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Transient Changes in the Size of the Extracellular Space in the Sensorimotor Cortex of Cats in Relation to Stimulus-induced Changes in Potassium Concentration*

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Summary. The time course of local changes of the extracellular space (ES) was investigated by measuring concentration changes of repeatedly injected tetramethylammonium (TMA^+) and choline (Ch^+) ions for which cell membranes are largely impermeable. After stimulus-induced extracellular $[K^+]$ elevations the $\Delta[TMA^+]$ and $\Delta[Ch^+]$ signals recorded with nominally K^+-selective liquid ion-exchanger microelectrodes increased by up to 100%, thus indicating a reduction of the ES down to one half of its initial size. The shrinkage was maximal at sites where the K^+ release into the ES was also largest. At very superficial and deep layers, however, considerable increases in extracellular K^+ concentration were not accompanied by significant reductions in the ES. These findings can be explained as a consequence of K^+ movement through spatially extended cell structures. Calculations based on a model combining the *spatial buffer mechanism* of Kuffler and Nicholls (1966) to osmolarity changes caused by selective K^+ transport through primarily K^+ permeable membranes support this concept.

Following stimulation additional iontophoretically induced $[K^+]_o$ rises were reduced in amplitude by up to 35%, even at sites where maximal decreases of the ES were observed. This emphasizes the importance of active uptake for K^+ clearance out of the ES.

Key words: Extracellular space – K^+ regulation – Spatial K^+ buffering – Epilepsy – Cerebral cortex

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Cell swelling has been observed in cerebral and cerebellar cortex during spreading depression (van Harreveld and Khattab 1967; Phillips and Nicholson 1979) and after superfusion of the cortex with strongly K^+-enriched media (Bourke and Nelson 1972; Lipton 1973). Due to the large depolarization the cortex will no longer be responsive under these experimental conditions. In this study we describe cell swelling in the absence of signs of spreading depression which arises as a consequence of more physiological variations in extracellular K^+ concentration ($[K^+]_o$) elicited by repetitive electrical stimulation and epileptiform afterdischarges. The concomitant reduction of the extracellular space (ES) will affect transport between cells, cerebrospinal fluid, and blood and may thus contribute to the destructive consequences of epilepsy.

To study changes in the ES we monitored the concentration of ions which do not pass the cellular membranes and are thus restricted to the extracellular space. As their concentration is inversely proportional to the volume fraction occupied by the ES (Nicholson et al. 1979) a relative volume change can be calculated from concentration changes of these ions. A further aim of this study was to clarify the role of active K^+ uptake in K^+ clearance from the ES. As shown by numerous authors (Lux 1974; Sypert and Ward 1974; Moody et al. 1974; Heinemann and Lux 1975, 1977) increases in $[K^+]_o$ in normal as well as in epileptic cortex are strictly limited to a ceiling level of 10 mM. After termination of any significant increase $[K^+]_o$ falls to subbaseline levels. Additional iontophoretically induced K^+ signals are reduced both during rises in $[K^+]_o$ and during subsequent undershoots as compared to control signals (Heinemann and Lux 1975, 1977). This finding can either be explained by enhanced active K^+ uptake into nerve cells or by an increase in the extracellular volume fraction. Contrary to the latter suggestion evidence will be presented for a local reduction of the ES during such changes, thus giving even more

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weight to active K^+ uptake in the limitation of the rise of $[K^+]_o$ and the generation of subsequent undershoots.

Material and Methods

Experiments were performed on adult cats anesthetized with 0.3–0.6 vol% Halothane and 65% N_2O, prepared and supervised as described before (Heinemann and Lux 1975). Stimulating electrodes were located in the nucleus ventroposterolateralis thalami (VPL) and on the surface of the sensorimotor cortex (CS) about 1–2 mm distant from the recording electrode.

Double-barrelled reference and K^+-selective electrodes (Corning 477317) and Na^+-selective electrodes (Simon) were prepared using the method described by Lux and Neher (1973). The K^+ ion-exchanger is very sensitive to tetralkylammonium ions (Neher and Lux 1973). Thus, two such compounds were chosen as probes for monitoring changes in extracellular volume: tetramethylammonium$^+$ (TMA^+) and choline$^+$ (Ch^+). Relative changes in volume were calculated according to:

$$\text{percentage shrinkage of ES} = \left(1-\frac{\Delta[TMA^+] \text{ before activity}}{\Delta[TMA^+] \text{ after activity}}\right)\times 100$$

To indicate changes of the ES the testing ions have to remain extracellular at least during the time of the measurement. Frog myelinated nerve membranes were shown to be impermeable to TMA^+ (Hille 1973) and this ion also appears to remain extracellular for at least 100 s in the cerebellum of rats (Phillips and Nicholson 1978). While this also applies to Ch^+ the distribution of this substance in the ES may additionally be influenced by active resorption (Kuhar and Murrin 1978). Injection into the brain was performed by series of short iontophoresis current pulses with bent double- barrelled iontophoresis electrodes. These electrodes were fixed parallel to the ion selective electrodes at tip intervals varying from 20–80 µm. One side of the iontophoresis electrode was filled with 1M KCl, while the second barrel contained 1M Ch- or 1M TMA-chloride. The iontophoretic injections were carried out using pulses between 20 and 150 nA for the ammonia compounds and up to 200 nA for K^+ lasting 0.5–5 s. As dilution of fluid in the iontophoresis pipettes can change the time course of the test signals (Purves 1979), we found it important not to apply backing currents, except when testing for spontaneous release of Ch^+, TMA^+ and K^+. Furthermore, for this reason the injected currents were applied at constant intervals. Recordings were only accepted when under resting conditions consecutively evoked TMA^+ and Ch^+ signals ($\Delta[TMA^+]$, $\Delta[Ch^+]$) remained identical. As only relative changes in $\Delta[TMA^+]$ and $\Delta[Ch^+]$ were measured, slight variations in tip separation which may occur while advancing the electrodes in the cortex do not affect the results.

The K^+ electrodes were calibrated by the fixed interference method (IUPAC 1976) with solutions of known concentrations at nearly constant ionic strength. Thus, the activity coefficient was kept constant and responses of such calibrated electrodes are convertible into concentration changes. Calibration solutions were prepared on the basis of 151 mM NaCl and 3 mM KCl solutions, adding different amounts of KCl, TMACl or ChCl respectively. The Na^+-selective electrodes were calibrated with solutions containing 25–200 mM NaCl. To improve and simplify conversion of measured voltages into concentrations the calibration values were fitted to the modified Nernst-equation

$$V = V_o + S \log (C_o + C_i)$$

with $C_i = [TMA^+], [Ch^+], [K^+]$

and $C_o = \sum_j k_{ij}\, c_j$.

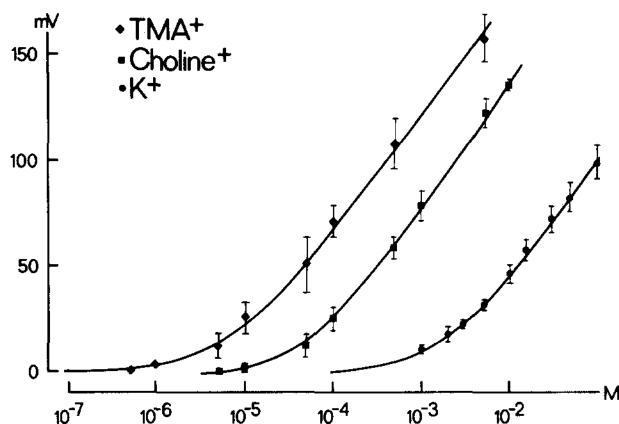

Fig. 1. Mean values measured for K^+-selective microelectrodes calibrated with solutions containing different amounts of TMA^+ (10 electrodes) Ch^+ (13 electrodes) and K^+ (15 electrodes). By a non-linear least square curve fit to the modified Nernst equation slopes S and interferences C_o were determined to be: $S_{K^+} = 57.3$ mV; $C_{oK^+} = 1.717$ mM; $S_{Ch^+} = 61.4$ mV; $C_{oCh^+} = 0.050$ mM; $S_{TMA^+} = 56.6$ mV; $C_{oTMA^+} = 0.007$ mM. Slopes of TMA^+ electrodes sometimes exceeded 61.4 mV. The curves shown were fitted to the mean values

The slopes S and the interference C_o, which describes the influence of the background ions in equivalents of the concentration of the primary ions, were determined with a non-linear least-square curve fitting programm (Marquardt 1963). Measured electrode responses and fitted curves are shown in Fig. 1. If empirical slope-values S different from 58 mV are allowed, the electrode responses can be excellently fitted to the modified Nernst-equation. The slowly reversible side effects which have been shown for high TEA^+ concentrations (Neher and Lux 1973) obviously do not occur in the investigated range of low TMA^+ concentrations.

Results

Repetitive stimulation of the cortical surface (CS) or the nucleus VPL evoked increases in $[K^+]_o$ from the baseline level of about 3 mM to values of up to 10 mM. Often such stimulation elicited self-sustained epileptiform afterdischarges during which $[K^+]_o$ remained elevated, as indicated by the recordings of field potentials and $[K^+]_o$ in Fig. 2. After the end of the stimulation and/or seizure $[K^+]_o$ fell to sub-baseline levels (Lux 1974, 1975; Heinemann and Lux 1975, 1977; Heinemann et al. 1978). During these undershoots additional $[K^+]_o$ changes evoked by phoretic injection of K-ions were reduced in amplitude by up to 35% as compared to control values. This is in accordance with previous observations (Heinemann and Lux 1975). Such reductions in iontophoretically induced $\Delta[K^+]_o$ were seen in all cortical layers.

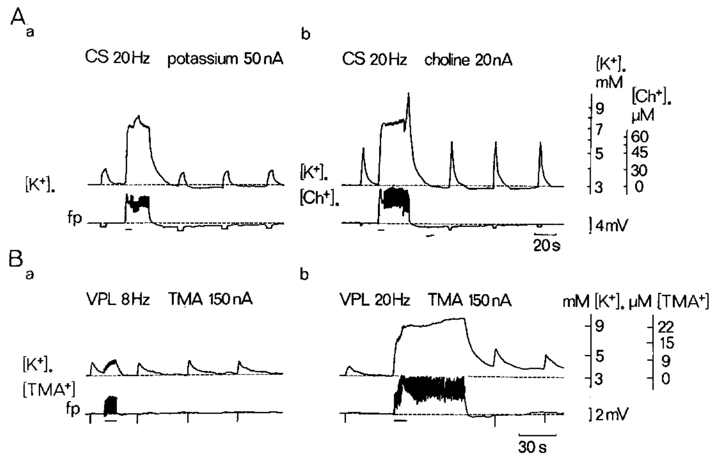

Fig. 2A, B. Extracellular K^+ concentration and superimposed iontophoretically induced choline$^+$ ($\Delta[Ch^+]$) or tetramethylammonium$^+$ ($\Delta[TMA^+]$) concentration signals as a function of time. Field potentials fp are shown in the lower traces; duration of stimulation is indicated by bars. During the sustained shifts in the field potential $[K^+]_o$ is elevated in consequence to neuronal discharges. The regular deflections in the $[K^+]_o$ trace are phoretic $\Delta[TMA^+]$, $\Delta[Ch^+]$ or $\Delta[K^+]$ signals; calibrations for these signals are shown on the extreme right. **A** Self-sustained afterdischarges were induced by cortical surface (CS) stimulation with 0.5 mA, 0.2 ms, 20 Hz for 6 s. During discharges $[K^+]_o$ was elevated by 4 mM and fell to subbaseline levels of 2.8 mM after the seizure. **Aa** Following the discharges phoretically induced K^+ signals were reduced in concentration by 5% as compared to the pulse before the discharges. Phoresis current: 50 nA for 5 s. **Ab** Ch^+ concentration signals following discharges were increased by 20% (phoresis current 20 nA for 2 s). Calibrations do not apply to phoresis pulses during seizure activity because of the altered $[K^+]_o$. In spite of a smaller voltage change at the electrode here the Ch^+ concentration was already enhanced. **B** Dependence of $\Delta[TMA^+]$ on preceeding stimulus strength and accompanying K^+ changes. **Ba** VPL stimulus of 0.5 mA, 0.1 ms, 8 Hz for 10 s: no afterdischarges and no $\Delta[TMA^+]$ changes were elicited. **Bb** VPL stimulus of 20 Hz evoked a seizure and the first $\Delta[TMA^+]$ signal was increased in concentration by 80%; control value was restored after 3.5 min

In contrast to these decreases in $\Delta[K^+]_o$ iontophoretically induced rises in $[TMA^+]$ and $[Ch^+]$ were enhanced in amplitude by up to 100% during this period (Fig. 2Ab, Bb, Fig. 3). Figure 2B shows the $\Delta[TMA^+]$ signals which were recorded after VPL stimuli of different frequencies (8 and 20 Hz). With the 8 Hz stimulus $[K^+]_o$ was elevated by only 1 mM and the rise in $\Delta[TMA^+]$ was insignificant. The 20 Hz stimulus evoked afterdischarges and a longlasting increase of $[K^+]_o$ by 5.8 mM. The first $\Delta[TMA^+]$ signal obtained following the afterdischarges was increased by 80%. 3.5 min passed until $\Delta[TMA^+]$ recovered to control value.

It is interesting to note that an increased $\Delta[TMA^+]$ accompanying $[K^+]_o$ elevations is not restricted to stimulus induced $[K^+]_o$ rises. As for an example, shortlasting increases in $[K^+]_o$ by up to 20 mM for 1–4 s, elicited by accidental destruction of

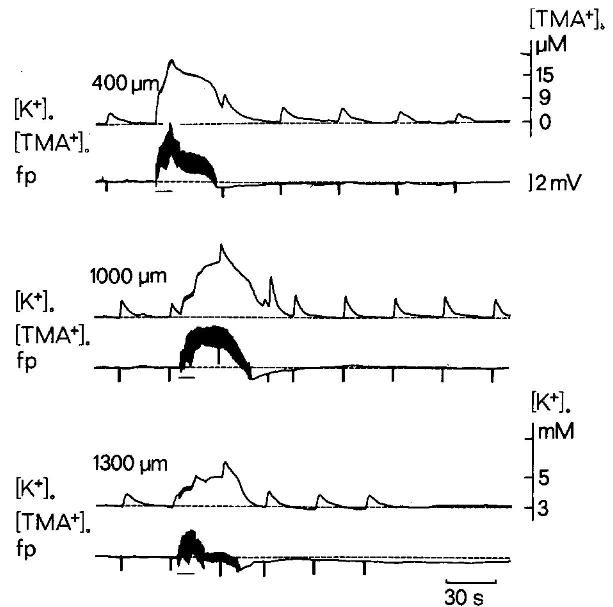

Fig. 3. $\Delta[TMA^+]$ changes elicited by identical VPL stimuli (20 Hz, 0.15 ms for 10 s) and recorded at different cortical depths. 400 µm below the cortical surface $\Delta[TMA^+]$ was elevated by 17%, at 1,000 µm depth by 80%, and at 1,300 µm depth by 10% after the seizure. TMA^+ phoresis pulses: 150 nA for 0.8 s

cells were also followed by increases in $\Delta[TMA^+]$. These could be larger than 100% and last up to 4 min.

These observations suggest that increases of $\Delta[TMA^+]$ or $\Delta[Ch^+]$, and thus reductions of the ES, are proportional to the stimulus induced increases in $[K^+]_o$. This was tested by comparing the amplitudes of the stimulus induced $[K^+]_o$ rises to $\Delta[TMA^+]$ changes in different cortical layers by advancing the electrode assembly into the cortex in steps of 200 µm. As previously reported (Moody et al. 1974; Sypert and Ward 1974; Heinemann and Lux 1975; Heinemann et al. 1979; Cordingley and Somjen 1978) stimulus- and seizure-related $[K^+]_o$ rises vary with cortical depth. These experiments as well as our present recordings show that during self-sustained epileptiform discharges $[K^+]_o$ is maximal at a depth of about 1,000 µm. In the six animals in which laminar cortical analyses were performed the largest $\Delta[TMA^+]$ changes were also found in this depth. The reductions of the extracellular space after stimuli of 20 Hz were calculated to be 30±19% (mean ± standard deviation) (n = 12) in depths between 900 and 1,100 µm. Between 300 and 600 µm significantly smaller decreases in ES were seen (14±11%; n = 16). In layers deeper than 1,100 µm (down to 1,400 µm) and between 600 and 800 µm decreases by 14±15% and 13±18% were recorded. Also the decay time of the ES change was longest in the layer

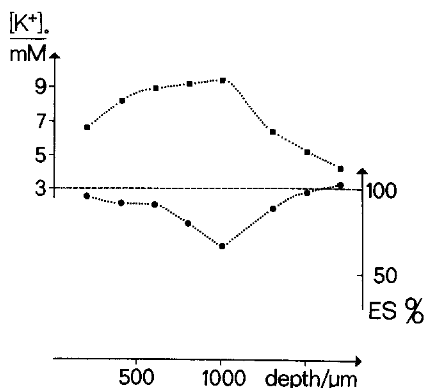

Fig. 4. Depth profile of $[K^+]_o$ rises (top curve) and concomitant changes in ES ($\Delta[TMA^+]$ before activity / $\Delta[TMA^+]$ after activity $\times$ 100) (lower curve). $\Delta[TMA^+]$ signals during the first 30 s after the neuronal discharges were averaged from 6 recordings in two different experiments

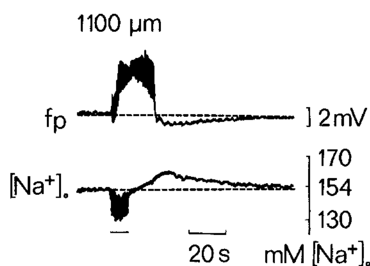

Fig. 5. Field potential and $[Na^+]_o$ before, during and after stimulation of the ventrobasal thalamus (0.1 ms, 20 Hz for 10 s); duration of stimulation indicated by bar; the electrode tip was 1,100 μm below the cortical surface

where the increases in $[K^+]_o$ and the changes in $\Delta[TMA^+]$ were maximal. The average half time of decay in this layer amounted to 60±21 s, which was 1.5 times longer than found in superficial or in deeper cortical layers. In view of the remarkable morphological changes in superficial layers attributed to swelling processes during spreading depression (van Harreveld and Schadé 1959) it was interesting to observe that under our experimental conditions hardly any changes of the extracellular space were seen in the upper 400 μm and at depths below 1,500 μm. In two animals we even recorded small but significant reductions of the $\Delta[TMA^+]$ signals at distances of more than 700 μm from the depth showing the maximal rise in $[K^+]_o$.

There was no indication that our microiontophoretic probing technique is less sensitive close to the cortical surface. Recordings at a distance of 500 μm from the stimulating electrodes on the cortical surface show a maximum rise in $[K^+]_o$ at a depth of 100–200 μm (Cordingley and Somjen 1978; Heinemann et al. 1979). Under this condition large increases in $\Delta[TMA^+]$ or $\Delta[Ch^+]$ were also observed in the upper cortical layers.

Depth profiles of stimulus-induced rises in $[K^+]_o$ and the calculated relative shrinkage of the ES are plotted in Fig. 4. Reductions of the ES were not observable in spite of the considerable rises in $[K^+]_o$ in the upper and lower cortical layers. Thus, the shrinkage in the ES seems to be more localized than the increase in $[K^+]_o$.

It has been reported that NaCl enters cellular elements during spreading depression (van Harreveld and Schadé 1959). To decide, whether the corresponding loss of Na^+ due to this entry contributes to the observed reductions in ES, additional measurements were carried out using Na^+-selective microelectrodes. At depths of about 1,000 μm, where maximal changes in the ES were recorded, $[Na^+]_o$ initially decreased by between 5–8 mM from control levels of about 150 mM. However, during stimulation and subsequent afterdischarges this reversed into an increase which could be as large as 5–7 mM above the baseline level. The increase in $[Na^+]_o$ reached a peak value at the end of the period of the enhanced neuronal activity. The elevation usually persisted for about 1 min (see Fig. 5). In superficial cortical layers only the initial decrease in $[Na^+]_o$ occurred. $[Na^+]_o$ usually started to return to the baseline level during the discharge period. At sites of maximal reductions of the ES the decrease in $[Na^+]_o$ was not found to exceed significantly the increase in $[K^+]_o$. This is in marked contrast to spreading depression where a loss in $[Na^+]_o$ by 90 mM is accompanied by an increase in $[K^+]_o$ by 40 mM (Kraig and Nicholson 1978). It should be noted that electrical signs of spreading depression were never observed in the present experiments. It appears that the swelling mechanism during normal neuronal activity has the beneficial effect of preventing larger decreases in $[Na^+]_o$. This may be essential to maintain neuronal activity.

Discussion

The observed increases of $\Delta[TMA^+]$ and $\Delta[Ch^+]$ signals following transient elevations in extracellular $[K^+]_o$ indicate a shrinkage of the extracellular volume fraction.

A mechanism explaining the depth profile of these changes can be derived from the *spatial buffer mechanism* for K^+ proposed by Orkand et al. (1966) combined with osmolarity changes caused by the selective K^+ transport through primarily K^+ permeable membranes (cf. Barry and Hope 1969).

Since the membrane potential of neurons and particularly of glial cells is established by the

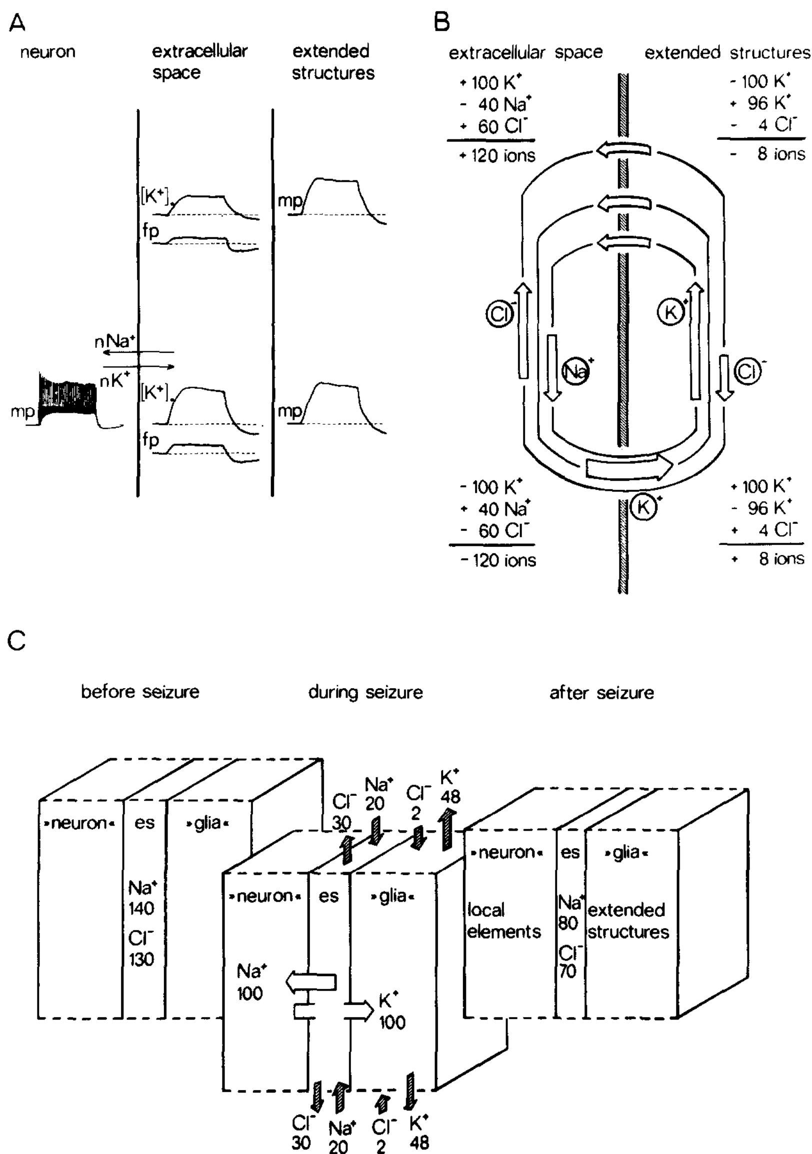

Fig. 6A–C. Schematic representation of three aspects of the proposed model. For the calculation of ionic dislocations in this example the following proportions have been assumed: ES 20%, cells involved in the buffering process (labelled 'glia'): 40%, uninvolved cells (labelled 'neurons'): 40%. **A** Illustration of processes occurring in the first 1,000 μm of the cortex: at a depth of about 1,000 μm extracellular $[K^+]_o$ is elevated by neuronal activity and depolarizes the adjacent cells. These are assumed to be sufficiently extended or electrically coupled such that the depolarization can propagate along their membranes. In upper cortical layers (lower layers are not shown here) the glial membrane potential (mp) now differs from the K^+ diffusion potential, forcing K-ions out of the cells. fp: field potential. See text for further explanation. **B** Current loop induced by a nonuniform extracellular K^+ distribution: across the membranes this current is carried exclusively by K-ions. Consequent changes in the numbers of particles in the extracellular and intracellular space are illustrated in the corners. This causes a water flow from the ES to the intracellular space at sites of maximal extracellular rise in $[K^+]_o$. The numbers of particles given in the picture were calculated for a shrinkage of the ES by 30% in a brain volume of 1,000 × 1,000 × 7.5 Å. **C** Survey of total ionic dislocations which interact to reduce the ES by 30% in a brain slice of 1,000 × 1,000 × 7,5 Å

extracellular K^+ concentration (Kuffler and Nicholls 1966) local elevation of $[K^+]_o$ will depolarize the adjacent membranes (Ransom and Goldring 1973b). If these cells are electrically coupled or spatially extended this depolarization propagates from the site of maximal $[K^+]_o$ to distant cortical layers. Coupling of glia cells by gap junctions has been proposed by Somjen (1973, 1975) and Cohen (1974) and it is known that many neurons are quite extended (Cajal 1911). At those sites where the membrane potential exceeds the potassium diffusion potential (E_k), K-ions will be driven out of the cells to restore electrochemical equlibrium (Fig. 6A).

Deviations of glial membrane potential from E_k have indeed been demonstrated (Futamachi and Pedley 1976). Thus, a circular current flow will be induced (Fig. 6B). Across the membranes this current will consist of K-ions whereas in the ES it will be carried about equally by the majority ions Na^+ and Cl^-. This extracellular current contributes to the generation of the slow potentials which occur concomitantly with $[K^+]_o$ elevations (Lux 1974; Somjen 1973; Castellucci and Goldring 1970; Gutnick et al. 1979; Heinemann et al. 1979). As a consequence of this current the ES will be depleted by an amount of cations and anions which is of the same order of magnitude as the number of K-ions that have crossed the membranes at the site of maximal $[K^+]_o$ elevation. In the intracellular compartment the current is carried by K-ions and mobile anions in proportion to their transport numbers. At the site of current entry into the cells some accumulation of K^+-anion pairs is expected, which will be limited, however, by the restricted mobility of the intracellular anions. The

effect will be an internal redistribution of KCl leading to a higher intracellular Cl^- content at the site of maximal ES-decrease, thus mimicking Cl^- conductivity of the membranes. An example for cell swelling as a consequence of these ionic movements is shown in Fig. 6C.

After the termination of efflux from active neurons $[K^+]_o$ falls to subbaseline levels and the cells become hyperpolarized (Ransom and Goldring 1973c; Sypert and Ward 1971). Thus, the current flow reverses during this phase. This is indicated by the positive field potential (Fig. 3). The reversion of the current contributes to the restitution of the initial volume fraction of the ES.

Transcellular K^+ movements underlying the mechanism described above can be inferred from transcortical current transport experiments in normal cortex (Gardner-Medwin 1977, 1979) and in gliotic tissue (Heinemann and Dietzel 1980). In both cases measurements with K^+-selective microelectrodes yielded transport numbers for K^+ that exceeded those assumed in the ES, thus providing evidence for the contribution of intracellular compartments to K^+ transport. Slow potential changes observed after superfusion of the cortical surface with K^+-enriched media (Heinemann and Lux 1977) or after local application of K^+ by iontophoresis (Heinemann et al. 1979) are further evidences for current flow resulting from extracellular K^+ gradients.

The above described redistribution of ions between the extracellular and intracellular compartments during enhanced neuronal activity can explain the observed depth profile of the shrinkage of the ES. As shown in the appendix the participating K^+ fluxes may significantly contribute to the equilibration of nonuniform extracellular K^+ distributions.

If glia cells are exclusively K^+-conductive (Lothmann and Somjen 1975) the spatial glial buffering mechanism implies that local accumulation of K^+ within one part of the glia is accompanied by a decrease of K^+ at sites where the current leaves the cells. As this mechanism cannot change the total amount of K^+ inside the glia it serves mainly to speed up the K^+ distribution and to decrease locally increased extracellular $[K^+]$ at sites of maximal neuronal activity.

The iontophoretically induced K^+ signals which were diminished during the time of undershoots in $[K^+]_o$ showed a significant decrease in amplitude in spite of a still reduced ES. In view of the preceding considerations this finding indicates enhanced neuronal K^+ uptake after seizures, which serves to restore resting $[K^+]_o$ and internal $[K^+]$.

This cell swelling probably also compensates to some extent for the decrease in $[Na^+]_o$ which results from Na^+ influx into neurons during excitation, as could be shown by direct measurements with Na^+-selective microelectrodes. The particularly large decrease of the ES in cortical layers III and IV could also account for the otherwise unexplained increase in $[Ca^{2+}]_o$ which was observed at these sites during stimulus induced epileptic activity using Ca^{2+}-selective microelectrodes (Heinemann and Konnerth 1979).

Cell swelling will also result if the membranes are somewhat permeable to Cl-ions. In this case a KCl influx over the depolarized membranes at the area of maximal $[K^+]_o$ and a loss of KCl out of the cells at more remote sites can be expected. However, this mechanism cannot explain the origin of slow field potentials, as no current loop will be induced. Nevertheless, we cannot exclude that it contributes to some extent to the observed changes in ES. A further mechanism leading to cell swelling could be an increase of intracellular particles produced by enhanced metabolism during excessive neuronal activity. The depth profile of the decreases of the ES should then be similar to the profile of the rises in $[K^+]_o$. The observed dissimilarities of both curves indicate that this mechanism is of minor importance.

Decreases of the ES have also been observed during spreading depression (van Harreveld and Khattab 1967; Phillips and Nicholson 1979). In this pathological condition increases in $[K^+]_o$ are not limited to a ceiling level of 10 mM. Spreading depression is additionally characterized by a drastic decrease of $[Na^+]_o$ and $[Cl^-]_o$ that exceeds more than two times the increase in $[K^+]_o$ (Kraig and Nicholson 1978) and by an opening of large anion channels (Phillips and Nicholson 1979). In this case cell swelling is probably mainly due to NaCl entry into cells. During normal neuronal activity, however, there is no indication that Na^+ influx significantly exceeds the K^+ efflux (Keynes and Lewis 1951). Thus cell swellings occurring under both conditions are not necessarily produced by identical processes.

Acknowledgement. We thank Mr. Zucker for the computer program with which the calibration curves were fitted. Prof. Simon, Zürich, kindly provided the Na^+ ion-exchanger.

Appendix

Decreases in ES caused by K^+ fluxes due to the above described *spatial buffer/specific transport mechanism* are calculated on the basis of the following data:

Volume fraction of the ES: about 25% (Tower 1967).

Concentrations of the involved extracellular ions: 3 mM K^+, 158 mM Na^+, 144 mM Cl^-, total osmolarity (including minority ions like Ca^{2+}, etc.): 330 mOsm (Ames et al. 1964)

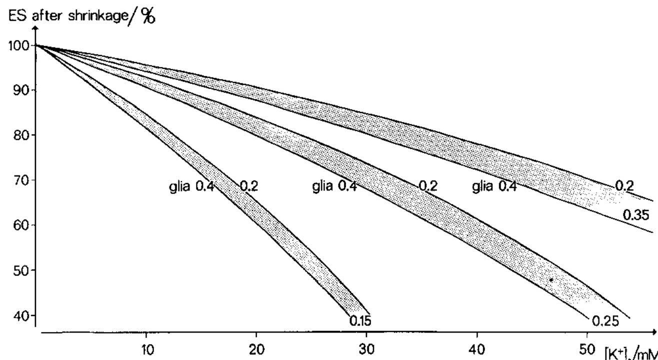

Fig. 7. Calculated reductions of the ES for different transmembrane K^+ displacements for initial extracellular volume fractions of 0.15, 0.25, 0.35. The part of the extended cells involved in the K^+ buffering was varied between 0.2 and 0.4 of total tissue volume (stippled fields)

Intraglial K^+ concentration is assumed to be about 110 mM (Nicholls and Kuffler 1964; Ransom and Goldring 1973a), and intracellular Cl^- to be 5 mM. This value is calculated from the total Cl^- content of 40 mM/kg wet weight of cortical tissue (Bourke et al. 1970) and a 25% volume fraction of the ES, where the Cl^- concentration is 144 mM (see also Grossmann 1972). Mobilities of the ions involved: $u(Na^+) = 5.2\ \mu\ s^{-1}\ V^{-1}$ cm, $u(Cl^-) = 7.9\ \mu\ s^{-1}\ V^{-1}$ cm, $u(K^+) = 7.6\ \mu\ s^{-1}\ V^{-1}$ cm (Conway 1952). Transport numbers of these ions are calculated by $t_i = z_i \times c_i \times u_i / \sum_i z_i \times c_i \times u_i$ where c_i denotes the concentration, z_i the charge and u_i the mobility of the corresponding ion. The values obtained are: $t(Na^+) \sim 0.42$, $t(Cl^-) \sim 0.58$ for the ES and $t(K^+) \sim 0.95$, $t(Cl^-) \sim 0.05$ for the intracellular transport. Transport numbers of the respective minority ions are neglected for the purpose of this estimate.

If n K-ions cross the membrane, as described in the Discussion, in a first approximation the current induced in the ES will be carried by 0.58 n Cl-ions and 0.42 n Na-ions according to their transport numbers. Thus, the ES will be locally depleted by n K- + 0.58 n Cl-ions while 0.42 Na-ions approach the site yielding a total loss of 1.16 n osmotically active particles. In the intracellular space the n K-ions will induce an increase in intracellular particles by $n_{(K^+)} - 0.95_{(K^+)} + 0.05_{(Cl^-)} = 0.10$ n ions.

If the differences in osmotic coefficents of the different ionic species (less than 1%, Robinson and Stokes 1968) are neglected, the ratio of the volume fraction of the intracellular space (α_{IS}) to that of the ES (α_{ES}) depends on the ratio of the intracellular to the extracellular number of particles. Thus, the amount of n K-ions, transferred from the ES to the cell interior, which causes the swelling, can be calculated by solving the equation:

$$\alpha_{IS} / \alpha_{ES} = (NI + AP) / (NE - LP)$$

where α_{IS} and α_{ES} are the values after neuronal activity; NI and NE are the initial numbers of intracellular and extracellular particles, calculated by multiplying the relative volume fractions with the assumed total osmolarity of 330 mOsm. LP is the number of particles lost from the ES (= 1.16 n) and AP is the number of particles added to the intracellular space (= 0.10 n). Calculated reductions of the ES are plotted in Fig. 7 versus transmembrane K^+ fluxes. Since there is some uncertainty about the glial/neuronal volume ratio and thus about the number of cells involved in the buffering process and also about the initial volume fraction of the ES, calculations were carried out for a wide range of probable values of these two parameters. The shrinkage turned out to be strongly dependent on the volume fraction of the ES and only marginally on the proportion of cells involved (Fig. 7). A 30% shrinkage of the ES, a commonly observed value in our experiments, is induced by K^+ displacements between 15 and 50 mM.

Estimation of the K^+ released during seizures shows good agreement with these values. The extracellular rise in $[K^+]$ evoked per action potential can be estimated by correlating the $[K^+]_o$ rise to the neuronal discharge rate measured during the first second of seizure activity. Evaluation of previously published data (Heinemann and Lux 1977) yielded a K^+ release of 0.01 to 0.02 mM per action potential, which is the same as reported by Syková et al. (1974). Thus, during a seizure of 30 s duration with an average firing rate of about 100 Hz and an extracellular volume fraction of 25% a total release in the order of 20 mM K^+ is estimated (per liter of brain volume).

The glial conductivity estimated from our experiments is comparable to values obtained by different methods. With a width of the ES of 100 Å and a seizure duration of 30 s we calculated a transmembrane K^+ flux of 1 to 10 × 10^{-12} M $cm^{-2}\ s^{-1}$. This is near to or at most one order of magnitude higher than the flux estimated by Lux and Neher (1973). From the membrane permeabilities of glia cells assumed by Trachtenberg and Pollen (1972) K^+ fluxes in the range of 10^{-10} M $cm^{-2}\ s^{-1}$ can be expected (Lux and Neher (1973). The glial conductivity may, however, be reduced for instance by the resistivity of the gap junctions which results in a smaller K^+ flux.

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Note added in proof

The use of choline$^+$ as extracellular marker was recently described by Hansen AJ and Olsen CE (1980) Brain extracellular space during spreading depression and ischemia. Acta Physiol Scand 108: 355–365

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