On line measurement of extracellular brain glucose using microdialysis sampling coupled with electrochemical detection.

P. G. Osborne. BAS Japan. Research and Development Laboratory.

Glucose is the main source of energy for the brain. Extracellular glucose concentration is dynamic and results from a balance between active transport from blood and utilization by brain cells. Both these mechanisms exhibit regional variation in response to stimuli.

The development of the microdialysis diffusion sampling technique by Ungerstedt in the early 1980's provided a means of easily obtaining, macro-protein free, dialysates of the extracellular fluid (ECF). At present, the concentrations of glucose, like lactate, pyruvate and glutamate in dialysates are most frequently assayed by HPLC systems which contain a column to temporally separate the analyte(s) of interest, a reactor column containing immobilized substrate specific enzyme(s) to generate H_2O_2 as per Eq. 1.

Eq. 1. Substrate + O_2 ------- Oxidized substrate + H_2O_2

Enzyme

and an electrochemical detector to quantify the product of the oxidation of H_2O_2 at 500 - 700 mV. However this technique, although extremely sensitive and reliable requires considerable technical outlay and expertise while the temporal resolution of the technique is dependent upon the retention time of the analyte in the column.

This talk will focus on recent studies at BAS that have resulted in the development of method for the on-line, real time measurement of brain ECF glucose from conscious, unrestrained rats. This new on-line technique is technically undemanding and as such represents a great simplification of the method used for measurement of glucose. Although the results presented are for glucose the methodology is transferable to the measurement of pyruvate, lactate and glutamate, provided their respective enzymes exhibit a high degree of substrate specificity.

This simplified method resulted from development of a 'bilayer' electrode and reconfiguration of the fluid circuit normally associated with microdialysis sampling of ECF. In brief, Glucose Oxidase immobilized onto the surface of a Os-polyvinylpyrridine wired HRP (Os-gel-HRP) glassy carbon electrode which was maintained at 0 mV and detected the current generated from the reduction of H2O2 produced by the Glucose Oxidase. Interference from ascorbic acid was eliminated by overcoating this 'bilayer' (Glucose Oxidase/Os-gel-HRP) electrode with a cellulose acetate film concurrent with the use of a platinum tube generator maintained at 250 mV. The microdialysis perfusate was mixed with a low pH phosphate buffer to minimize the oxidation of ascorbic acid, buffer the detector from variations of pH and increase the enzyme efficiency.

In invivo experiments were performed in rats during the 4 days immediately after a microdialysis probe (CMA 11 - 1mm dialysing membrane) was chronically implanted into the striatum. A catheter was also implanted into the intraperitoneal

cavity and exteriorized on the head adjacent to the microdialysis probe. Using the BAS on-line system striatal glucose in the conscious rat was measured to increase by 71 +/- 3% (n = 4 rats) in response to glucose load of 1.5 ml of 10%glucose in ringer. Anesthesia (40 mg/kg Nembutal IP) increased striatal glucose by 69 +/- 3%(n = 5 rats). In contrast perfusion of the microdialysis probe with Veratridine 50µM for 12.5 min reversibly decreased basal striatal glucose by 94 +/- 2 %(n = 4 rats). These results are all consistent with published data and confirm that ECF glucose levels are influenced by neural activity and body glucose levels. Additional behavioral experiments demonstrated that ECF glucose increased by 50%to restraint stress (n = 1 rat) but was unchanged during experience of an open field test (n = 2 rats). In these experiments each 'bilayer' electrode could be used for up to 4 invivo experiments.

These results demonstrate a reliable and easy to use on-line system that because of it's high sensitivity and stability will facilitate trouble free on-line measurement of ECF glucose. Currently we are endeavoring to adapt this procedure for the measurement of lactate, pyruvate and glutamate.