and Jorge R. Barrio

Department of Molecular and Medical Pharmacology, The David Geffen University of California, Los Angeles School of Medicine, Los Angeles, CA 90095-6948

The articles by Vaishnavi et al. (1) and Vlassenko et al. (2) in PNAS demonstrate regional variation in aerobic glycolysis vs. oxidative phosphorylation in the human brain and then link aerobic glycolysis to amyloid β (Aβ) deposition on the basis of concordant spatial distributions in Alzheimer's disease (AD). As referenced in these articles, the molar ratio of O_2 to glucose consumption by the brain is never 6 (the ratio for complete oxidation of glucose to CO_2 and H_2O). Because the experimentally determined molar ratio is normally ≈5.3, the major portion of glucose flux proceeds through the tricarboxylic acid (TCA) cycle to meet the high ATP demands of the brain, as well as other TCA cycle functions. The term "aerobic glycolysis," as defined by the authors, refers to flux from glucose to pyruvate without entering the TCA cycle, as well as glucose providing substrates for the pentose phosphate shunt and glycogen synthesis, in conditions whereby oxygen availability is not limiting. Although much less energy efficient than mitochondrial oxidative phosphorylation (36 ATP/glucose), aerobic glycolysis (2 ATP/glucose) can rapidly provide ATP and substrates for biosynthetic pathways.

Raichle and colleagues were the first to clearly demonstrate that task-based activation of local regions of the human brain associated with increases in glucose metabolism determined with 2-deoxy-2-[^{18}F] fluoro-D-glucose (FDG) and PET (3) occur with little to no increases in oxygen metabolism, even though local availability of oxygen is sufficient for oxidative phosphorylation (references in ref. 1). These findings indicate that, in execution of these tasks, the involved brain tissue switches to aerobic glycolysis.

These "switch points" that alter glucose metabolic flux through various biochemical pathways occur in a number of transitions from one cellular state to another, in normal and pathologic conditions. As stated by the authors, the oldest and most cited switch point in aerobic glycolysis is the "Warburg effect," as part of malignant transformation. In further support of the examples cited by Vaishnavi et al. (1), other demand-driven transitions to aerobic glycolysis have been demonstrated, exemplified in the developing embryo (4), the transition from the resting state to activation in the immune system (5, 6), and during viral infection (7).

Vaishnavi et al. (1) and Vlassenko et al. (2) show that higher levels of aerobic glycolysis are concentrated in the default mode network (DMN), an anatomically defined brain network—involving the medial temporal lobe and the medial prefrontal subsystems that converge for integration in the posterior cingulate gyrus—preferentially active when individuals are not focused on the external environment (8). Intriguingly, the regions within the DMN exhibiting high resting glucose metabolism in normal subjects are the ones most heavily affected in AD. Moreover, Buckner et al. (9) have previously reported increased 2-[4′-([^{11}C]methylamino)phenyl]-6-hydroxybenzothiazole ([^{11}C]PIB) deposition in the DMN.

Vlassenko et al. present a case for involvement of aerobic glycolysis with amyloid deposition in the brain.

Brain regional correlations between markers for amyloid tau deposition, glucose hypometabolism, and neuronal losses and corresponding alterations in neuropsychiatric measures were demonstrated in humans with AD and mild cognitive impairment (MCI) (10). These results strengthened earlier suggestions that clinical symptoms characteristic for AD stem from the disruption of major neuronal circuits caused by the extensive loss of neurons forming these circuits associated with early pathological Aβ and τ deposition initially affecting the entorhinal cortex. With advancing stages of AD, the death of cortical neurons increasingly spreads to regions beyond the hippocampus, one of the critical initial sites of neuronal damage. As part of disease progression, glucose metabolic deficits become increasingly widespread, with worsening in clinical parameters of AD. This specific pattern of regional hypometabolism, as assessed with FDG PET, distinguishes AD from other pathologic processes that can cause dementia, as evidenced by autopsy-confirmed diagnoses (11).

Vlassenko et al. (2) present a case for involvement of aerobic glycolysis with amyloid deposition in the brain—which is considered causative according to the Aβ hypothesis of AD—under the assumption that [^{11}C]PIB provides an in vivo assay of Aβ as established by other investigators (references in ref. 2). This conclusion is reached through a carefully executed spatial registration of PET images of glucose and oxygen metabolic rates with [^{11}C]PIB images. Although the observations presented by Vlassenko et al. (2) on the spatial correlation between brain aerobic glycolysis and amyloid deposition are experimentally consistent, a different interpretation of the results may be considered.

Whereas brain glucose metabolism decreases in AD patients, as determined with FDG PET, and this metabolic deficit increases and spreads to an increasing number of brain structures in a pattern predictive of disease progression, [^{11}C]PIB binding can be identified in approximately 30% of control subjects in the pattern seen in symptomatic AD patients, and AD patients can present with [^{11}C]PIB scans identical to those of controls (12). In at least two studies, negative [^{11}C]PIB results were reported in AD patients with positive autopsy evidence of amyloid aggregates in the brain (13, 14). In addition, [^{11}C]PIB has not shown a pattern of accumulation consistent with progressive Aβ accumulation across the trajectory of the disease, as would be expected from autopsy data (15). For example, MCI patients seem to group into two categories: [^{11}C]PIB binding pattern that is negative (similar to controls) or positive to the level of signal intensity and cortical localization comparable to symptomatic AD patients (16). Regarding the latter, this has been interpreted as due to the accumulation of AD-level amyloid load by the time the

Author contributions: M.E.P. and J.R.B. wrote the paper.

Conflict of interest statement: J.R.B. is a coinventor of a patented UCLA imaging probe, FDDNP, which tests for amyloid in the brain. There is no commercial license for FDDNP, and no revenue has been received. M.E.P. has no conflict of interest.

See companion articles on pages 17757 and 17763.

[1]To whom correspondence should be addressed. E-mail: mphelps@mednet.ucla.edu.

patients meet MCI clinical criteria (16). Further, [^{11}C]PIB binding in the precuneus has been reported to be the highest of all brain cortical areas (12), yet autopsy studies have not found higher amyloid deposition in the precuneus compared with other cortical areas (17). Moreover, [^{11}C]PIB binding in the medial temporal lobe is not present in AD patients at all levels of cognitive impairment, even though Aβ is rarely absent in medial temporal lobe substructures of AD patients at autopsy (17, 18).

Spatial correlations of [^{11}C]PIB images with brain Aβ deposition at autopsy do not establish causation. In vivo target specificity of [^{11}C]PIB and binding reversibility have not yet been demonstrated by other means (e.g., with blocking or displacement determinations with competitive molecules known to specifically target Aβ). Although in vitro dilution experiments with nonradioactive PIB (19) can establish the presence of saturable targets, they will not determine amyloid target specificity because all tissue targets would be affected.

The specificity of [^{11}C]PIB for Aβ in vivo thus remains in question. An alternative explanation for [^{11}C]PIB images is that [^{11}C]PIB brain accumulation is mediated at least in part by estrogen sulfotransferase (SULT1E1) (20). SULT1E1 is an enzyme involved in estrogen homeostasis that is present in the rat brain (21) and other species. In the rat brain, for example, there is SULT1E1-mediated transformation of [^{11}C]PIB to its 6-O-sulfate product that is trapped in tissue and accumulates in proportion to the rate of this enzyme-mediated reaction (20), similar to the way hexokinase mediates FDG accumulation as its 6′-phosphate product (3). [^{11}C]PIB-6-sulfate has also been determined to be present in human plasma as a major peripheral metabolite, as an indication that [^{11}C]PIB metabolic transformation is also possible in humans.

Estrogens are known to exert neuroprotective effects (22), and increased expression of SULT1E1 is also known to suppress estrogen effects, which are associated with inflammation in various tissues, including in human cancers. Considerable evidence gained over the past decade has implicated neuroinflammation as a possible prelude and consequence of AD pathology, with components including microglia and astrocytes as well as elevated cytokines and chemokines (23).

Further biochemical experiments will be needed to establish the reaction mechanisms underlying the retention of [^{11}C]PIB in the human brain in vivo, and from this, to explore the mechanisms that underlie the spatial correspondence of aerobic glycolysis and the biochemical process reflected in [^{11}C]PIB images.

1. Vaishnavi SN, et al. (2010) Regional aerobic glycolysis in the human brain. *Proc Natl Acad Sci USA* 107:17757–17762.
2. Vlassenko AG, et al. (2010) Spatial correlation between brain aerobic glycolysis and amyloid-β (Aβ) deposition. *Proc Natl Acad Sci USA* 107:17763–17767.
3. Phelps ME (2000) Positron emission tomography provides molecular imaging of biological processes. *Proc Nat Acad Sci USA* 97:9226–9233.
4. Kondoh H, et al. (2007) A high glycolytic flux supports the proliferative potential of murine embryonic stem cells. *Antioxid Redox Signal* 9:293–299.
5. Maciver NJ, et al. (2008) Glucose metabolism in lymphocytes is a regulated process with significant effects on immune cell function and survival. *J Leukoc Biol* 84: 949–957.
6. Radu CG, Shu CJ, Shelly SM, Phelps ME, Witte ON (2007) Positron emission tomography with computed tomography imaging of neuroinflammation in experimental autoimmune encephalomyelitis. *Proc Natl Acad Sci USA* 104:1937–1942.
7. Delgado T, et al. (2010) Induction of the Warburg effect by Kaposi's sarcoma herpesvirus is required for the maintenance of latently infected endothelial cells. *Proc Natl Acad Sci USA* 107:10696–10701.
8. Buckner RL, Andrews-Hanna JR, Schacter DL (2008) The brain's default network: Anatomy, function, and relevance to disease. *Ann N Y Acad Sci* 1124:1–38.
9. Buckner RL, et al. (2005) Molecular, structural, and functional characterization of Alzheimer's disease: Evidence for a relationship between default activity, amyloid, and memory. *J Neurosci* 25:7709–7717.
10. Kepe V, et al. (2006) Serotonin 1A receptors in the living brain of Alzheimer's disease patients. *Proc Natl Acad Sci USA* 103:702–707.
11. Silverman DHS, et al. (2001) Positron emission tomography in evaluation of dementia: Regional brain metabolism and long-term outcome. *JAMA* 286:2120–2127.
12. Mintun MA, et al. (2006) [^{11}C]PIB in a nondemented population: Potential antecedent marker of Alzheimer disease. *Neurology* 67:446–452.
13. Leinonen V, et al. (2008) Assessment of beta-amyloid in a frontal cortical brain biopsy specimen and by positron emission tomography with carbon 11-labeled Pittsburgh Compound B. *Arch Neurol* 65:1304–1309.
14. Cairns NJ, et al. (2009) Absence of Pittsburgh compound B detection of cerebral amyloid beta in a patient with clinical, cognitive, and cerebrospinal fluid markers of Alzheimer disease: A case report. *Arch Neurol* 66: 1557–1562.
15. Braak H, Braak E (1991) Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol* 82: 239–259.
16. Okello A, et al. (2009) Conversion of amyloid positive and negative MCI to AD over 3 years: an ^{11}C-PIB PET study. *Neurology* 73:754–760.
17. Nelson PT, et al. (2009) Alzheimer's-type neuropathology in the precuneus is not increased relative to other areas of neocortex across a range of cognitive impairment. *Neurosci Lett* 450:336–339.
18. Nelson PT, et al. (2009) Brains with medial temporal lobe neurofibrillary tangles but no neuritic amyloid plaques are a diagnostic dilemma but may have pathogenetic aspects distinct from Alzheimer disease. *J Neuropathol Exp Neurol* 68:774–784.
19. Klunk WE, et al. (2004) Imaging brain amyloid in Alzheimer's disease with Pittsburgh Compound-B. *Ann Neurol* 55:306–319.
20. Cole GB, et al. (2010) Specific estrogen sulfotransferase (SULT1E1) substrates and molecular imaging probe candidates. *Proc Natl Acad Sci USA* 107:6222–6227.
21. Miki Y, et al. (2002) Systemic distribution of steroid sulfatase and estrogen sulfotransferase in human adult and fetal tissues. *J Clin Endocrinol Metab* 87: 5760–5768.
22. Brann DW, Dhandapani K, Wakade C, Mahesh VB, Khan MM (2007) Neurotrophic and neuroprotective actions of estrogen: basic mechanisms and clinical implications. *Steroids* 72:381–405.
23. Tuppo EE, Arias HR (2005) The role of inflammation in Alzheimer's disease. *Int J Biochem Cell Biol* 37:289–305.