Experimental Evidence of Ischemic Thresholds and Functional Recovery

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**Background:** Impaired blood flow below certain critical levels and an insufficient supply of energy-rich substrates cause failure of neuronal function, triggering biochemical disturbances that eventually lead to ischemic cell damage.

**Summary of Review:** This article reviews experimental studies that attempted to define those ischemic thresholds using functional and histological markers and, more recently, chemical markers of ischemic damage.

**Conclusions:** The duration of ischemia causing irreversible cell damage still is ill defined. Reestablishment of sufficient perfusion must be induced very early after an ischemic attack to ameliorate the potentially harmful biochemical sequelae of transient ischemia. *(Stroke 1992;23:1668-1672)*

**KEY WORDS** • cerebral ischemia • neuronal damage • perfusion

Normal function of the central nervous system requires a steady supply of the major substrates for energy metabolism. When blood flow is decreased below certain levels and substrate supply becomes insufficient, neuronal function fails. In the 1970s, experimental studies concentrated on defining those ischemic thresholds using electrophysiological (and occasionally neurological) methods or histology (reviews in References 1 and 2). More recently, however, research efforts were directed mainly toward the determination of biochemical markers of reversible or irreversible ischemic damage. Since impaired blood flow, no matter how transient, is the cause and/or trigger of longer lasting biochemical derangements eventually leading to ischemic cell damage, this brief review attempts to summarize those changes with respect to critical levels of perfusion.

**Functional and Histological Markers of Ischemic Damage**

Experimental work on the ischemic flow thresholds of brain tissue demonstrated the existence of two critical levels of decreased perfusion: first, a level representing the flow threshold for reversible neuronal failure (functional threshold); second, a lower threshold below which irreversible membrane failure and morphological damage occur. The range of perfusion values between those limits was called the “ischemic penumbra,” which was characterized by the potential for functional recovery without morphological damage, provided that local blood flow can be reestablished at a sufficient level and within a certain time window. The functional threshold was demonstrated in ischemic monkeys gradually developing a neurological deficit progressing from mild paresis at 22 ml/100 g per minute to complete paralysis at 8 ml/100 g per minute (Figure 1a). Concurrently, the electrocorticogram and evoked potentials (EPs) were abolished at 15–20 ml/100 g per minute (Figure 1b and 1c), the spontaneous activity of cortical neurons disappeared at approximately 18 ml/100 g per minute. The large variability of the functional thresholds of individual neurons (6–22 ml/100 g per minute) indicates selective vulnerability even within small cortical sectors. This interpretation is commensurate also with the observed gradual development of neurological deficits. Furthermore, although single-cell activity is already altered at blood flow levels above the threshold, below which grouped or regular discharges occur at a high rate, clear threshold relations could be demonstrated; after short ischemic episodes, spontaneous cellular activity as well as EPs were restored when blood flow was normalized (Figure 1c and 1d).

Whereas neuronal function is impaired immediately when blood flow drops below the threshold, the development of irreversible morphological damage is time dependent. Of course, once morphological damage becomes apparent, the initially reversible functional deficit turns into a persistent defect. Therefore, numerous studies were performed to investigate for how long brain tissue or individual cells tolerate ischemia of a given density. Hossmann and coworkers repeatedly demonstrated that even 1-hour, complete cessation of blood flow to the whole brain can be followed by recovery of electrophysiological function, and also by survival and recovery of neurological function in a few animals. EPs are reliable markers of cortical function and its disturbance, as long as the respective sensory cortex is included in the core of ischemia. Recordings of evoked responses in experimental middle cerebral artery (MCA) occlusion showed that EPs recover even after dense ischemia of up to 1 hour. Conversely, EPs...
disappear during focal ischemia, with the sequence of failure of multimodal responses differing among brain regions. Typically, that decrease is instantaneous in the core of cortical ischemia and slower and more gradual in the vicinity of a focal flow disturbance. In areas outside the MCA territory, EPs are abolished despite nearly normal cortical blood flow values, thus indicating an ischemic effect on afferent pathways in the white matter. Differences between cortical and white matter ischemia were studied in greater depth, using simultaneous recordings of flow and electrophysiological activity in thalamic relay nuclei, cerebral white matter, and primary cortex. During the ischemic period, the EPs were abolished both in auditory and in somatosensory cortex. After 1-hour ischemia at a local flow rate of 8.5 ml/100 g per minute, the amplitude of auditory cortical EPs attained only 18% of its preischemic level during reperfusion. However, in somatosensory cortex, where blood flow remained close to normal at all times, EPs recovered to 94% of their starting amplitude. That
transient blockade was caused by ischemia of 6.4–7.6 ml/100 g per minute in the afferent pathways, while no flow changes were observed in related thalamic nuclei. These findings shed some doubt on the reliability of EPs as markers of neurological function and cortical damage, since they are also altered by (reversible) interruption of white matter tracts. The reversibility of that blockade, in turn, attests not only to the fact that white matter can take longer periods of severe ischemia than cortex, but it also suggests that functional impairment due to deafferentation has a better prognosis than cortical damage caused by direct cortical ischemia.

The interaction of severity and duration of ischemia in the development of irreversible cell damage can be studied by simultaneous recordings of cortical neuronal activity and local blood flow.8 The ischemic tolerance of neurons obviously is quite variable, as could be demonstrated with simultaneous recordings using up to eight closely spaced microelectrodes.14 On the basis of a large number of neurons assessed during and after ischemia of varying degree and duration, it was possible to construct a discriminant curve representing the worst activity and local blood flow.8 The ischemic tolerance of cortex, but it also suggests that functional impairment can take longer periods of severe ischemia than cortex, with different mechanisms responsible for immediate and delayed destruction of cells.

Chemical Markers of Ischemic Damage

Of the many interrelated biochemical mechanisms contributing to ischemic cell damage, various markers can be used as indicators of ischemic thresholds and of reversible or irreversible functional deficits. In accordance with the critical role that the supply of energy-rich substrates plays in brain function, cerebral ATP content (Figure 1g)2 and cerebral metabolic rate of glucose (Figure 1h) exhibit a threshold dependency similar to the electrophysiological variables. Actually, the mean thresholds for ATP depletion (18.5 ml/100 g per minute) and spontaneous neuronal activity (18 ml/100 g per minute) are identical. However, it should be noted that the decrease in ATP content sets in at a slightly higher flow value, at which point glucose consumption is increased in an attempt to compensate for the loss of oxygen. At that flow level, local hypoxia induces anaerobic glycolysis, resulting in excessive lactate production. Using proton magnetic resonance spectroscopy, Behar et al25 could demonstrate that increased lactate concentration is an indicator of progressive tissue destruction. In animals recovering from total cerebral ischemia, phosphocreatine and ATP soon return to normal, whereas lactate and pH, after some delay, normalize only slowly. Conversely, further deterioration of those biochemical markers was found in animals eventually showing no recovery after 1-hour total ischemia.

In this context it should be mentioned that protein synthesis does not follow the usual thresholding pattern, with definite changes occurring at a flow rate of approximately 20 ml/100 g per minute.26 Its function of flow dependence (Figure 1i) rather indicates that normal protein synthesis requires a significantly higher level of blood flow (mean value, 55 ml/100 g per minute), which is considered to be without effect on regular neuronal function and morphology. Therefore, protein synthesis may not be a critical factor in the pathophysiology of ischemic cell damage. It rather may be involved in mechanisms of neuroplasticity because larger portions of the brain outside the ischemic region proper typically are deactivated. However, since blood flow and protein synthesis in those experiments were determined autoradiographically only once, at 1 hour after MCA occlusion, short-lasting, transient perfusion abnormalities may have gone undetected. Such severe ischemic episodes of short duration may then have triggered biochemical changes in the neighborhood of the ultimate infarct, which in turn may have caused depression of protein synthesis and selective neuronal loss. Single cell necroses outside the areas of gross infarction actually could be demonstrated and their density correlated with persistent flow decreases, thus reemphasizing the close relation between cell count and metabolic demand.27

Ionic homeostasis and tissue water content are heavily affected by ischemia, with different mechanisms responsible for immediate and delayed destruction of cells. For example, it is generally accepted that irreversible neuronal damage is indicated by increases of extra-
cellular potassium (Figure 1e)\textsuperscript{28} and intracellular sodium concentrations (Figure 1e) by the water content of the tissue.\textsuperscript{2} In recent years, the Ca\textsuperscript{2+} influx into cells was also found to be one of the central mechanisms of immediate as well as of delayed cell damage.\textsuperscript{23} As shown by Greenberg et al.,\textsuperscript{27} the intracellular Ca\textsuperscript{2+} concentration again follows a flow threshold relation similar to the electrophysiological variables (Figure 1f), which may be reversible after reperfusion. Using severe, transient ischemia, two distinct time courses of Ca\textsuperscript{2+} accumulation were observed. In some areas, Ca\textsuperscript{2+} concentrations further increased during reperfusion, showing serious damage on subsequent histological examination. In contrast, the tissue remained histologically intact when Ca\textsuperscript{2+} concentrations became normal after onset of reperfusion. By administration of the Ca\textsuperscript{2+} entry blocker nimodipine the detrimental accumulation of Ca\textsuperscript{2+} was reduced, and electroencephalographic recovery as well as histological changes were improved in comparison with the control group. Moreover, labeled nimodipine can be used as a marker of activated Ca\textsuperscript{2+} channels,\textsuperscript{30} in regions with dense ischemia that later were found infarcted, [\textsuperscript{3H}]nimodipine binding increased earlier than in areas of milder hypoperfusion. When binding declined in a region where it was previously increased, infarction was likely to develop. Therefore, nimodipine binding permits assessment of the severity of ischemic tissue damage at an early stage and may help to identify essentially viable tissue. From their results on time-dependent cyclic changes of receptor activation and deactivation, the authors conclude that the penumbra is not an anatomic space in ischemic brain but rather a dynamic phase through which ischemic regions pass in their progression to infarction. The delay in onset of this cycle depends on the severity and duration of the ischemia.

During the past few years, yet another mechanism has attracted much attention and research effort: the release of excitatory amino acids and the activation of the respective receptors and receptor-operated ionic channels during ischemia. First results were obtained in cell cultures,\textsuperscript{31,32} but increases in glutamate and aspartate were also demonstrated in vivo, namely in hippocampal structures.\textsuperscript{29,31} During global as well as during focal ischemia, Shimada et al\textsuperscript{34} found even larger increases in glutamate and aspartate concentrations in cerebral cortex. Those changes as well as the increased concentration of the inhibitory amino acid y-aminobutyric acid (GABA) followed the typical flow threshold relation (Figure 1j and 1k). Blood flow–dependent changes were observed mainly for transmitter and, to a lesser extent, for modulator amino acids but not for essential amino acids without synaptic action, thus indicating that amino acids are released by depolarization of excitatory membranes and not by an unspecific effect on cell metabolism. The observed reversibility of the increase in glutamate concentration after transient ischemia emphasizes the importance of early intervention to counteract the neurotoxicity of excitatory amino acids. The inhibitory amino acid GABA, the concentration of which is also increased during ischemia, obviously is not a very efficacious internal antie excitotoxic compound for two reasons: first, the excess of excitatory agents render its inhibitory action insufficient; and second, in contrast to glutamate receptors that are resistant to ischemia,\textsuperscript{35} GABAergic receptors are impaired by ischemia.\textsuperscript{36}

However, another mechanism of counteracting excitotoxins may be of some import. The increases in adenosine and purine catabolites exhibit threshold characteristics (Figure 1l) similar to those of glutamate, but their blood flow threshold (25 ml/100 g per minute) is slightly higher than that for amino acids.\textsuperscript{37} Whereas the ischemia-induced elevation of adenosine was found to be only transient, that of inosine, hypoxanthine, and glutamate persisted during the entire ischemic period. The higher flow threshold for the induction of adenosine release, which exerts inhibitory action and therefore has the potential to ameliorate glutamate release and its receptor-mediated effects, may represent an inherent but time-limited protective mechanism against excitotoxicity.

**Therapeutic Window**

All evidence indicates that there is a clear ischemic threshold for the production of functional impairment and for the triggering of biochemical disturbances eventually leading to cell destruction. However, the duration of ischemia causing irreversible cell damage, i.e., the point of no return, still is ill defined. It depends on the level of residual blood flow and on specific cell properties determining their ischemic vulnerability. Moreover, its estimate is also strongly affected by the experimental design of a study. Several experiments indicate that the actual time window for the restoration of blood flow may be much narrower than the interval left for effectively reversing or inhibiting biochemical processes initiated during the ischemic episode, which otherwise persist and contribute to ischemic cell damage. Even after 5-minute ischemia, the number of viable neurons in the gerbil's hippocampus can be significantly increased by halothane anesthesia.\textsuperscript{38}

To be of benefit reestablishment of sufficient perfusion must be induced very early after an ischemic attack. In contrast, treatments aimed at late improvement of blood flow may have little effect because at that time reperfusion of still viable tissue often already has occurred spontaneously. Therefore, it may be more rewarding to interfere with the various complex biochemical changes that essentially determine the fate of the tissue in the long run. No matter how controversial the efficacy of such strategies still may be, at least they should follow all conventional measures taken to reestablish perfusion to ameliorate the potentially harmful biochemical sequelae of transient ischemia.

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