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Measuring *In Vivo* Metabolite Levels in Brain

Commentary on Dworak et al. Sleep and brain energy levels: ATP changes during sleep. Journal of Neuroscience 2010;30:9007-16.

David F. Wilson, PhD

Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA

The paper presented several interesting but surprising experimental results. Most important are measurements showing large increases in ATP, up to more than 3 times that of awake animals, during sleep. Reports of large changes in ATP in the brain, except in severe hypoxia/ischemia, are rare.

The author's description of the procedure used to prepare the samples includes:

"The rats were killed by decapitation, and brains were removed. Coronal slices (2 mm thick) were carefully placed on a dry ice (−78.5°C)-containing covered Petri dish for rapid freezing and subsequent dissection." and "Extreme care was exercised to complete this process rapidly, with an average time of 80 ± 9 s for tissue collection…"

The rat brain consumes approximately 77×10^{-9} moles O_2/g sec.[1] Assuming most of this is used for oxidative phosphorylation and 6 ATP are made per O_2, ATP is being synthesized/consumed at 0.46×10^{-6} moles/g sec. The brain has approximately 2.6×10^{-6} moles ATP/g,[2,3] so on the average all of the ATP in the brain is consumed and resynthesized (turned over) every 6-10 seconds. Interruption of oxygen delivery results in loss of consciousness in about 10 sec.

For trapping the metabolic state in accessible tissues, such as heart or liver, Wollenberger clamps[4] have been used. These are a pair of tongs about 2 feet long with flat aluminum plates with much greater mass than the tissue sample. The aluminum plates are cooled in liquid N_2 (−196°C), and then the tissue, without prior interruption of perfusion or blood flow, is clamped between the plates, compressing the tissue to 1 and 2 mm thick. The low temperatures, high rate of transfer of heat to the metal plates, and the thin samples results in freezing (quenching) times of < 1 sec. Krebs and coworkers[5,6] and Williamson and coworkers,[7-9] as examples, used this method when studying metabolism in liver and heart, were able to measure metabolite changes occurring within a few seconds.

Brain is a challenge because the Wollenberger clamps crush the bone and soft tissue together. To overcome this limitation, other methods were developed. Veech and coworkers,[2,3] for example, designed a "brain blower" in which pressurized gas blew the soft brain tissue out through a burr hole in the skull and onto an aluminum plate at −196°C. Quenching occurred in < 1 sec. Four different methods for rapid quenching of metabolism in brain were compared by Lust et al.[3] It was concluded that the brain blower, with quenching in < 1 sec, resulted in tissue samples giving significantly more physiological metabolite measurements than could be attained using the somewhat slower quenching methods.

The method Dworak and coworkers used to prepare the brain tissue is suitable for measuring gene expression and protein levels, but not for trapping metabolic intermediates such as ATP or enzyme phosphorylation at *in vivo* levels. The reported values represent metabolism occurring in the brain after decapitation and not of the brain *in vivo*.

DISCLOSURE STATEMENT

Dr. Wilson has indicated no financial conflicts of interest.

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Address correspondence to: David F. Wilson, Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA 19104