2001 Mihara Prize Awardee's Summary

NEURONAL DEPOLARIZATION AND MICROVASCULAR DERANGEMENT -CLINICAL RELEVANCE-

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Stroke is proving intractable. In particular, cerebral infarction, despite the fact that its mortality in Japan has been greatly reduced over the last 20 years, still has a high incidence, and the victims' disabilities continue to create social problems. To clarify some aspects of this multifaceted entity, I have focused on two points: neuronal depolarization causing ischemic cell swelling, and microvascular derangement. I would like to emphasize initially that neuronal depolarization during the early phase of ischemia ignites a huge osmotic potential due to unbalanced intracellular colloid osmotic pressure, which in turn causes an increase in intracranial pressure, and irreversible microvascular derangement due to compression. Since this potential has not been reported in the literature, I will describe this in detail. When activated, neurons have been reported to swell (2). Similarly, when the brain is made ischemic, brain cells, especially the perivascular endfeet of glia have also been reported to swell (1). The colloid osmotic pressure of brain cells was measured by us using brain tissue homogenate by separating it with a copper ferrocyanide semipermeable membrane from mock CBF solution (3). We found that the intracellular colloid osmotic pressure was 213 mm Hg, which is higher than the blood pressure. The osmotic potential, defined as the pressure preventing volume flow across a leaky membrane separating two osmotically different solutions, and therefore the pressure to prevent volume flow across brain blocks soaked in isotonic saline solution, was also found to be 578 mm Hg (4). Employing an in vitro two-compartment model in which NaCl and colloid solutions were separated isoosmotically by an artificial electronegatively charged membrane, we demonstrated that fluid shift through the membrane began immediately following elimination of the negative charge of the membrane (5). The relevant mechanisms in the brain were considered by us to be as follows. From the thermodynamic viewpoint, living brain cells (neurons and glial cells, which swell in ensemble) constitute nonequilibrium, open systems in which ionic trafficking occurs all the time. The high intracellular colloid osmotic pressure is counterbalanced by a steep osmotic gradient

developed by sodium ions, which are excluded intermittently by the sodium pump. Contrary to common belief, I thus believe that the cell under normal conditions is fundamentally very unstable. The cell can maintain its small size only through continuously endeavoring to overcome ionic leakage at the membrane from the environment. When ischemia occurs, with interruption of fuel and oxygen supply, the sodium pump loses its metabolic support, leading to elimination of the osmotic gradient. The cell membrane becomes permeable to small ions and osmosis will take place. However, such osmosis has hardly been predicted hitherto, since there are no gradients of osmotic pressure ($\Delta\pi$), hydraulic pressure (Δp) and temperature across the cell membrane. Why do cells swell? This can be explained by nonequilibrium thermodyamics as $Jv = Lp(\Delta p - \{\sigma_\iota \Delta\pi_\iota - \sigma_{pr}\Delta\pi_{pr}\}$ where Jv is the volume flow, Lp the mechanical filtration rate, σ the Staverman's reflection coefficient, postfix i denotes the ions (here sodium ions) and postfix pr the proteins. Under the conditions that $\Delta p = 0$, $\sigma_{pr} = 1$, change in σ_ι from 1 to <1 causes Jv>0. This may be called isothermal, isobaric, isoosmotic osmosis, which has puzzled researchers. When the potential exerted by the intracellular colloid osmotic pressure becomes uncontrollable owing to the loss of membrane integrity with ischemia, the brain cells swell (cytotoxic edema). Unlike other organs, the swelling of tissue would cause a serious outcome, since the brain is encased in a rigid container, the skull. This is a spontaneous process requiring no extrinsic energy, but occurring just through dissipation of the intrinsically stored energy at the plasmic membrane as a double ionic layer. In a sense, a cell controls polarization/depolarization for function and cell volume by just adjusting the ionic pore size; opening or closing of ionic channels like a carburetor. We found that membrane depolarization and therefore osmotic gradient formation would cause optical changes like scattering or transmission. A recent study has suggested that neurons and glia behave in close association through metabolic collaboration (6) and both types of cells must therefore be involved in brain cell swelling as a neuro-glial (N-G) complex. To maintain such a high potential at the membrane for the uninterrupted function of neurons, the N-G complex needs to be kept continuously supplied with fuel materials from the capillary blood. When neuronal function is elevated, the relevant capillary flow must be increased. If the fuel supply becomes limited or is interrupted, such as in the case of arterial occlusion, the stored potential would soon be depleted. Accordingly, the N-G complex would swell. If the swelling of the tissue is restricted in the rigid skull, an increase in intracranial pressure would ensue.

The microvascular flow changes occurring along with such depolarizing neurons in the ischemic tissue have remained unexamined because of a lack of appropriate

methods. We developed a new optical technique for measuring the capillary-level microflow in a small region of the sensory motor cortex in small animals (7) based on a modification of our previous method (8,9). During continuous recording of cerebral blood volume with transmitted light at 550 nm, one of the isosbestic points of hemoglobin, a blood tracer was injected into the internal carotid artery. From the resultant hemodilution curves, the mean transit times (MTT) in all pixels of a 50 x 50 matrix were calculated with the help of Matlab software and the reciprocal values were expressed on 2-dimensional microflow maps. Microcirculatory flow parameters were extracted from each flow histogram based on moment analysis. When KCl was topically injected into the sensory motor cortex, we observed wave-ring spreads of flow changes (ischemia followed by hyperemia) moving along with induced spreading depression. This proved that the depolarizing neurons can somehow influence the adjacent capillary flow (10). Employing the same technique, the microflow changes in a lesion were examined after occlusion of a small pial artery of ca. 50 □m in diameter. The average flow decreased, but moment analysis of the flow histogram revealed a very low flow component. The sluggish flow component or near-stasis of blood in the microvasculature disappeared with time, presumably gradually shifting to an irreversible state of no-reflow (11). Reperfusion was able to restore flow from stasis with or without tissue injury. In previous experiments, we had found that reactive hyperemia upon reperfusion began to occur at 1 min after occlusion, increased in magnitude with time, peaked at around 10 min to 1 h, decreased again thereafter, and finally disappeared at several hours after occlusion (12). We observed marked edema in the final state. The clinical relevance is that the ischemic flow values yielded by conventional flow measurement techniques must be interpreted cautiously, since they are hardly able to represent the microvascular state in the ischemic focus: stasis (reversible flow) or no-reflow.

In conclusion, neuronal depolarization during ischemia causes swelling of the neuro-glial complex. The swelling, driven by the huge thermodynamic potential of intracellular colloid osmotic pressure, can result in a high intracranial pressure, since the brain is encased in the rigid skull. We measured pressures amounting to 213-576 mmHg, which are far higher than arterial pressure. This could stop the blood circulation through the brain, killing patients with a large infarct.

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