Differential Behavior of Glial and Neuronal Cells Exposed to Hypotonic Solution

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Summary

The comparative modes of swelling of glial and neuronal cells were examined by video-enhanced differential interference contrast (VEC) microscopy. When exposed to hypotonic solution, C6 cells swelled slowly to 5 times their normal volume and burst, whereas N18 cells swelled more rapidly, forming blebs, and then burst partially. The time to burst was 410.6 ± 45.7 s (mean ± SD, n = 5) for C6, and 69.3 ± 10.4 s (n = 5, p < 0.01) for N18, respectively. The present findings suggest a great difference in physical strength of the cell membrane between glial and neuronal cells: the former cell membrane appears to be elastic and tolerant to high tension, while the latter cell membrane is relatively weak.

Keywords: Glial swelling; neuronal swelling; VEC microscopy.

Introduction

The most pronounced histopathological changes occurring at the early stage of cerebral ischemia are swelling of astrocytes in the perikarya and perivascular processes2-4. In contrast, the morphological changes in the neurons are not regular, involving swelling, no change or even shrinking, and usually occur later4,5. Advances in technology based on video microscopy and digital image processing have made it possible to visualize cultured glial and neuronal cells with a spatial resolution which is greatly increased from that of conventional light microscopy. The present study compares the modes of swelling of glial and neuronal cells, as revealed by such video-enhanced differential interference contrast (VEC) microscopy1,3,6.

Materials and Methods

In a previous communication7, we demonstrated that the modes of swelling of cells under hypotonic conditions (osmosis) and swelling due to full opening of all ionic channels at the cell membrane (osmosis with no osmotic gradient) were quite similar in terms of their speed and the magnitude of the fluid shift. This suggests that investigations of cell swelling by hypotonic shock could provide a clue for gaining a better insight into the dynamics of specific astroglial and neuronal alterations occurring in the cerebral cortex during ischemia.

Cultured glial cells (C6; astrogloma) and cultured neuronal cells (N18; neuroblastoma) were inoculated into a flask containing 10% Dulbecco’s modified Eagle’s medium plus 10% Gibco newborn calf serum (DMEM), and then incubated at 37°C in 5% CO2 plus oxygen. Two days before the experiment, cells were harvested from the flask, reseeded and cultured further on a collagen-coated thin coverglass placed in a small dish containing the DMEM medium. On the day of the experiment, the cells were observed and videotaped in a microscopic system devised by one of the authors (S.T.): the coverslip on which the cells grew was taken out, and secured watertightlly with grease on a slide glass having a square window so that a microscopic observation chamber with the cells at the bottom was created. The cells were maintained at 37°C in the microscopic observation chamber by superfusion with either DMEM or distilled water. The morphological changes of the cells were observed during exposure to hypotonic solution under an inverted Nomarski microscope equipped with a x100 DIC objective lens and a x2.5

Fig. 1. Dye concentration curve. Optical density changes were monitored using a small silicon photodiode placed on the monitor screen at the center of the cell. At the downward arrow, a concentrated dye solution of chlorophenol red was introduced into the tip of the feeding tube for the superfusion fluid. Approximately 20 s was required for the dye to reach the cell in the observation chamber. This time lag was subtracted from the total time taken for the cell to burst
Fig. 2. Morphological changes of C6 cells at the control stage (left column) and at just about the time to burst (right column). Note the full moon appearance of the C6 cells in which all intracellular structures appear to be stretched with cell membrane enlargement.

Fig. 3. Morphological changes of N18 cells at the control stage (left column) and at just about the time to burst (right column). Note the hydropic changes of the cell membrane which contains only liquid fluid.

Results and Discussion

Both the C6 and N18 cells contained a large nucleus, mitochondria, lysosomes, and numerous fine granules which were clearly visible in the cells as dark or bright spots moving slowly or rapidly. Frequently, the granules exhibited gliding or travelling movements over fairly long distances at a various but roughly individually constant velocity.

When the hypotonic solution was introduced into the superfusing fluid, the C6 cells (n = 5) swelled slowly. The nucleus as well as the intracellular organelles, such as mitochondria and granular particles, also swelled together with the cell body. Gradual development of liquid fluid areas was observed in the cytoplasm which contained rapidly moving particles (Brownian movement). The cells swelled to 5 times their normal volume, and became like a full moon. The shape changes of each of the C6 cells from the control (left column) to maximum swelling (right column) are illustrated in Fig. 2. The time from onset of hypotonic stress to bursting was 470 s for #1, 324 s for #2, 527 s for #3, 257 s for #4 and 475 s for #5, respectively. The mean ±