Brain microvessel hexokinase: Kinetic properties

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Summary. Kinetic differences between brain capillary and parenchymal hexokinase in the presence of glucose, ATP, fructose, potassium, sodium and different pH were established. Parenchymal hexokinase is more susceptible to glucose inhibition, can tolerate greater variations in the ATP concentration, is inhibited by increasing concentrations of fructose and potassium, and showed greater activity on the lower pH values. The data suggest that in brain parenchyma and endothelial cells of brain microvessels, there are 2 different enzymes with regard to the kinetic properties.

Brain microvessels are unique with respect to their barrier mechanism; selective transport of substrates from the blood to brain has been attributed to the specific biochemical organization of the endothelial cells of cerebral capillaries. In an attempt to establish some of the metabolic properties of brain microvessels at the cellular level, we have begun to study capillaries isolated from the brain. Recently, we have proposed the possible role of hexokinase (HK) in carrier-mediated glucose transport from the blood into the brain parenchyma.

In the hexokinase (ATP: D-glucose-6-phosphotransferase, EC 2.7.1.1) reaction, glucose-6-phosphate (glucose-6-P) is formed from glucose and adenosine triphosphate (ATP). Glucose-6-P can be directed towards a) glycogen synthesis, b) pentose phosphate pathway, and/or c) glycolysis. The glycolytic pathway is the main energy source in a number of tissues, particularly in the brain, where HK acts not only at the point of 'distribution' of glucose-6-P into different metabolic pathways but is regulatory enzyme in glycolytic flux as well.

In the previous study, noticeable HK activity was found in isolated brain microvessels in comparison with the brain parenchyma; it was striking to find that the activity of phosphofructokinase (EC 2.7.1.11; PFK), the key enzyme of glycolysis, was low in brain microvessels when compared with the HK activity. Hence, there is great difference in the HK/PFK ratios between brain microvessels and parenchyma indicative for possible distinct roles of HK in these 2 compartments. Therefore, we made an attempt to establish kinetic differences between 2 HK's (capillary and parenchymal) in the presence of glucose, ATP, fructose, potassium, sodium and different pH.
Materials and methods. Adult male Wistar rats were sacrificed by decapitation, skulls were quickly removed, and forebrain homogenized in 5 vol. of the ice-cold homogenizing medium as described elsewhere. Hexokinase activity was determined following the appearance of NADPH at 340 nm in Beckman DB-G spectrophotometer with thermostopped cuvette holder (37 °C). The assay mixture used for the determination of the baseline activity and for the effect of Na⁺ and K⁺ concentrations was composed as follows (final concentrations): Imidazole-Cl buffer, 50 mM, pH 7.1; MgCl₂, 10 mM; dithiotetrol, 0.1 mM; NADP⁺, 0.2 mM; glucose, 0.5 mM; ATP, 0.5 mM; glucose-6-P-dehydrogenase (EC 1.1.1.49), 0.2 U/ml. When the effects of glucose concentrations were studied, ATP concentration was 4 mM; in the study of the effect of ATP, glucose concentration was 4 mM and imidazole buffer was replaced with the HEPES buffer, 50 mM, pH 7.1. For the fructose studies, ATP concentration in the assay mixture was 4 mM and phosphoglucoisomerase (EC 5.1.3.9), 0.2 U/ml was added. Reaction was initiated by the sample adding, and reaction velocities were linear with respect to the time and protein concentrations. Protein concentrations were estimated according to Lowry et al., with bovine serum albumin as the standard. Michaelis-Menten constant were obtained from the double reciprocal plots of Lineweaver-Burk in a non-inhibitory range of the substrate concentrations.

Results and discussion. The HK activity was 147±6 nmoles of glucose/mg protein/min (mU/mg protein) and 56±2 mU/mg protein in brain parenchyma and microvessels, respectively.

Figure, A. When glucose concentration was varied up to 8 mM, parenchymal HK responded with high increase in activity to about 2 mM of glucose being inhibited after that by the substrate. In microvessels, the inhibition was less pronounced and the increase in activity was observed up to 4 mM of substrate. Michaelis-Menten constant (K_m) for 2 HKs were 0.106 mM and 0.147 mM for the parenchymal and microvessel enzyme, respectively. Hence, capillary enzymes can tolerate relatively great changes in glucose concentration (approximately from 0.5 to 4 mM) without significant changes in activity; concentration range is more narrow for the parenchymal HK.

Figure, B. Variations in ATP concentrations up to 4 mM resulted in slight inhibition of microvessels HK, while apparently there was no inhibition of parenchymal enzyme; respective K_m's for this substrate were 0.366 mM and 0.554 mM. These findings may be related to the lower enzyme metabolism of the endothelial cells in comparison with the parenchymal cells, and consequent lower intracellular ATP concentration in capillaries.

Figure, C. When fructose was used instead of glucose as the substrate, in parenchyma the activity was about half of that with glucose exhibiting inhibitory effects with concentrations greater than 1 mM; K_m-value was 0.081 mM. In brain capillaries, with the increasing concentrations of fructose, HK activity reached the activity found to be maximal for the glucose; K_m-value was 0.196 mM. In general, curves for fructose followed those for glucose, except that the parenchymal enzyme is more substrate specific.

Figure, D and E. Parenchymal enzyme activity decreased with the increase in potassium concentration. Microvessel HK responded biphasically: up to 50 mM potassium stimulated HK, but further increase inhibited it. Increase in sodium concentrations caused also biphasic respond of the parenchymal HK: up to 70 mM of sodium concentration caused inhibition of HK, while further increase in sodium