Introduction

Ion concentration gradients provide the basis for neuronal activity, and neuronal activity in turn leads to changes in extracellular ion concentrations which are very significant during seizures and seizure-like events although lower than in case of spreading depolarization or anoxic depolarization. These changes are mostly due to transmembrane ion fluxes mediated by voltage and ligand gated ion channels. Ligand gating may occur from the intracellular or/and from the extracellular side of the membrane. Transmembrane transporters in most cases contribute only marginally to fast changes in extracellular ion concentrations, since transport protein density and transport capacity are much smaller than those of ion channels. In the long run, of course, the transporters must sustain the transmembrane ion concentration gradients. The transmembrane ion concentration gradient for potassium, and to a lesser extent for sodium and other ions, sets the resting membrane potential to approximately \(-60\) mV. This resting membrane potential level, in turn, determines the availability of voltage gated ion channels and thereby strongly influences neuronal excitability. For example, at a membrane potential of \(-50\) mV the voltage-gated \(\text{Na}^+\) channels transiently open and allow the entry of cations into the intracellular space generating an action potential. At the peak of the AP they inactivate and neurons are therefore no longer excitable. The difference between resting membrane potential and the equilibrium potential for a given ion sets the driving force for transmembrane ion flux. For example, at a resting potential of \(-60\) mV, the driving force for \(\text{Ca}^{++}\) influx into the cell is close to \(170\) mV due to the equilibrium potential for \(\text{Ca}^{++}\) ions beyond \(+120\) mV.

Changes in transmembrane ion concentration gradients therefore affect membrane potential and the transmembrane ion currents induced by opening of voltage- and ligand-gated ion channels. During and following periods of increased neuronal activity, ion transporters must restore the normal transmembrane ion concentration gradients by using (directly or indirectly) ATP. The brain’s dependence on this function accounts for most of the 15% of the whole organism’s oxygen consumption.

Background

The extracellular space is narrow and very tortuous and it accounts for about 15% of the overall brain volume. This space is filled with extracellular fluid containing proteins and electrolyte, its colloid osmotic pressure is low. In this extracellular milieu, significant concentrations of the amino acids \(\text{GABA (20 \mu M)}\), \(\text{taurine (20 \mu M)}\), and \(\text{glutamine (500 \mu M~2 mM)}\) are present. \(\text{GABA and taurine may activate high affinity GABAA, GABAB, and glycine receptors, generating tonic inhibition. Glutamine is generated in glutamine synthetase-containing astrocytes from glutamate, and is exported into the extracellular space; neurons then take up glutamine for GABA and glutamate synthesis. Lack of glutamine contributes to hepatic coma. The extracellular space also contains glucose which is vital for brain metabolism. The pH of the extracellular space is 7.4. The electrolyte composition in the extracellular space is similar,
but not identical, to that of the cerebrospinal fluid. The potassium concentration ([K⁺]₀) is between 2.7 and 3.2 mM at rest, the sodium concentration ([Na⁺]₀) is about 155 mM, the chloride concentration ([Cl⁻]₀) 135 mM, the calcium concentration ([Ca²⁺]₀) 1.6 mM, and the magnesium concentration ([Mg²⁺]₀) about 1–1.2 mM. The bicarbonate concentration is about 21 mM. There is iso-osmolarity between the interstitial and intracellular space, and the sum of cation concentrations equals the sum of anion concentrations. Salts are not fully dissociated in the extracellular electrolyte (as described by their activity coefficients). Unfortunately, we do not know all ion interacting partners, so that our view of ionic concentrations is based on comparison to calibration solutions. In the case of calcium, this point is particularly important, as Ca²⁺ binding proteins exist in the extracellular space and bicarbonate can interact with Ca²⁺ to form Ca(HCO₃)₂ whose solubility depends on the pCO₂. The 1.6 mM Ca²⁺ concentration given earlier compares to a calibration solution containing also 21 mM bicarbonate. In absence of bicarbonate this would correspond to 1.2 mM. The transmembrane ion movement through ion channels is determined by the driving force and the conductance. The relevant formula is Iₙ = gₙ (Eₘ – Eₙ), with I being the transmembrane current carried by a defined ion x, Eₘ being the actual membrane potential, gₙ the conductance for the ion x, and Eₙ being the equilibrium potential for the ion species x. The resultant ion changes in the extracellular space depend not only on the transmembrane ion fluxes themselves but also on the size of the extracellular space, and on regulatory processes such as diffusion and transport. An open blood–brain barrier can be a confounding variable in trying to measure extracellular ion concentrations; in such conditions, the Ca²⁺ and Mg²⁺ binding of plasma proteins will lower apparent concentrations of these ions, while the higher and more variable K⁺ concentration in plasma will affect baseline extracellular K⁺ concentrations. In fact, in many conditions with epilepsy, tumors, inflammations, bleeding and ischemia, the blood–brain barrier is open.

As indicated earlier, the magnitude of ion shifts is influenced by extracellular space (ES) size changes. Shrinkage of the ES due to water uptake into cells (and subsequent cell swelling) will increase baseline ion concentrations, while widening of the ES (eg, due to increases in colloid osmotic pressure as a consequence of protein exudation from the plasma into the interstitial space) will lower baseline concentrations. During seizures, shrinkage of the ES by up to 30% has been reported. This change would elevate Na⁺ and Cl⁻ concentration to near 200 mM, while K⁺, Ca²⁺, and Mg²⁺ concentration would be much less affected. For quantitative estimates of the transmembrane ion fluxes, therefore, ES size changes must be taken into account.

The intracellular space is, of course, shared between neurons and the different types of glial cells. This space is always larger than the extracellular milieu and hence the ionic changes are usually thought to be smaller than indicated by ion concentration changes in the extracellular spaces – but this is not necessarily the case. Assume for a moment that extracellular Na⁺ decreases by 10 mM, but the ES shrinkage is 30%. As a result of the ES change, baseline sodium would increase to near 200 mM. For that change to occur, 60 mM Na⁺ would have to move across membranes into the cell. If the interneuronal volume fraction is 40%, then the increase in intracellular Na⁺ concentration would amount to about 25 mM; however, due to the extracellular space shrinkage, the Na⁺ concentration declines only by 10 mM (instead of the projected 60 mM). Thus, the ES size change compensates, in part, for the large transmembrane movements. Shrinkage of the ES has a further consequence. It increases the extracellular resistivity, thereby augmenting extracellular potentials and potential gradients generated by glial cell and nerve cell transmembrane currents. This influence is particularly important in conditions where (eg, due to trauma or hypoxia) the ES is already reduced, or in areas such as rodent hippocampal CA1 pyramidal cell layer where the ES size is about 10% and thus smaller than elsewhere in cortical structures. Indeed, ionic changes and extracellular space size changes are not uniform in cortical structures. The largest ionic changes are usually seen in layer IV/V of neocortex and in the cell layers of the hippocampus (granule and pyramidal cell layers). At those sites, ES shrinkage during seizure like events and/or prolonged repetitive stimulation are also maximal, while at remote sites (such as in superficial cortical layers or in stratum molecular of the hippocampus) a widening of the extracellular space has been seen.

Methods

Changes in ion concentrations, inside or outside neurons, can be measured with ion selective microelectrodes. These electrodes are filled at their very tips with a resin which can resolve the activity of specified ions (eg, K⁺, Ca²⁺, Na⁺, Cl⁻, or protons). Within the electrode (above the tip), this resin is superfused by a defined solution containing the ion of interest. When the electrode tip is immersed into an ionic solution, the ion for which the resin is selective will move into the resin and create a ‘border potential.’ The difference between the border potential inside the electrode (at the resin–electrolyte border) and outside the electrode tip (at the border between the resin and the outside medium) responds in a Nernstian manner to a change in ionic concentration (provided the measurements are done within the range of concentrations at which the ion-sensing microelectrode is selective). Such an electrode measures field potentials also. Therefore, a second electrode is required to measure the local field potential, and that field is then subtracted from the signal of the ion selective microelectrode. In practice, we use double barreled microelectrodes (one barrel filled with resin, the other to measure field potentials), the sensitivity of which is determined before and after the experiment. For such measurements, specialized amplifiers with a high input impedance, negative capacitance compensation, and DC potential correction are required. Intracellular ion concentrations can be measured by different methods. Most popular are fluorescent dyes which are available for protons, Ca²⁺, Mg²⁺ and Na⁺ as well as Cl⁻. Changes in ES size can be measured by monitoring the apparent increase in concentration of a substance which cannot enter cells by transport processes or movement through ion channels. In practice, we use tetrapropylammonium or tetraethylammonium. These substances may be applied locally by pressure pulses or ionophoresis, or may be present in the ES due to experimental perfusion. An increase in concentration of such a marker substance is inversely correlated with the decrease in ES size.
Results

Changes in Extracellular Na\(^+\) Concentration

Repetitive stimulation, seizures, seizure-like events and spreading depolarizations usually lead to biphasic changes in \([\text{Na}^+]_0\) with an initial decline of about 10 mM and a subsequent increase above baseline. Such alterations have been seen in different species and cortical regions. Similar changes in \([\text{Na}^+]_0\) were also observed when AMPA or kainate were focally applied. The second, overshoot component of this response is probably due to shrinkage of the ES. The consequences of these changes for Na\(^+\)-dependent uptake processes – for glutamate and GABA or glucose into glial cells – are probably minimal, as the transmembrane Na\(^+\) gradient will not be strongly affected. In neurons, the Na\(^+\) equilibrium potential might decline by about 20 mV (from 60 to 40 mV), thereby reducing driving force for Na\(^+\) inward currents. This change will also reduce Na\(^+\)-dependent secondary transport for glucose and amino acids into neurons. In addition, the intracellular Na\(^+\) accumulation will activate the electrogenic sodium–potassium pump, which will transport excess Na\(^+\) out of the cell and shift the membrane potential in hyperpolarizing direction. Activation of the sodium–potassium pump thereby contributes to seizure termination. In addition, intracellular Na\(^+\) accumulation may lead to activation of potassium channels of the slick or slo family. Voltage gated Na\(^+\) channels are also found in NG2 cells which seem to be precursor cells for oligodendrocytes.

Changes in Extracellular Cl\(^-\) Concentration

As is the case with Na\(^+\) concentration changes, Cl\(^-\) signals are also usually biphasic in response to repetitive stimulation, seizures, and seizure-like events. Following an initial decrease in Cl\(^-\) concentration, a small overshoot (by about 10 mM) take place. Based simply on the decrease in ES size, one would expect a larger increase (overshoot) in Cl\(^-\) concentration. Consequently, some 15 mM of Cl\(^-\) will accumulate within neurons, shifting the \(E_{\text{Cl}}\) in a depolarizing direction to levels beyond or equal to the membrane potential. This intracellular Cl\(^-\) accumulation contributes to the depolarizing shift of the equilibrium potential for GABA\(\alpha\)-mediated synaptic potentials. A reduced electrochemical gradient for Cl\(^-\) will also increase the contribution of the transmembrane bicarbonate gradient to the GABA\(\alpha\)-mediated synaptic potentials. As glycolysis and respiration are increased during seizures, the bicarbonate equilibrium potential may be shifted in a depolarizing direction, thus accounting for depolarizing inhibitory potentials during seizures. Cl\(^-\) is maintained relatively low in most adult neurons. This is due to export of Cl\(^-\) by the KCC2 transporter which uses the transmembrane K\(^+\) gradient for the export. In some conditions, Cl\(^-\) equilibrium potential is set to depolarizing values above resting membrane. This can be due to expression of the bumetanide sensitive NKCC1 which imports Cl\(^-\) into cells. Another possibility is that intracellular bicarbonate is exchanged against Cl\(^-\) by for example the anion exchanger AE3.

The question arose which processes of neuronal activity are responsible for oxygen consumption. In the hippocampus 20% of oxygen consumption was ascribed to action potential generation and 80% to synaptic activity with about half for postsynaptic processes and half for presynaptic processes such as removal of Ca\(^{++}\) transmitter uptake, filling and recycling of vesicles.

Changes in Extracellular K\(^+\) Concentration

Increases in \([\text{K}^+]_0\) as a function of action potential generation were first noted by Frankenhaeuser and Hodgkin in 1956; they saw that hyperpolarization after an action potential (AHP) became smaller with each action potential during a train of action potentials. It was, therefore, no surprise that changes in \([\text{K}^+]_0\) in the central nervous system occur during motor system activation, during sensory stimulation and during different states of mentation. These changes do not exceed 2 mM above baseline. During pathophysiological states, much larger changes in K\(^+\) concentration have been noted. In particular, during seizures in adult neocortex and hippocampus, potassium can increase to near 12 mM, a level that is surpassed during seizures in developing cortex and hippocampus. In adult tissue of monkeys, cats and rodents, this ceiling level of 12 mM is usually preserved, unless blood flow or metabolism is disturbed or albumin mediated astrocytic activation has occurred. If K\(^+\) accumulates to between 15 and 18 mM, it is likely that spreading depression (SD) will be induced, which is frequently observed in cortical structures during early postnatal development, but also during migraine, after brain trauma and subarachnoid hemorrhage. The increased probability for induction of spreading depression during ontogenesis may be due to delayed maturation of the Na\(^+\)/K\(^-\)-ATPase, the enzyme which removes Na\(^+\) from the intracellular space in exchange against K\(^+\) and to delayed maturation of glial cell properties. Disturbances in ion regulation due to compromised blood flow, disturbances in mitochondrial function, or reduced availability of ATP later in life will increase the likelihood of the induction of spreading depression. If blood flow is interrupted or synthesis of ATP is stopped, then a rapidly spreading depolarization develops which – if not rapidly reversed – leads to cell death. During SDS, K\(^+\) increases to about 60 mM, while Na\(^+\) and Cl\(^-\) (and extracellular Ca\(^{++}\)) concentrations strongly decrease. The ion movements across the membrane lead to a strong reduction of the extracellular space size.

Elevation in \([\text{K}^+]_0\) has a number of different effects. In most situations, K\(^+\) accumulation leads to depolarization of neurons. In axons, the hyperpolarization mediated by voltage activated K\(^-\) currents will be reduced, leading not only to prolonged action potentials but also to changes in firing rate. In presynaptic terminals, elevation in K\(^+\) causes augmented spontaneous and evoked transmitter release. This is particularly impressive in case of GABA release. Postsynaptically, in neurons, the \(E_{\text{EPSP}}\) and also the \(E_{\text{IPSP}}\) is shifted in depolarizing direction changing the synaptic balance between excitation and inhibition in favor of excitation. The reduction of outward K\(^+\) currents will also contribute to increased excitability. GABA\(\beta\)-mediated inhibitory currents decline in amplitude;
Indeed, part of the depolarization observed during repetitive stimulation can be due to the activation of GABAB receptors. K\(^+\) accumulation may also interfere with chloride transport into or out of cells. The depolarizing effect of K\(^+\) accumulation onto neurons is mostly mediated by two-pore-domain potassium channels but K\(_{ir}\) channels will also contribute.

K\(^+\) accumulation in the ES also leads to depolarization of passive (or simple) astrocytes. These astrocytes are also described as glutamate transporter cells (they express high levels of Na\(^+\)-dependent glutamate transporters) and are usually strongly coupled by gap junctions and form an electrical and metabolic syncytium. Depolarization of astrocytes by local K\(^+\) accumulation will spread within the glial syncytium, leading to facilitated K\(^+\) uptake at sites of maximal K\(^+\) accumulation and facilitated K\(^+\) release at remote sites. At sites of maximal K\(^+\) accumulation, this K\(^+\) uptake contributes to shrinkage of the ES, whereas at remote sites, spatial K\(^+\) buffering can lead to increases in the ES. The spatial K\(^+\) buffering is associated with generation of slow field potentials, which are (at least in part) due to glial cells.

The spatial K\(^+\) buffer mechanism under pathophysiological conditions may contribute to vasodilatation with subsequent increased blood flow. This effect is due to an increase of K\(^+\) conductance in K\(_c\) channels which are expressed in smooth muscle cells. These cells have a rather depolarized membrane potential which is paradoxically shifted in the hyperpolarizing direction when K\(^+\) concentration increases. The widening of the ES at sites of K\(^+\) release, presumably at astrocytic endfeet, may contribute to increased blood flow by reducing transepithelial pressure. Depolarization of astrocytes has effects on Na\(^+\)-dependent glutamate and GABA uptake, as the transmembrane electrochemical Na\(^+\) gradient necessary for effective uptake is reduced in depolarized glial cells.

This effect will further lead to the accumulation of transmitters in the extracellular space. Whether glutamate transport can be reversed under conditions of anoxia and spreading depression depends on the transmembrane Na\(^+\) gradient; in such conditions, as the Na\(^+\) concentration gradient declines the transport may be slowed (but probably not reversed). It is generally believed, that K\(^+\) accumulation in the extracellular space is due to K\(^+\) release from neurons, mediated by voltage-dependent K\(^+\) conductance. That this assumption is not really true is shown by experiments in which A-type and delayed rectifier K\(^+\) currents are blocked by TEA and 4-AP – leading to increased extracellular K\(^+\) accumulation. Most of the K\(^+\) release is therefore due to K\(^+\) movement through two pore domain (2P) K\(^+\) channels, that are sensitive to quinine, quinidine, and bupivacaine. These agents reduce stimulus induced K\(^+\) signals considerably. Interestingly, blockade of 2P K\(^+\) channels or of K\(_{ir}\) channels augments iontophoretically induced K\(^+\) signals, presumably due to reduced K\(^+\) uptake into glia and prevention of spatial K\(^+\) buffering. Down-regulation of glial K\(_c\) 4.1 channels in neuronal tissue is an early reaction after lesions to the brain and after opening of the blood–brain barrier. This downregulation causes a facilitated accumulation of potassium in the extracellular space, and leads to early transformation of physiological responses into epileptiform events during normal processing of information in the brain. In such conditions K\(^+\) can accumulate to more than 20 mM during epileptiform events and induce spreading depressions.

K\(^+\) release from cells can also be mediated by the Cl\(^-\) transporter KCC2. It uses the transmembrane outward K\(^+\) concentration gradient for removal of Cl\(^-\). This implies that with K\(^+\) elevation chloride export is hampered.

Experiments on mature neocortex have shown that K\(^+\) accumulation does not trigger seizure activity. However, in the hippocampus both in vivo and in vitro studies have shown that elevation of extracellular K\(^+\) concentration can trigger generation of seizures. This sensitivity to extracellular K\(^+\) was also seen in human tissue from drug refractory patients. In such patients, elevation of K\(^+\), in dentate gyrus and subiculum or in neocortex when combined with bicuculline, could trigger seizure-like events. Elevations in [K\(^+\)]\(_e\) concentration may support seizure generation by associated shrinkage of the extracellular space and a resultant increase in extracellular resistivity. Transmembrane currents will then cause larger extracellular potentials. Potential gradients in the order of 8 mV mm\(^{-1}\) have been shown to affect neuronal excitability. Spatial buffering of potassium causes elevation of potassium at sites which are not yet recruited into seizures. Spread of K\(^+\) by diffusion and spatial K\(^+\) buffering seems to underlie the slow spread of low Ca\(^{2+}\)-induced seizure-like events in rat hippocampus. The speed of spread in this condition compares to spreading velocities of Jacksonian march, of experimentally induced seizures caused by lowering Mg\(^{2+}\) concentration, and by the application of 4-aminoypyridine. Although no clear K\(^+\) threshold for induction of seizures was found in neocortex, this does not exclude contribution of extracellular K\(^+\) accumulation to induction and spread of seizures also in neocortex.

### Changes in Extracellular Mg\(^{2+}\) Concentration

The interstitial Mg\(^{2+}\) concentration is probably close to 1 mM, and changes in extracellular Mg\(^{2+}\) concentration are difficult to detect due to the lack of appropriate sensors. In favorable conditions, small decreases in Mg\(^{2+}\) concentrations have been observed. Mg\(^{2+}\) is intracellularly bound to ATP but is free when ATP is consumed. Hence, the intracellular magnesium concentration may fluctuate, and in some condition may be as large as 1 mM. Such an intracellular level would result in a Mg\(^{2+}\) equilibrium potential close to 0 mV, and provide a driving force for Mg ions to move in the inward direction. The major pathway for Mg\(^{2+}\) ions to enter cells is through NMDA receptors. Indeed, measurement of Mg\(^{2+}\) concentration changes in response to iontophoretic application of NMDA under conditions of zero Ca\(^{2+}\) (when measurement of Mg\(^{2+}\) loss is facilitated) indicates that Mg\(^{2+}\) enters neurons. This observation would suggest that the Mg\(^{2+}\) blocking site within the NMDA channel is deep within the pore. Larger changes in Mg\(^{2+}\) concentration, in both plasma and CSF, may occur during eclamptic seizures, when a sudden and large increase in blood pressure opens the blood–brain barrier. Lowering Mg\(^{2+}\) concentration causes facilitated transmitter release, due to the removal of the Mg\(^{2+}\) block on voltage-dependent Ca\(^{2+}\) channels. In addition, the activation of NMDA type glutamate receptors is facilitated by lower Mg\(^{2+}\). Both effects cause a rapid accumulation of Ca\(^{2+}\) within neurons. High intracellular Ca\(^{2+}\) may be responsible for the activation of enzymatic processes leading to excitotoxicity. Lowering Mg\(^{2+}\) concentration will also affect surface charge screening, thereby augmenting neuronal excitability.
Lowering Mg$^{++}$ concentration has also been investigated and found to trigger seizure-like events in juvenile hippocampus and in juvenile and adult neocortex (‘zero Mg$^{++}$’ solution). However, the concentrations required are probably lower than achieved under physiological conditions. Whether Mg$^{++}$ can decline during eclampsia to levels sufficient to trigger seizures is unclear. However, intravenous Mg$^{++}$ substitution is as effective as phenytoin in blocking seizures under these conditions.

**Changes in Extracellular Ca$^{++}$ Concentration**

Ca$^{++}$ concentration declines whenever neurons are activated. The decreases are about 0.1–0.2 mM during physiological activity. However, repetitive electrical stimulation of the thalamus or the cortex can induce decreases by up to 0.4–0.6 mM. As is the case with changes in K$^{+}$ concentration, the amplitude of decreases in Ca$^{++}$ concentration is layer-dependent in cortical structures. Maximal Ca$^{++}$ decreases occur in layer II and IV/V of cat somatosensory cortex. In the hippocampus, decreases in Ca$^{++}$ concentration are largest in the pyramidal cell layer, followed by changes in stratum granulare of the dentate gyrus. During seizures, Ca$^{++}$ has also been shown to decline, and these decreases are generally similar to the decreases seen with repetitive stimulation. However, in some species, decreases in Ca$^{++}$ can be much larger; for example, in the neocortex of the baboon *Papio papio*, decreases in Ca$^{++}$ concentration reach about 1 mM during seizures induced by photic driving – changes only observed during spreading depression and anoxic depolarization in other animals. The unique cellular arrangements in the hippocampus permit separation of pre- and postsynaptic Ca$^{++}$ signals. Repetitive stimulation of the Schaffer collaterals causes Ca$^{++}$ concentration decreases due to activation of P/Q type Ca$^{++}$ channels (N type Ca$^{++}$ channels inactivate relative rapidly during repetitive stimulation). K$^{+}$ channel blockers such as TEA and 4-AP augment presynaptic Ca$^{++}$ entry. Antidromic stimulation permits analysis of postsynaptic Ca$^{++}$ entry; P/Q type and also L type Ca$^{++}$ channels are activated during antidromic stimulation. NMDA receptors mediate postsynaptic – and when present –also presynaptic Ca$^{++}$ entry.

While Ca$^{++}$ declines monotonically to a new steady state level during the application of NMDA, glutamate or aspartate, Ca$^{++}$ only transiently declines when AMPA and kainate receptor agonists are applied; under this latter condition, the initial decline in Ca$^{++}$ concentration is followed by an overshoot. The reasons for these kinetic differences are not fully understood. The overshoot in Ca$^{++}$ concentration during kainate or AMPA application, however, is removed when the Na$^{-}$–Ca$^{++}$ exchanger is blocked.

The decrease in Ca$^{++}$ concentration increases neuronal excitability by effects on surface charge screening and facilitation of action potential generation. In addition, the decrease in Ca$^{++}$ concentration facilitates activation of NMDA receptors. It is expected that decreases in Ca$^{++}$ concentration also lead to reduced transmitter release.

Studies in the hippocampus have revealed that during washout of Ca$^{++}$, stimulus-evoked synaptic inhibition disappears before synaptic excitation; the loss of synaptic inhibition was noted at about 0.9 mM extracellular Ca$^{++}$, while postsynaptic excitatory potentials were lost at about 0.2 mM. The release of transmitters such as glutamate and GABA depends on the fourth power of Ca$^{++}$ entry. In disynaptic pathways, therefore, the effects of lowering Ca$^{++}$ concentration will be cumulative. The loss of feedback and feed-back inhibition will therefore be particularly sensitive to decreases in extracellular Ca$^{++}$ concentration.

Surprisingly, lowering Ca$^{++}$ can also directly activate nonselective cation currents, as originally reported for dorsal root ganglion cells and more lately for central neurons. Whether this effect involves a unitary class of channels or includes different ion channels is presently unclear. The activation of these channels requires only relatively small decreases in extracellular Ca$^{++}$ concentration. Lowering of Ca$^{++}$, similar to lowering of proton or Mg$^{++}$ concentration, can increase neuronal excitability by effects on surface charge screening; it can trigger epileptiform activity in rodent hippocampus, provided that K$^{+}$ is elevated to 5 mM or more. This effect is not seen, however, in human hippocampus from patients with epilepsy.

Since decreases in extracellular Ca$^{++}$ concentration are due to Ca$^{++}$ inward currents, effects of changes in extracellular Ca$^{++}$ concentration on neuronal activity are not always easy to predict. The net consequences for excitability depend on the intracellular Ca$^{++}$ kinetics and the different effects which Ca$^{++}$ can have on activation of ion channels, pumps, secretion, etc. With regard to effects of intracellular Ca$^{++}$ accumulation, it must be noted that Ca$^{++}$ can activate cation channels, and different types of K$^{+}$ channels. However, in some cells, Ca$^{++}$ may also inhibit activation of K$^{+}$ channels. Recent data suggest that certain types of voltage gated Ca$^{++}$ channels co-localize with either BK or SK type Ca$^{++}$-activated K$^{+}$ channels, thus permitting generation of locally limited domains of intracellular Ca$^{++}$ concentration changes. Intracellular Ca$^{++}$ changes may have effects on metabolism as well. Three enzymes in the tricarboxylic acid cycle respond to increases in intramitochondrial Ca$^{++}$ concentration. However, larger increases in intracellular Ca$^{++}$ concentration lead to mitochondrial depolarization, with potentially impaired synthesis of ATP and increased production of radical oxygen species.

**Alterations in pH**

Usually, seizure-like events are initially accompanied by alkalosis and then by acidosis. Initial alkalosis may be due to exchange of bicarbonate against Cl$^{-}$ with bicarbonate generated due to increased metabolism during seizures. Since bicarbonate is exported in exchange for Cl$^{-}$ this leads to intracellular Cl$^{-}$ accumulation and may be a factor for loss of inhibitory efficacy. Part of the acidosis developing during seizures and outlasting into the postictal episode may be due to shrinkage in ES size, but also to lactate accumulation in the tissue. Some of the lactate may be released from astrocytes due to depolarization. While acidosis reduces neuronal excitability, alkalosis increases neuronal excitability. The increase in excitability can, in part, be attributed to reduced surface charge screening and in part to effects on inhibition.
Combined Changes in Extracellular Electrolytes due to Blood Brain Barrier Impairment

As mentioned previously in many neurological disorders the integrity of the blood brain barrier may be impaired. In some conditions this would lead to apparent decreases in Ca$^{2+}$ and Mg$^{2+}$ concentration and increases in K$^+$ concentration. In case of seizure like events induced in slices this will lead to shortened but frequent epileptiform discharges which are insensitive to standard antiepileptic drugs such as carbamazepine, phenytoin, barbiturate and phenobarbital. Such effects may lead to very severe forms of status epilepticus.

Contribution of Different Neuronal Compartments to Changes in Extracellular Electrolytes

It is not yet fully understood how much ion movements across neuronal membrane in the different compartments of a soma contribute to changes in extracellular ion concentrations. In the hippocampus such studies are relatively simple due to the separation of axons from soma and dendrites. In stratum radiatum of rat hippocampus it was found that mild to moderate repetitive stimulation causes significant changes in ion concentrations. Blocking synaptic transmission from Schaffer collaterals to pyramidal cells by application of glutamate receptor antagonists leads to a near 50% reduction of ionic changes and of oxygen consumption. If transmitter release is blocked from presynaptic terminals only about 10% of the initial ion changes persists suggesting that on the presynaptic site only 10% of ion changes and of oxygen consumption can be allocated to activity in axons and that a large part of ionic changes are due to processes related to transmitter release such as Ca$^{2+}$ extrusion, uptake of transmitters and glucose or lactate, uptake of transmitters into vesicles and vesicle turnover.

Directions for Future Research

Obviously, changes in extracellular ion concentrations and changes in transmembrane ion concentration gradients have profound effects on neuronal activity, and may often be relevant for seizure induction and seizure propagation. When seizures spread, the ‘front’ of seizure spread is preceded by an avalanche of strongly increased inhibition. Recruitment of ‘normal’ brain regions into seizure activity then depends on the break-down of this inhibition. The precise mechanisms by which this break-down occurs are not yet fully understood, but changes in transport efficacy for Cl$^-$, as well as changes in bicarbonate concentration, may be involved. In order to maintain, and particularly to recover, relatively normal ion concentration gradients after a seizure or a seizure-like event, the nerve cells must increase production of ATP so that the ion pumps can work efficiently. This demand for ATP requires increases in blood flow and more delivery of oxygen and glucose to sites of ictogenesis. The roles played by ionic changes in neurovascular coupling are far from being understood. The same applies to the precise mechanisms by which neuronal metabolism is adapted to the needs for ATP synthesis in very active neurons. Another still-to-be resolved question is how ionic changes are altered when the blood–brain barrier is open, or when seizure activity develops in an area with compromised blood–brain barrier. Particularly important are the effects of alterations in glial cell properties on the kinetics of ionic changes in the nervous system since these kinetics may influence the conditions under which seizure progresses into status epilepticus and/or induces damage to the nervous system.

Further Reading


