Ionic and Volume Changes in Neuronal Microenvironment

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Neuronal elements are separated by only narrow intercellular spaces, which are rich in ions, transmitters, proteins, neuropeptides and enzymes. The intercellular space is known as the extracellular space (ECS) or neuronal microenvironment, and its existence as well as disturbances caused to it by external environmental changes were neglected by scientists for many years.

The modern conception of the neuronal microenvironment has been developed recently and is based on research results of the last 25 years. Since then, scientists have made major contributions to our understanding of:

a) the function of glial cells, including their influence on the composition and size of the microenvironment of nerve cells,

b) the function of the ECS, in terms of its dynamic ionic and volume changes and the physiological significance of these.

These findings gave rise to the view that the ECS functions as a communication and modulation channel, whose ionic and chemical composition and anatomical structure depend on neuronal activity and glial cells function. In turn, its composition significantly influences the complex function of the neurones and glial cells in nervous tissue and in the sensory organs. Czech scientists – Jan Bureš, Pavel Hník, Norbert Kříž, Eva Syková, Evžen Ujec, Ladislav Vyklický and František Vyskočil – contributed to this research from 1970 by modifying ion-selective microelectrodes for use in neurophysiology. Together with Canadian scientists K. Krnjevic and M. Morris (1972), they determined as first the dynamic extracellular changes in potassium concentration ([K+]e) during neuronal activity in the brain cortex, the mesencephalic reticular formation, and the spinal cord, in skeletal muscle, and in the organ of Corti (Hník et al. 1972, Vyklický et al. 1972, Vyskočil and Kříž 1972, Vyskočil et al. 1972, Syková et al. 1974, Johnstone et al. 1989).

This review deals with major ionic changes in the microenvironment of neuronal and receptor cells (including potassium, calcium and pH changes) with the mechanisms of changes, with redistribution of ions (homeostasis), and with the physiological significance of these changes. It deals also with new findings on dynamic changes in the volume of the ECS and their possible physiological significance. It is not surprising that various transmembrane fluxes of ions that are produced by neuronal and fibre activity modify the ionic composition of the fluid in the ECS. During action potential or during excitatory postsynaptic potential (EPSP), Na+ and/or Ca2+ enter the intracellular compartment and K+ leaves it. In addition to the typical Na+ and K+ exchange, there are dynamic changes in the
concentration of Ca\textsuperscript{2+}, Cl\textsuperscript{-}, HCO\textsubscript{3}\textsuperscript{-}, and H\textsuperscript{+}. The ionic changes which occur during and long after neuronal activity are accompanied by movement of water into the cellular elements, resulting in cell volume increase and ECS volume decrease. This research was made possible primarily by the use of ion-selective microelectrodes, which allow the measurement of the dynamic ionic changes in living tissue in the immediate vicinity of nerve and glial cells.

**Ion-selective microelectrodes**

An ion-selective microelectrode (ISM) that consists of a liquid membrane (liquid ion-exchanger, ion-carrier) placed in the tip of a glass micropipette is a miniaturized potentiometric sensor. When introduced into tissue or solution where the activity of the respective ion is to be measured, a Nernst potential develops across the ion-exchanger membrane, i.e. one measures a potential that changes logarithmically with the activity of the ion for which the ion-exchanger is selective. However, when the microelectrode is inserted into neural tissue, it records not only electrical potential differences generated by free ion concentrations, but also all types of electrical activity, including membrane potentials, action potentials, and synaptic potentials. This disturbing factor can be abolished if the reference electrode is in the immediate vicinity of the tip of ISM. This requirement is best fulfilled by a double-barrelled microelectrode (see Fig. 1) which has a liquid ion-exchanger in one channel, while the other channel serves as the reference microelectrode and is therefore filled with saline or some other indifferent solution (Kříž et al. 1974, 1975, Syková et al. 1974). Since the reference microelectrode also records electrical activity in the tissue, the signal from it is used to cancel out the undesired component.

![Fig. 1](attachment:fig1.jpg)

Schematic cross-section of a double-barrelled ion-selective microelectrode.
Nowadays we have at our disposal ion-exchangers (natural and synthetic ionophores) for $H^+$, $K^+$, $Li^+$, $Na^+$, $Ca^{2+}$, $Mg^{2+}$, $Cl^-$ and $HCO_3^-$. The ISMs are widely used in electrophysiology and the theory underlying their operation and their preparation has been described in several monographs (Syková 1976, Thomas 1978, Koryta 1980, Syková et al. 1981, Ammann 1986).

**Extracellular $K^+$ changes**

The resting level of extracellular $K^+$ can significantly differ from that in cerebrospinal fluid as well as in different parts of the nervous system. It has been demonstrated in the rat and frog spinal cord (Syková et al. 1983, Svoboda et al. 1988), in the rat mesencephalic reticular formation (Syková et al. 1974) and in the rat cortex (Moghaddam and Adams, 1987) that the $K^+$ level can vary between 2.6 and 5.0 mM in the unstimulated animal, apparently in structures with high spontaneous activity level (Fig. 2).

![Fig. 2](image)

**Fig. 2**
Top line: Activity-related extracellular potassium changes ($K^+$) in unstimulated reticular formation of rat associated with burst of firing of cell. Bottom line: Neuronal firing recorded by reference barrel. (Modified from Syková et al. 1974).

Activity related changes in $[K^+]_e$ have been found in brain, spinal cord, peripheral nerves (for review see Somjen 1979, Nicholson 1980, Syková 1983), receptor organs (Karwoski et al. 1989) and in organ of Corti (Johnstone et al. 1989). Almost all kinds of stimulation – artificial or adequate – lead to increases in $[K^+]_e$. The main sources of $K^+$ are stimulated neurons, unmyelinated fibres, and unmyelinated terminals of myelinated axons. An increase of 0.1 to 0.5 mM has been found after a single electrical stimulus applied to peripheral input or after a single adequate stimulus (light touch or pinch) applied to the skin (Fig. 3A). Repeated stimulation leads to summation of responses from a baseline level of about 3 - 4 mM to as much as 10 - 12 mM. When stimulation is continued, no further changes in $[K^+]_e$ are found because a steady state is established, which is the result of concurrent release and clearance of $K^+$ (Fig. 3C). This so-called "ceiling level" (Kříž et al. 1975, Heinemann and Lux 1975) is only broken through by pathological events,
A long-term increase in $[K^+]_e$ in the spinal dorsal horn of the rat is evoked by chemical and thermal injury of the hind paw (Svoboda et al. 1988). The increase evoked by injury begins several minutes after injury and persists for many minutes or even hours.

Fig. 3
Transient changes in extracellular potassium concentration (A) and in extracellular pH (B) in the L4 spinal segment in response to light touch (touch), to application of pressure to the toes (press), and to application of about 1 ml of hot water onto the hind paw (heat). C: Extracellular potassium and pH changes in response to repetitive electrical stimulation at frequency 100 Hz. Horizontal bars indicate stimulus duration. Recorded at a depth of 600–700 µm from dorsal spinal surface. (Modified from Svoboda et al. 1988 and Sykova and Svoboda 1990).

Active neurones that lose K$^+$ and thereby induce a change in the ionic composition of the extracellular fluid can significantly affect the function of neighboring neurones. Although this may be an important way in which neurones interact and integrate their activity, stability of nervous tissue function requires prompt renewal of the neuronal microenvironment composition. Many observations support the conclusion that the changes in $[K^+]_e$ are cleared by active uptake across neuronal membranes and by glial cells.

The conclusion that K$^+$ is cleared by means of active transport is well-supported. With repetitive stimulation, $[K^+]_e$ falls from the ceiling level during stimulation and when stimulation stops, it decreases below resting level, i.e. there is a post-stimulation "undershoot" (Fig. 3C). In particular, the lowering of $[K^+]_e$ below
baseline points to the participation of active transport processes in the redistribution of accumulated $K^+$. The decrease of $[K^+]_e$ during stimulation and the undershoot can be blocked by ouabain, a known inhibitor of Na/K pump, or by anoxia, ischaemia and anaesthetic drugs.

**Fig. 4**
Stimulation-evoked changes in extracellular potassium and pH in spinal dorsal horn of rats 3 and 10 days old. Note that stimulation at a frequency of 10 Hz evoked an alkaline shift in the 3-day-old pup, which was accompanied by an increase in extracellular potassium. When stimulation was discontinued, the post-stimulation acid shift appeared, which was accompanied by a $K^+$-undershoot. In the 10-day-old pup the potassium increase was smaller; there was a small initial alkaline shift, which was followed by an extracellular acid shift (compare with Fig. 3C). (Modified from Jendelová and Syková 1991).

Glial cells contribute to $K^+$ clearance by two types of uptake processes: uptake of $K^+$ driven by Na/K pump (Hertz 1965) and spatial buffering (Orkand et al. 1966). It has been demonstrated (see Fig. 4) that the $[K^+]_e$ ceiling level in rats is closely related to gliogenesis (Jendelová and Syková 1991).

**Extracellular $Ca^{2+}$, $Na^+$ and $Cl^-$ changes**

In addition to $[K^+]_e$ changes, neuronal activity is accompanied by the changes in the concentration of other ions in the microenvironment of the nerve and receptor cells. In the case of $Na^+$ and $Cl^-$ it is difficult to measure small
extracellular changes since the sensitivity of the relevant ion-selective microelectrodes is small in the context of the high extracellular activity of these ions. However, the dramatic changes in $[\text{Na}^+]_e$ and $[\text{Cl}]_e$ have been found during anoxia, epilepsy and spreading depression (for review see Somjen 1979, Nicholson 1980, Syková 1983).

Decrease in $[\text{Ca}^{2+}]_e$ has been observed during activity in different parts of the brain and spinal cord, during stimulation of afferent input, as well as in pathological states. It has a time course similar to $[\text{K}^+]_e$ increase. Ceiling level is achieved during repetitive stimulation. When stimulation is discontinued, recovery takes place quickly, as in the case with $[\text{K}^+]_e$ and there is an "overshoot" to concentrations higher than the original resting level. Repetitive stimulation causes a $0.1-0.5$ mM drop in $[\text{Ca}^{2+}]_e$, i.e. it falls from $1.2-1.6$ mM to less than $1.0$ mM (Chvátal et al. 1988). During pathological states, $[\text{Ca}^{2+}]_e$ falls to very low values of about $0.1$ mM. The decrease in $[\text{Ca}^{2+}]_e$ is caused by movement of Ca$^{2+}$ into the neurones and presynaptic endings and it may be an essential factor for modulation of transmission in CNS.

Extracellular pH changes

The use of pH-sensitive microelectrodes has proven to be the best method to study activity-related dynamic pH changes in nervous tissue in vivo. Since the extracellular pH ($\text{pHe}$) depends on depolarization of the neuronal membranes, on the metabolic activity of the nerve cells and on ion shifts across the cell membrane (for review see Thomas 1988, Syková 1989, Chesler 1990), neuronal activity can be expected to be accompanied by dynamic changes in pH.

The pH$_e$ resting levels studied so far in CNS vary between $7.10-7.35$, i.e. pH is slightly alkaline. In spinal cord of the rat, pH$_e$ was most acid in the lower dorsal horn — about $7.15$, i.e. about $0.2$ pH units more acid than the pH of the cerebrospinal fluid (Syková and Svoboda 1990). Transient biphasic (alkaline-acid) or triphasic (alkaline- acid-alkaline) pH$_e$ shifts were found in cortex, hippocampus, cerebellum, spinal cord, and peripheral nerves and in the retina during electrical or adequate stimulation of afferent input (Fig. 3B,C), seizures, anoxia, ischaemia and spreading depression. It has been found that polarized neurones change their surface pH (see Thomas 1988), their surface pH becoming alkaline. Repetitive stimulation results typically in an initial alkaline shift which is followed by acidification which builds up during the stimulation, reaches ceiling level and drops when stimulation is discontinued with the same time course as $[\text{K}^+]_e$. It drops below the original pH baseline, i.e. there is a pH post-stimulation undershoot associated with the post-stimulation K$^+$ undershoot, which can also be blocked by ouabain (Fig. 3C, Syková and Svoboda 1990). These results show that the recovery of the activity-related $[\text{K}^+]_e$ and pH$_e$ changes may have a common mechanism, the most important being Na/K pump activity and presumably also glial cell spatial buffering. The mechanisms of activity-related transient pH$_e$ changes and the mechanisms by which pH$_e$ is stabilized are not yet clear. However, it has been shown that active neurones, primary afferent fibres and polarized glial cells are responsible for the observed changes.

In neonatal rat spinal cord during the first 6 postnatal days, a stimulation of the afferent input evokes large alkaline changes, which are followed by slow and
small acid shifts after stimulation has been discontinued. The alkaline shifts progressively decrease with development and the acid shift becomes dominant about 10 days after birth, i.e. at the same time as the [K+]e ceiling level starts to decrease (Fig. 4). Since gliogenesis to a great extent occurs postnataUy with the peak at about 10 days and later, it can be concluded that in spinal cord (Jendelová and Syková 1991) as in the optic nerve (Ransom et al. 1985), the stimulation-evoked acid shifts are related to gliogenesis. When gliogenesis is blocked, e.g. by postnatal X-irradiation, the acid shifts are also blocked, while the alkaline shifts persist (Syková et al. in press). It is therefore evident that activity-related alkaline shifts in pH_e, as well as the [K+]e ceiling level, are effectively buffered by glial cells.

To clarify the mechanisms of the observed pHe changes, we studied the effects of various inhibitors of enzymatic activity and of ion transport mechanisms across cell membranes. The initial alkaline shift was blocked by Ca2+ and H+ channel blockers. The stimulation-evoked acid shifts were blocked by amiloride, SITS, DIDS and furosemide. They, therefore, have a complex mechanism which includes Na+/H+ exchange, Cl-/HCO3- exchange, Na+/Cl-/H+/HCO3- antiport or Na+/HCO3- cotransport and K+/Cl- cotransport (Sykovi and Svoboda 1990), transport mechanisms described recently in neuronal and glial cell membranes (for review see Walz 1989, Syková 1989).

Dynamic changes in the extracellular space volume

Ion-selective microelectrodes made it possible to follow diffusion of ions in tissue. Diffusion in the extracellular space in the nervous tissue is influenced by its size, i.e. volume fraction (alpha) and by its tortuosity (lambda). Dynamic changes in the size of the extracellular space can be studied by means of the iontophoretic application of ions which do not cross the cell membranes but remain in the extracellular space. Their concentration is therefore in inverse proportion to the size of the space (see Nicholson and Rice 1988). This method is used for the determination of diffusion coefficients, volume fraction, tortuosity and cellular uptake. It has been demonstrated recently that the ECS volume in CNS amounts to about 0.23 and tortuosity to about 1.6.

Activity-related dynamic changes in the extracellular space volume (ESV) occur locally, in concert with the already described ionic changes. Reversible increases in size of the cells, which are accompanied by decreases in the ESV have been demonstrated in the cerebral and cerebellar cortex of rats (for review see Nicholson and Rice 1988), in spinal cord (Svoboda and Syková in press) and in the optic nerve (Ransom et al. 1985) during direct electrical stimulation, as well as during pathological events. Under more physiological conditions, dynamic changes in the ESV were found in rat spinal cord after peripheral injury (Svoboda and Syková in press). The ESV changes in CNS vary according to stimulation intensity, duration and frequency. The ESV decreases by as much as 30–50 % (i.e. to about 12 % of total tissue volume) and this occurs after electrical stimulation as well as after peripheral injury. ESV changes develop more slowly than ionic changes, but they can last for many minutes or even hours after the cessation of stimulation or injury. Even larger decreases in the ESV (to about 4 % of total ESV) have been found during terminal anoxia and spreading depression.
The mechanisms of ESV changes can be deduced from changes in tissue osmolarity, from metabolic changes, and from ionic shifts across the cell membranes. The ion shifts across cell membranes accompanied by intake of water are likely to be most significant in determining of the neuronal, glial cell and extracellular space volume.

Functional significance of activity-related ionic and volume changes

It has been proposed that transient ionic and volume changes in the extracellular microenvironment might be a powerful mechanism in the regulation of nervous tissue function. Changes in extracellular K⁺ and Ca²⁺ activity can influence neuronal excitability, transmitter release, intercellular communication, glial cell function, etc. and can therefore act as a signal. Ionic changes (K⁺, Ca²⁺, H⁺) will simultaneously, but independently, stimulate metabolism and they act as a strong vasodilatory stimuli. The changes may have important effects on manifestation of diseases and on reversibility of pathological states. Moreover, K⁺ accumulation has been accepted as one of the causal factors (together with GABA) in primary afferent depolarization, which is the mechanism underlying presynaptic inhibition (Kříž et al. 1975, Syková and Vyklický 1977, Vyklický et al. 1978).

Changes in pHₑ accompanying neuronal activity as well as changes during pathological states have been shown to influence impulse transmission (for review see Thomas 1988, Syková 1989, Chesler 1990). Changes in pHₑ of only a few tenths of a pH unit have been shown to evoke a marked effect on neuronal Na⁺ and Ca²⁺ channels and on neuronal excitability. Moreover, pHₑ changes can influence cell metabolism, proliferative capacity, cell-to-cell coupling, regulation of cell volume and glial cell function. The activity-related pHₑ changes can, therefore, be an important modulating and controlling factor in CNS.

Even when significant changes in ESV occur in CNS, we must admit that its functional significance is far from clear. However, a regional inhomogeneity in ESV and a very low ESV would predispose a brain region to synchronous firing via enhancement of electric field excitation (see MacBain et al. 1990). The changes observed in the shape and dimension of postsynaptic elements may provide a morphological basis for the facilitation of transmission in nervous tissue (Van Harreveld and Físková 1975). Uptake of water by dendritic spines can be the basis for long-term potentiation of transmission. An increase in synaptic transmission efficacy may be one of the mechanisms leading to plastic neuronal changes and it can, therefore, influence the formation of short-term memory and furthermore, by its effect on proteosynthesis, also long-term memory.

Conclusions

I have described various changes in the composition and volume of the CNS microenvironment, their possible mechanisms and functional significance. Some of the studies were carried out by scientists in Prague. However, many of the important findings about dynamic ionic and volume changes in the CNS microenvironment, and especially about glial cells properties and function were obtained elsewhere. There are still many questions to be answered. It remains to ascertain to what extent ionic and volume changes in extracellular space ensure intercellular interaction and
integration. Do they provide a kind of "wireless" connection in CNS, alongside the well-described and familiar connection via nerve fibres and synapses? What is the role of ionic and volume changes in pathological processes, during injury, and in various diseases including inflammatory and demyelinating ones? Further research on ionic and volume changes, together with research on glial cells function, may disclose other functional consequences of activity-related changes in the microenvironment of nerve and receptor cells.

References


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