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Stimulus-induced Changes in Extracellular Na^+ and Cl^- Concentration in Relation to Changes in the Size of the Extracellular Space*

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Summary. Extracellular Na^+- and Cl^--concentrations ($[Na^+]_o$, $[Cl^-]_o$) were recorded with ion-selective microelectrodes during repetitive stimulation and stimulus-induced self-sustained neuronal afterdischarges (SAD) in the sensorimotor cortex of cats. In all cortical layers $[Na^+]_o$ initially decreased by 4–7 mM. In depths of more than 600 μm below the cortical surface such decreases usually turned into increases of 2–6 mM during the course of the SADs, whereas in superficial layers $[Na^+]_o$ never rose above its resting level. $[Cl^-]_o$ always showed an increase in the course of the SADs often preceded by an initial small decrease. The average increase at a depth of 1,000 μm was about 7 mM. $[Cl^-]_o$ reached peak values at about the end of the ictal period, whereas $[Na^+]_o$ reached its maximum shortly after the end of the SAD, at times when $[K^+]_o$ was still elevated above the baseline concentration.

These data indicate that the extracellular osmolarity can increase during SAD by up to 30 mM. Such an increase in osmolarity can be explained by an increase in the number of intracellular particles, caused by cleavage of larger molecules during enhanced metabolism. This could lead to cell-swelling due to passive water influx from the extracellular space (ES). However, the resulting reduction of the size of the ES is calculated to be less than 10% for an increase in intracellular osmolarity by 30 mOsm. This value is too small as compared to previously measured ES-reductions under similar conditions (i.e., 30% reduction at 1,000 μm; Dietzel et al. 1980). Reductions of the size of the ES that accompany the observed changes in the ionic environment, are quantitatively explained on the basis of the extended glial buffering mechanism described in the preceding paper.

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In earlier investigations self-sustained neuronal afterdischarges (SADs) evoked by electrical stimulation have been shown to be accompanied by changes in the size of the extracellular space (ES) (Dietzel et al. 1980, 1982) and by rises of the extracellular K^+ concentration ($[K^+]_o$) by maximally 7 mM (Lux 1974; Sypert and Ward 1974; Moody et al. 1974; Heinemann and Lux 1975, 1977). In most cortical layers decreases in the size of the ES occur which show a maximum of 30% on average at a depth of about 1,000 μm below the cortical surface. In upper and lower cortical layers, however, smaller rises in $[K^+]_o$ occur in association with much smaller decreases in the ES. Sometimes even a slight increase in ES is observed in very superficial and deep areas. Such changes of the ES could well be explained by selective K^+ transport through coupled cellular networks, as predicted by an extension of the glial-buffering hypothesis proposed by Orkand et al. (1966). The involved mechanism permits not only accelerated re-equilibration of the stimulus induced $[K^+]_o$ rise but should also serve to keep the extracellular Na^+- and Cl^--concentrations constant.

The predictions of this theory concerning the maintenance of $[Na^+]_o$ and $[Cl^-]_o$ during repetitive neuronal activity have yet to be tested. Also lacking are estimates of the magnitude of a possible contribution by metabolic activity to the reduction of the ES. An increase in metabolism during epileptic activity has been observed (King et al. 1973; Siesjö 1978) and the resulting decomposition of larger molecules into several smaller ones would be expected to increase

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intracellular osmolarity. One manifestation of enhanced metabolic activity is an increase in glucose utilisation. It is noteworthy that the cortical depth profile of local glucose consumption induced by natural afferent stimulation (Hand et al. 1979) shows qualitative similarities to the depth profile of the amplitudes of $[K^+]_o$ and the ES changes (Dietzel et al. 1980).

The aim of this study was to estimate the possible contribution of metabolic activity to the decreases of the ES and to further examine the predictions of the K^+ transport/glial buffering hypothesis with respect to $[Na^+]_o$ and $[Cl^-]_o$ homeostasis.

Methods

$[Na^+]_o$, $[Cl^-]_o$, $[K^+]_o$, $[Ca^{2+}]_o$ and field potentials were measured with double barrelled ion-selective/reference microelectrodes, prepared by using a modification of the method of Lux and Neher (1973). To observe the time course of concentration changes of Cl^-, Na^+, and K^+ simultaneously, combinations of bent microelectrodes were glued in parallel with tip separations of 5–20 μm. K^+ and Ca^{2+} electrodes were prepared with K^+ ion exchanger Corning 477317 and Ca^{2+} ion exchanger ETH 1001, as previously described in Lux and Neher (1973) and Heinemann et al. (1977). The reference barrels of Cl^--selective microelectrodes were filled with 100 mM Na_2SO_4. The tip of the Cl^--selective barrel, which contained 150 mM NaCl, was first silanized with a solution of 5% dichlorodimethylsilane in CCl_4 and then filled with Corning 477315 Cl^- ion exchanger. Sometimes, single-barrelled electrodes were filled with Orion Cl^- ion exchanger and glued to double-barrelled Na^+-selective/reference electrode pairs. The Orion exchanger usually also entered the unsilanized reference-barrel and was therefore only used in single-barrelled electrodes. The Na^+-selective microelectrodes contained 150 mM NaCl and their tips were filled with Na^+ ion exchanger ETH 227 (Steiner et al. 1979). The reference barrels were filled with 150 mM NaCl or tetramethylammoniumchloride (TMACl) solutions. Calibrations were carried out with Ringer solutions containing different amounts of NaCl. From a baseline concentration of 150 mM both Cl^- and Na^+ electrodes usually responded to a change in $[Na^+]$ or $[Cl^-]$ to 15 mM with a voltage change of about 59 mV. Since the minimal voltage change resolvable was 0.1–0.2 mV the smallest detectable concentration change was about 0.6 mM in the physiological concentration range. In the presence of 151 mM NaCl no voltage change of the Na^+ electrodes could be seen with a K^+ change in the range of 1–60 mM. Also no voltage response of the Ca^{2+}-electrodes was observed if 150 mM NaCl was replaced by a mixture of 80 mM NaCl and 70 mM KCl. However, the Na^+-electrodes also responded to changes in $[Ca^{2+}]$ between 0.5–5 mM in the presence of 150 mM NaCl with about a 7 mV change. To correct for possible errors of the $[Na^+]_o$ measurement due to interference with $[Ca^{2+}]_o$ changes, comparative measurements were carried out with juxtapositioned Na^+/Ca^{2+} electrode pairs. In the range of the small $[Ca^{2+}]_o$ changes ($[Ca^{2+}]_o' - [Ca^{2+}]_o''$) which occur during SADs (Heinemann et al. 1981), the voltage response (ΔV) of the electrodes is proportional to $\log [Ca^{2+}]_o' - \log [Ca^{2+}]_o''$. Thus, at constant $[Na^+]_o$ the voltage-change of the Na^+ electrode due to a $[Ca^{2+}]_o$ change is $\Delta V_{Na} = A (\log [Ca^{2+}]_o' - \log [Ca^{2+}]_o'')$, while the response of the Ca^{2+}-electrode is $\Delta V_{Ca} = B (\log [Ca^{2+}]_o' - \log [Ca^{2+}]_o'')$. Thus, the voltage response of the Na^+ electrode induced by the $[Ca^{2+}]_o$-change can be compensated by subtracting $\Delta V_{Ca} \cdot A/B$ from ΔV_{Na}. For this purpose simultaneous $[Na^+]_o$ and $[Ca^{2+}]_o$ measurements were stored on magnetic tape. The A/B ratio was obtained from the individual calibration curves. The $[Ca^{2+}]_o$ records were attenuated by the factor A/B, subsequently subtracted from the corresponding $[Na^+]_o$ signals using a differential amplifier, and finally plotted with a chart recorder.

The experiments were performed on adult cats, anesthetized with 0.3–0.9 Vol.% Halothane and 80% N_2O, prepared and supervised as described before (Heinemann and Lux 1975). Recordings were performed in the sensorimotor cortex, which was stimulated through Ag/AgCl balls placed on the cortical surface (CS-stimulation) or by aid of concentric electrodes stereotactically positioned into the corresponding thalamic projection nuclei (nucleus ventroposterolateralis thalami, nucleus ventroposteromedianus: VB-stimulation). Stimuli of 0.1–0.2 ms and 0.1–1.0 mA at 15–20 Hz were applied for 10 s to elicit SADs which varied in duration between 2 and 50 s. The electrodes were advanced in steps of 100 μm into the cortex with a calibrated motor-driven micromanipulator. During each of these laminar traces stimulus parameters were kept constant.

Results

Changes in Extracellular Sodium Concentration

Baseline $[Na^+]_o$ was determined to be 146.4 ± 7.0 mM ($\bar{x}$ ± SD, n = 16). This is slightly less than the Na^+ concentration of 158 ± 4 mM determined by Ames et al. (1964) in the cisterna magna fluid of cats. During the stimulation period $[Na^+]_o$ always showed an initial fast decrease in all cortical layers (Fig. 1). The minimum was usually reached before the end of the 10 s of stimulation. The latency to the $[Na^+]_o$ minimum ($t_{Na,min}$) seemed to be independent of the stimulation site, and on average no dependence on the cortical depth was seen. The average $t_{Na,min}$ of all measurements was 8.8 ± 5.8 s (n = 342) before correction for Ca^{2+}-interference. In some cases the $[Na^+]_o$ minimum was already reached within 2 s after onset of stimulation. In only 53 of 342 measurements did the $[Na^+]_o$ minimum occur following the cessation of stimulation (i.e., during the beginning of the SADs).

From 0–500 μm below the cortical surface, only these decreases were seen, with a return to the baseline value often beginning while SADs were still in progress. As previously described (Dietzel et al. 1980, 1982), these decreases in $[Na^+]_o$ usually turn into slight increases above baseline in deeper cortical layers. The depth profile of the $[Na^+]_o$ changes is illustrated in Fig. 1. At depths between 900 and 1,100 μm, an initial decrease of 5.6 ± 2.8 mM on the average (n = 45) occurred after the onset of the stimulation. During the afterdischarges this decrease inverted into an increase over the baseline value by 2.3 ± 2.3 mM (n = 45). $[Na^+]_o$ usually reached its maximum after the cessation of SADs. Slow field

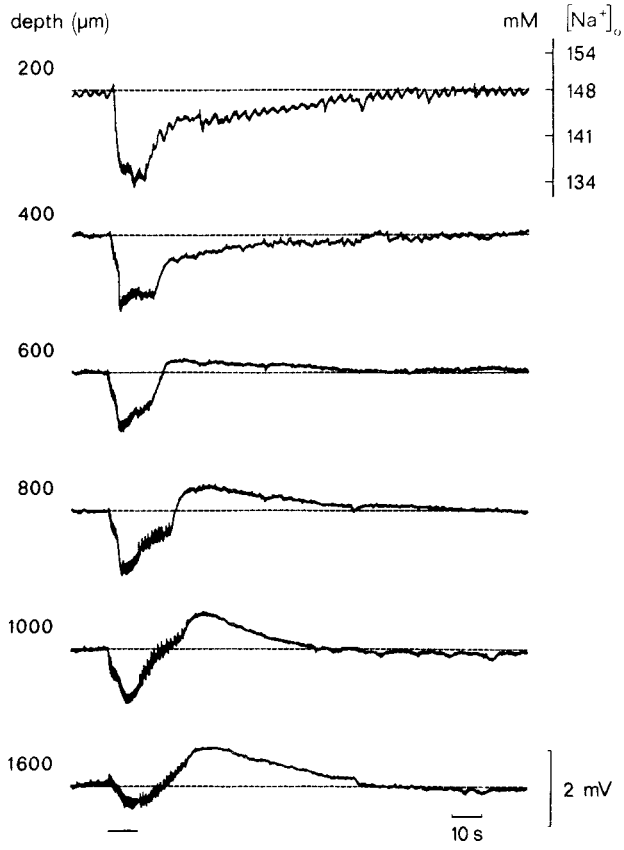

Fig. 1. Laminar profile of $[Na^+]_o$ changes induced by stimulation of the cortical surface with 20 Hz, 0.2 ms, 0.5 mA, applied for 10 s (stimulation period indicated by bar). Amplitude and time calibrations are valid for all records

potentials usually changed from negative to positive voltage deflections at the end of SADs (Figs. 2, 4B, 6, 7). The latency of the $[Na^+]_o$ maximum ($t_{Na,max}$) coincided with the time of the maximal positivity of the field potential (t_{+max}), the average time interval ($t_{Na,max}$–t_{+max}) being only 0.14 ± 8.5 s (n = 201). In the traces corrected for the Ca^{2+} sensitivity of the Na^+-electrodes $t_{Na,max}$ was usually retarded in comparison to t_{+max}($t_{Na,max}$–t_{+max} = 3.7 ± 19 s, n = 20). In all simultaneous measurements of $[Na^+]_o$ and $[K^+]_o$ the maximum of $[Na^+]_o$ clearly preceded the undershoots in $[K^+]_o$ (Fig. 4). In layers between 800 and 1,700 μm $t_{K,min}$–$t_{Na,max}$ was 30.6 ± 14 s (n = 14). This value will be reduced by about 4 s if the $[Na^+]_o$ records are corrected for Ca^{2+} interference. The difference between $t_{K,min}$ and $t_{Na,max}$ is, however, so large, that no special care has to be taken to reduce low frequency noise in order to obtain a less scattered estimation of the flat extrema. Since an excess transport of Na^+ ions out of the cells will change the field potential to positive values (see also Gutnick et al. 1979), the correspondence between $t_{Na,max}$ and t_{+max} and the much longer latency of $t_{K,min}$ suggest a possible contribution by the electrogenic Na^+/K^+ pump to the field potential.

Simultaneous Na^+/Ca^{2+}-measurements with combined Na^+/Ca^{2+} selective microelectrodes (tip intervals of 5–20 μm) are shown in Fig. 2 together with the corrected $[Na^+]_o$ records (see Methods). The amplitude of the $[Na^+]_o$-increase, as measured from the baseline level, was reduced to up to half of its size. In Fig. 3 the laminar profiles of the amplitudes of uncorrected and corrected $[Na^+]_o$ decreases from a typical experiment are shown. While the uncorrected $[Na^+]_o$ decreases are maximal in upper cortical layers, there is much less variation in the corrected traces. In the upper cortical layers, decreases in $[Na^+]_o$ by more than 10 mM were observed in the uncorrected $[Na^+]_o$ measurements. Since $[K^+]_o$ increases are limited to maximally 7 mM (Lux 1974; Moody et al. 1974; Heinemann and Lux 1977), such large $[Na^+]_o$ decreases would indicate that the Na^+ entry into cells significantly exceeds the K^+ efflux. However, after correction for Ca^{2+} interference, the decreases in $[Na^+]_o$ were reduced to 4–7 mM, which is in line with an initial 1 : 1 Na^+/K^+ exchange during stimulus induced activity. The time courses of $[Na^+]_o$ and $[K^+]_o$ changes were compared by simultaneous measurements of both ions (Figs. 4 and 5). So far we did not succeed in measuring more than two ionic species simultaneously. Thus, only uncorrected Na^+-signals could be directly compared to their corresponding K^+-signals. At a depth of about 1,000 μm there are no fast initial Ca^{2+}-changes so that the amplitudes and time courses of $[K^+]_o$ and $[Na^+]_o$-signals can be directly related to one another (Fig. 5). The initial close correspondence of $[K^+]_o$ and $[Na^+]_o$ further confirms the concept of a 1 : 1 Na^+/K^+ exchange. While the initial increases of $[K^+]_o$ mirrored the corresponding $[Na^+]_o$ decreases, the kinetics of reequilibration of these ions differed significantly. Since $[Na^+]_o$ returned to baseline faster than $[K^+]_o$, which still applies after correction of the $[Na^+]_o$-signals, the sum of the external cation concentrations usually increased during the course of the SAD. In the upper cortical layers this sum showed only small increases of about 2 mM and in some cases was unchanged altogether. In layers deeper than 600 μm, however, increases of up to 6 mM were observed (Fig. 5). Their maxima were attained shortly before $t_{Na,max}$, and coincided roughly with the termination of the SADs. For reasons of electroneutrality it is to be expected that such a rise in the sum of the cation concentrations is accompanied by a rise in anion concentrations as well. To test this hypothesis, measurements of changes in $[Cl^-]_o$, which is the main extracellular anion, were performed.

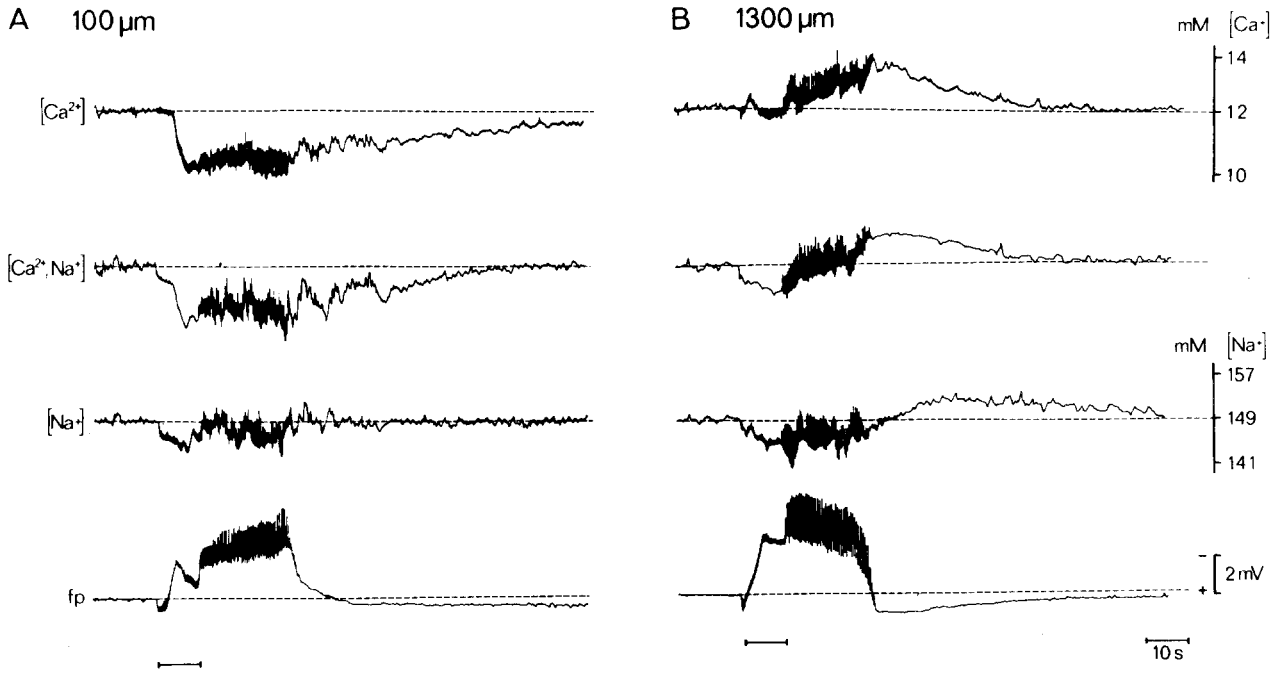

Fig. 2A, B. Correction of $[Na^+]_o$ measurements for interference with $[Ca^{2+}]_o$ changes at two depths in the cortex. The Ca^{2+}-induced component of the $[Na^+]_o$ record was eliminated by subtracting the $[Ca^{2+}]_o$ record, multiplied with the appropriate correction factor, from the $[Na^+]_o$ record (see Methods). fp = field potential (CS stimulation: 20 Hz, 0.2 ms, 0.5 mA applied for 10 s)

Changes in Extracellular Chloride Concentration

Baseline $[Cl^-]_o$ was found to be 148.7 ± 7.8 mM (n = 9), which is close to the value of 144 ± 2 mM reported by Ames et al. (1964).

In most cases stimulus induced SADs were accompanied by elevations in $[Cl^-]_o$ (Figs. 6 and 7) (see also Lehmenkühler et al., in press). In contrast

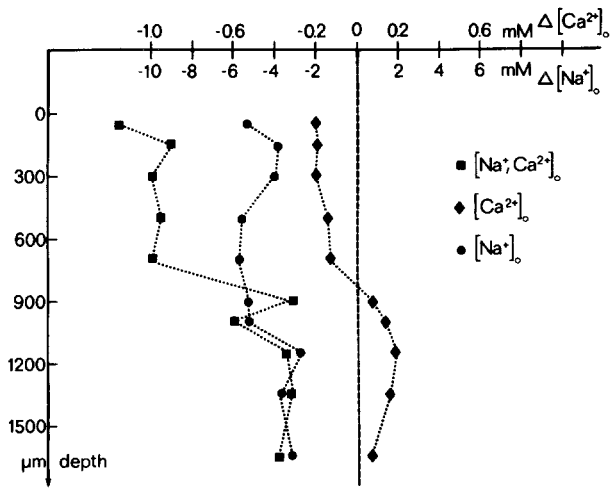

Fig. 3. Depth profile of the amplitudes of uncorrected and corrected $[Na^+]_o$ decreases and $[Ca^{2+}]_o$ changes. The subsequent $[Na^+]_o$ increases in deeper cortical layers are not shown. Data were taken from the experiment shown in Fig. 2

to the steep slopes of the $[K^+]_o$ rises and $[Na^+]_o$ decreases, $[Cl^-]_o$ rose gradually during the discharge period with half times of about 10.5 ± 3.9 s (n = 135) (Figs. 6 and 7). Sometimes $[Cl^-]_o$ did not start to rise during the 10 s of stimulation prior to the development of SADs. In 22% of all records, small $[Cl^-]_o$ decreases (during both cortical and thalamic stimulation) preceded the $[Cl^-]_o$ increases. These decreases, which occurred in all depths, had an average amplitude of 2.9 ± 2.3 mM (n = 41). Such decreases in $[Cl^-]_o$ might be due to Cl^- entry into cells caused by enhanced inhibition during the stimulation. Altogether an initial NaCl influx into depolarized cells may occur. Though our evaluations point to an average 1 : 1 exchange between K^+ and Na^+ at the onset of enhanced neuronal activity we cannot exclude that in some cases the initial Na^+ influx can exceed the K^+ efflux. To definitely clarify the origin of the decreases in $[Cl^-]_o$ simultaneous measurements of more than two ionic species are necessary.

The average amplitude of the $[Cl]_o$-rises was 6.3 ± 3.0 mM (n = 151). In most cases the maximal value was reached shortly before or at the end of the SADs. If SADs were shorter than 10 s, the time of the $[Cl^-]_o$ maximum ($t_{Cl,max}$) coincided with the end of the discharges. With increasing discharge duration $t_{Cl,max}$ also increased, however, by a smaller factor than the duration of SADs. If SADs exceeded 10 s, $[Cl^-]_o$ reached its maximum before the discharges

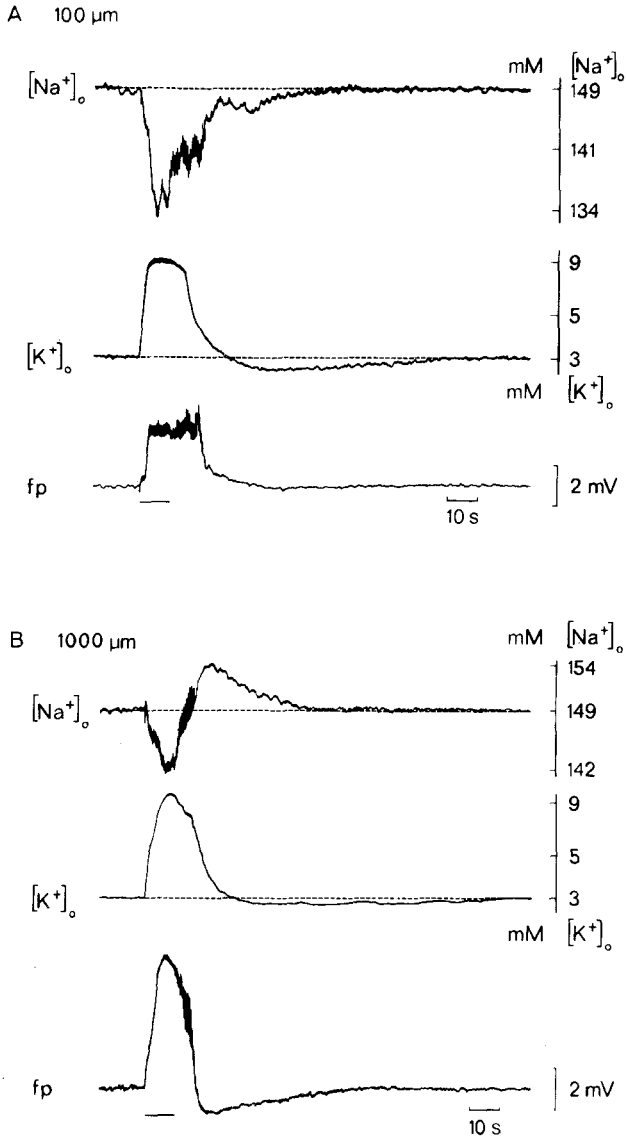

Fig. 4A, B. Simultaneous measurements of $[Na^+]_o$, $[K^+]_o$ and field potential (fp) at a depth of 100 and 1,000 µm below the cortical surface (CS-stimulation, 20 Hz, 0.1 ms, 0.6 mA, duration indicated by bars)

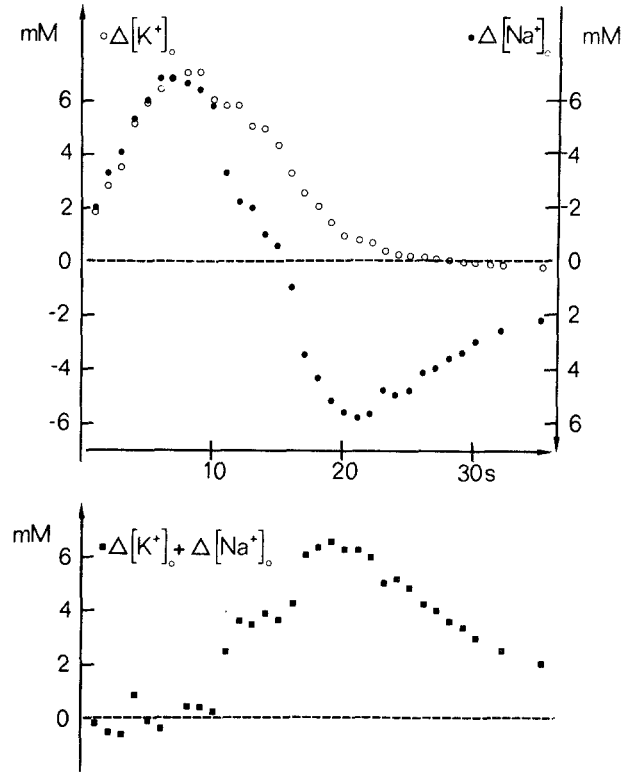

Fig. 5. To compare the kinetics of $\Delta[K^+]_o$ and $\Delta[Na^+]_o$, the time courses of $[K^+]_o$- and the inversed $[Na^+]_o$-changes ($\Delta[K^+]_o$, $-\Delta[Na^+]_o$) were calculated from records in Fig. 4B using the individual calibration curves and displayed on a linear scale. The sum of both concentrations is shown in the lower part of the figure. Note that the maximum of the sum precedes the peak of $[Na^+]_o$

terminated. Thus the time between $t_{Cl,max}$ and the end of SADs also increased with increasing discharge duration (Fig. 8). Sometimes $[Cl^-]_o$ started to decline before the end of the seizure activity. Half times of decay, measured from $t_{Cl,max}$, were 16.3 ± 13.3 s (n = 121). These results did not depend on the kind of ion exchanger used for the Cl^--selective electrodes.

The kinetics of the sum of the cation concentrations (Fig. 5) is comparable to the kinetics of the $[Cl^-]_o$ measurements (Figs. 6 and 7). There is a gradual increase during the SADs, and $t_{Cl,max}$ precedes $t_{Na,max}$. However, $[Cl^-]_o$ on the average exceeds the sum of the cation-increases. This is especially prominent in upper cortical layers where cation-increases of less than 2 mM correspond to increases in $[Cl^-]_o$ of about 5 mM. At depths of about 1,000 µm, $[Cl^-]_o$ increases of about 7 mM are registered concomitant with cation-increases of about 6 mM. This discrepancy may be explained by several effects: First, we have not yet succeeded in measuring more than two ionic species at a time; and the separation between the electrode tips, as well as the low sensitivity of the electrodes for Na^+ and Cl^- in extracellular fluid, may introduce considerable error. Second, there may be a concomitant extracellular $[HCO_3^-]$-change (Howse et al. 1974) which compensates possible unbalanced increases in anion concentrations. This is in accordance with suggestions by Janus and Lehmenkühler (1981) who have shown $[Cl^-]_o$ increases, but no increases in total cation concentration in a spinal cord preparation of the rat. Such decreases in $[HCO_3^-]$ would, however, affect the measurement of $[Cl^-]_o$ (Deisz and Lux 1978), resulting in a reduced electrode response to $[Cl^-]_o$ increases. This would lead to an underestimation of the increases in $[Cl^-]_o$.

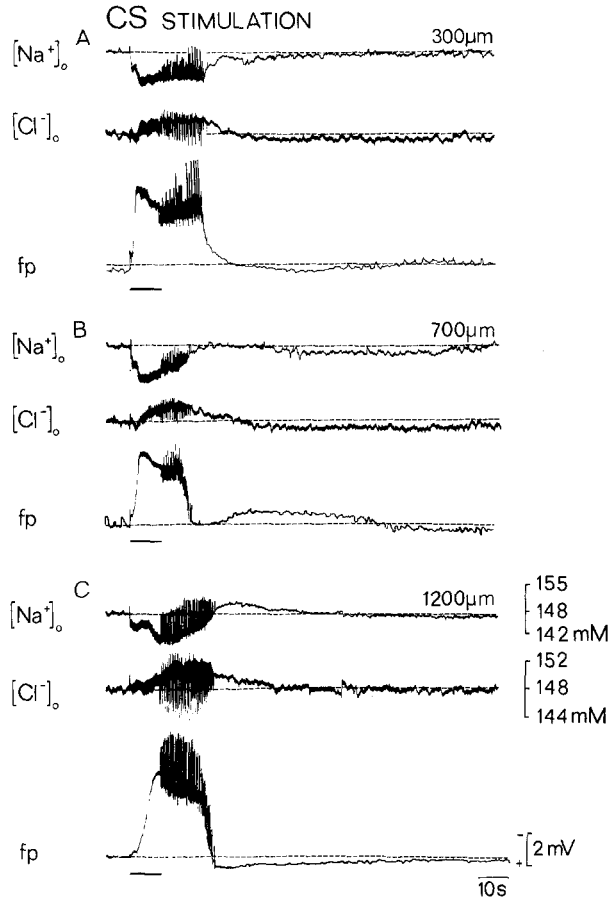

Fig. 6A–C. Simultaneous $[Na^+]_o$, $[Cl^-]_o$ and field potential (fp) recordings during CS-stimulation induced neuronal afterdischarges at different cortical depths; stimulus parameters: 20 Hz, 0.1 s, 0.5 mA, duration 10 s. Note the similarity of the kinetics of the $[Cl^-]_o$ rises to the calculated sum of cations (Fig. 5)

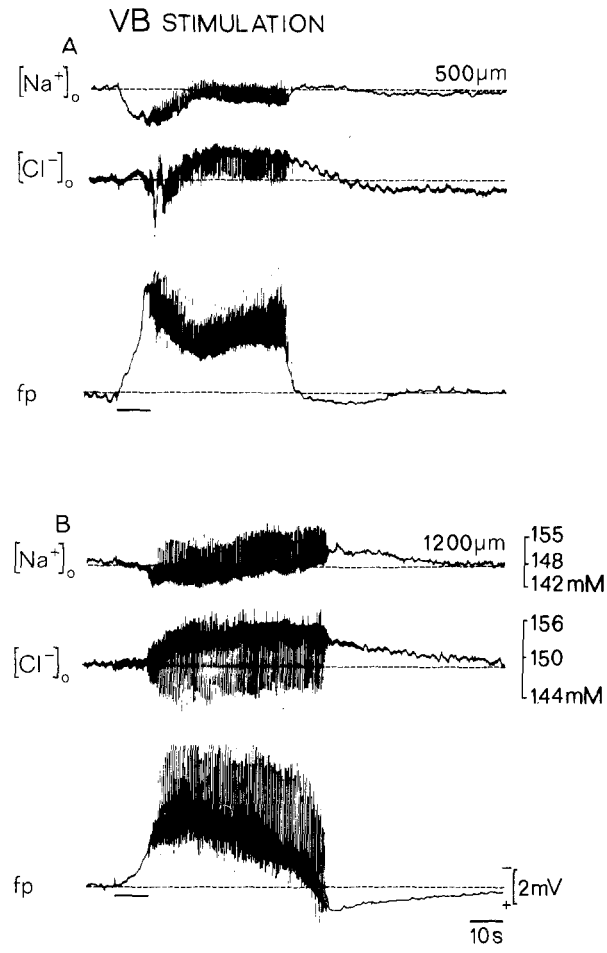

Fig. 7A, B. Simultaneous $[Na^+]_o$, $[Cl^-]_o$ and field potential (fp) recordings during VB-stimulation induced neuronal afterdischarges at different cortical depths; stimulus parameters: 20 Hz, 0.1 s, 0.5 mA, duration 10 s

Discussion

In a preceding paper we have described decreases of the ES by on average 30% at a cortical depth of about 1,000 µm which occured under experimental conditions comparable to those employed in the present investigation.

The method is based on the following: The concentration of a substance is inversely proportional to the volume within which it can distribute. Thus, relative changes of that volume can be ascertained by measuring the accompanying concentration changes. Nominally K^+-selective microelectrodes (Corning 477317) are extremely sensitive to tetramethylammonium and choline-ions (Neher and Lux 1973) for which cellular membranes are largely impermeable within the time required for the measurements (Phillips and Nicholson 1978; Hansen and Olsen 1980). Thus, repeated iontophoretic injection of constant amounts of these ions permits local monitoring of relative changes of the ES within time intervals of less than 30 s. In the present investigation we have shown, that $[Cl^-]_o$ rises by about 7 mM and that $[Na^+]_o + [K^+]_o$ increases by about 6 mM at the end of SADs.

The concentration of extracellular particles besides Na^+, K^+, and Cl^- is estimated to be about 32 mM under control conditions. A decrease of the ES by 30% will condense this solution of impermeable molecules thus increasing its concentration by 14 mM. Altogether, an increase in tissue osmolarity by maximally 27 mOsm can be expected under these conditions. This increase in osmolarity can be explained by a delay in the clearance of smaller molecules produced intracellularly, during enhanced metabolism, by cleavage of larger molecules such as glycogen, ATP, etc. An increase in the number of intracellular particles is assumed to induce a water

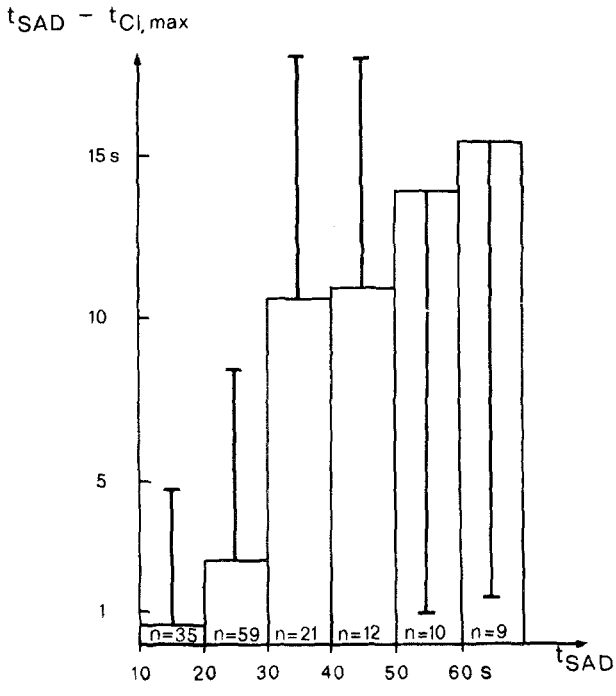

Fig. 8. Average time interval between the end of self-sustained afterdischarges (t_{SAD}) and the latency of the $[Cl^-]_o$ maximum ($t_{Cl,max}$). All $[Cl^-]_o$ measurements (including CS- as well as VB-stimulation induced $[Cl^-]_o$ rises) were classified according to the duration of the corresponding SADs. $t_{Cl,max}$, as well as t_{SAD}-$t_{Cl,max}$ (represented by columns) increased with the increasing SAD-duration. (Standard deviations are indicated by bars)

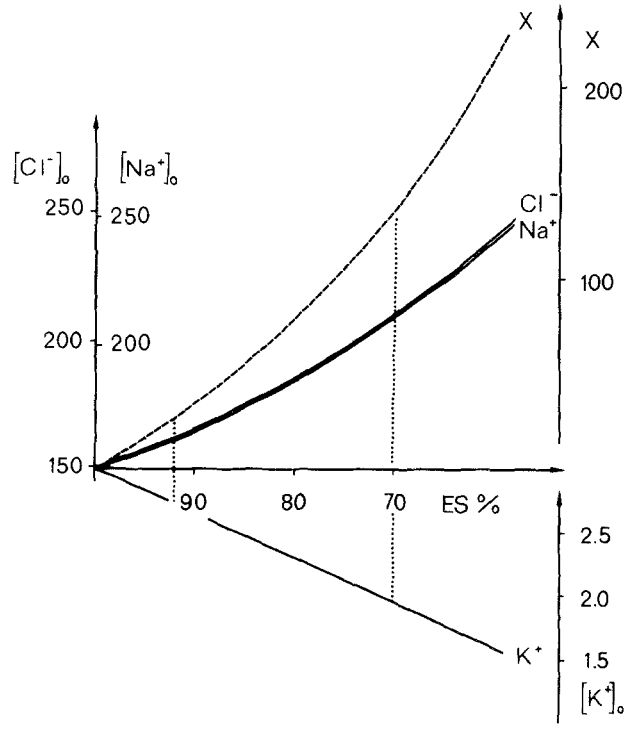

Fig. 9. Solution of Eqs. (1)–(9) describing changes in $[K^+]_o$, $[Cl^-]_o$ and the size of the ES exclusively due to an increase in intracellular osmolarity (y = 0). Initial values were chosen as follows: $[K^+]_o$ = 3 mM, $[Na^+]_o$ = 146 mM, $[Cl^-]_o$ = 149 mM, $[K^+]_{n,g}$ = 100 mM, $[Cl^-]_{n,g}$ = 4.5 mM, c_1 = 80.6, c_2 = 33.4, c_3 = 38.2, c_4 = 6.4, $c_5 = c_6$ = 90.2, Ai = 0.2, Bi = 0.4, z = x/2, n = 330. The first dotted line shows the consequences of an increase in osmolarity by x = 27 mOsm, as estimated from the present data. A decrease in $[K^+]_o$ from 3 to 2.8 mM, a $[Cl^-]_o$ increase from 149 to 161 mM, a $[Na^+]_o$ increase from 146 to 158 mM occurs concomitant with an ES reduction by only 8%. The second dotted line shows an osmolarity increase, x = 137 mOsm, which explains the ES reduction of 30% of its initial size (Dietzel et al. 1980). The accompanying ionic changes are much larger than those observable: $[Na^+]_o$ = 209 mM, $[Cl^-]_o$ = 211 mM, $[K^+]_o$ = 1.81 mM. Concentrations are calibrated in mM

flow from the ES into the cells, thus reducing the size of the ES and increasing the concentration of the extracellular ions. Unless the water permeability of brain cells is markedly less than that of large invertebrate axons, equilibration of osmotic gradients between adjacent cells is expected to be accomplished on a time scale of seconds or less (House 1980). Thus, it can be assumed that the osmolarity of the ES and adjacent cells does not differ significantly at the end of a seizure of 30 s duration. To determine whether decreases of the size of the ES and ionic changes in the observed order of magnitude can be explained by such increases in osmolarity, model calculations were carried out under the assumption that the ratio of extra- to intracellular space is determined by the ratio of the number of extra- to intracellular particles. Additionally, it was assumed that under resting conditions, that exist after the termination of SADs, Donnan-equilibration determines the ratio of extra- and intracellular Cl^- and K^+ concentrations, whereas membrane permeabilities to the remaining ions are low. The assumption that the KCl distribution across glial membranes may be described by Donnan equilibration is justified by measurements of Bührle and Sonnhof (1981). Whether possible Cl^- shifts across the glial membranes are due to active or passive processes has no influence on the model discussed here. After the end of SADs neuronal membranes are hyperpolarized by active pumping and in a first approximation we may assume that KCl is Donnan-distributed at that time. It has been claimed that under these conditions an increase in the number of intracellular particles by enhanced metabolism may lead to a reduction of the size of the ES, accompanied by an increase in $[Cl^-]_o$ and a decrease in $[K^+]_o$ (Gardner-Medwin 1980). To test this hypothesis the following equations were solved: Before and after the addition of intracellular particles, $[K^+]$ and $[Cl^-]$ are determined according to the Donnan equation:

$$[Cl^-]_n[K^+]_n = [Cl^-]_o[K^+]_o \quad (1)$$

$$[Cl^-]_g[K^+]_g = [Cl^-]_o[K^+]_o \quad (2)$$

where $[Cl^-]_{o,n,g}$ and $[K^+]_{o,n,g}$ stand for the extracellular, intraneuronal and intraglial concentrations after the addition of intracellular particles. During the short time of discharge activity, about 30 s, no K- or Cl-ions are assumed to be cleared out of or added to the tissue through blood capillaries. This is supported by the observation that $[K^+]_o$ changes in brain slices are not different from those seen in animals with intact circulation (Heinemann and Schubert, unpubl. observ.). So the total numbers of K- and Cl-ions are assumed to be conserved:

$$[K^+]_oA+[K^+]_gB + [K^+]_n(1-A-B) = c_1 \quad (3)$$

$$[Cl^-]_oA+[Cl^-]_gB + [Cl^-]_n(1-A-B) = c_2 \quad (4)$$

where c_1 and c_2 are the total tissue contents (calculated from the initial values) of Cl- and K-ions. A, B, and (1–A–B) are the volume fractions of the extracellular, glial and neuronal spaces. Conservation of electroneutrality is described by

$$[Cl^-]_o = [K^+]_o + [Na^+]_o \quad (5)$$

where we assume, for simplicity, that the remaining extracellular positive charges ($2 \cdot [Ca^{2+}]_o+2 \cdot [Mg^{2+}]_o$) are approximately equal to the negative charges ($[HCO_3^-]_o$). Small changes in the extracellular cation concentration like the $[Ca^{2+}]_o$ decreases (by maximally 0.4 mM; Heinemann et al. 1981) and possible $[HCO_3^-]$ changes are neglected. Electroneutrality in glial cells is described by

$$B([K^+]_g - [Cl^-]_g) = c_3 \quad (6)$$

where c_3 is the fixed number of net charges besides Cl^- and K^+. The metabolically induced increase in osmotically active particles in the tissue (x) can be calculated with

$$n+x = [K^+]_o+[Cl^-]_o+([Na^+]_{oi}A_i-y)/A+c_4/A \quad (7)$$

where $[Na^+]_{oi}$ and A_i are the initial extracellular Na^+ concentration and volume fraction; y is the number of Na-ions transferred from the ES to the neurons; c_4 is the total number of the remaining extracellular particles (Ca^{2+}, Mg^{2+}, HCO_3^-, neutral molecules), and n the tissue osmolarity. From x particles added into 1 liter of brain tissue, x–z particles are assumed to be added into neurons while the remaining z particles are added into glial cells, which are in osmotic equilibrium with the ES:

$$n+x = [K^+]_g+[Cl^-]_g+(c_5+z)/B \quad (8)$$

where c_5 is the initial number of intraglial particles besides K^+ and Cl^-. The last equation describes the osmotic equilibrium of the neurons with the ES and the glial cells:

$$n+x = [K^+]_n+[Cl^-]_n+(c_6+y+x-z)/(1-A-B) \quad (9)$$

where c_6 is the number of intraneuronal particles besides K^+ and Cl^-, including the Na^+ content before the stimulation.

The solutions of these equations, under the assumption that no Na ions are displaced across any membranes (y = 0), are shown in Fig. 9. Hence an increase in tissue osmolarity by 27 mOsm is predicted to be accompanied by an increase in $[Cl^-]_o$ from 149 to 161 mM, by a decrease in $[K^+]_o$ from 3 to 2.8 mM and by a decrease in the size of the ES by 8%. $[K^+]_o$ undershoots from 3 to 2.8 mM have been observed following SADs (Heinemann and Lux 1975) but $[Cl^-]_o$ changes were usually smaller than 12 mM and the average ES change in 1,000 μm depth was several times larger than the predicted 8%. If we assume that an ES reduction by 30% is produced by metabolic activity, then an increase in osmolarity by 137 mOsm, a decrease of extracellular $[K^+]_o$ to 2.0 mM and an increase of $[Cl^-]_o$ to 210 mM is to be expected. Calculations were also carried out for initial values of the ES varying between 10–30%, baseline $[Cl^-]_o$ = 144 mM and $[Na^+]_o$ = 158 mM. The results showed negligible differences from those shown here. All calculated values are far from those ever observed. Hence we can conclude that a metabolically increased number of intracellular particles alone can at best explain a decrease in the size of the ES by 8%.

In an ES decreased by only 6–9%, the metabolic mechanism could explain the $[K^+]_o$ undershoots observed after termination of seizure activity (Heinemann and Lux 1975) (see also Fig. 4). That is, a metabolically induced increase in osmolarity, which is an aftereffect of active transport, can contribute to the efficacy of the pump, but does not explain the K^+ undershoots which occur in an ES reduced by 30%.

If we assume that NaCl influx into the depolarized neurons is responsible for the reductions of the ES, and that no increase in osmolarity occurs (x = 0), solution of the Eqs. (1)–(9), leads to a reversed distribution of the extracellular ions (Fig. 10): In this case a reduction of the ES by 30% is accompanied by a decrease in $[Na^+]_o$ by 13 mM and of $[Cl^-]_o$ by 7 mM, while $[K^+]_o$ rises by 7 mM (Fig. 10). Hence it may be suggested that a combination of both mechanisms might explain the present results. Figure 11 shows the solution of Eqs. (1)–(9) for an ES reduction by 30%

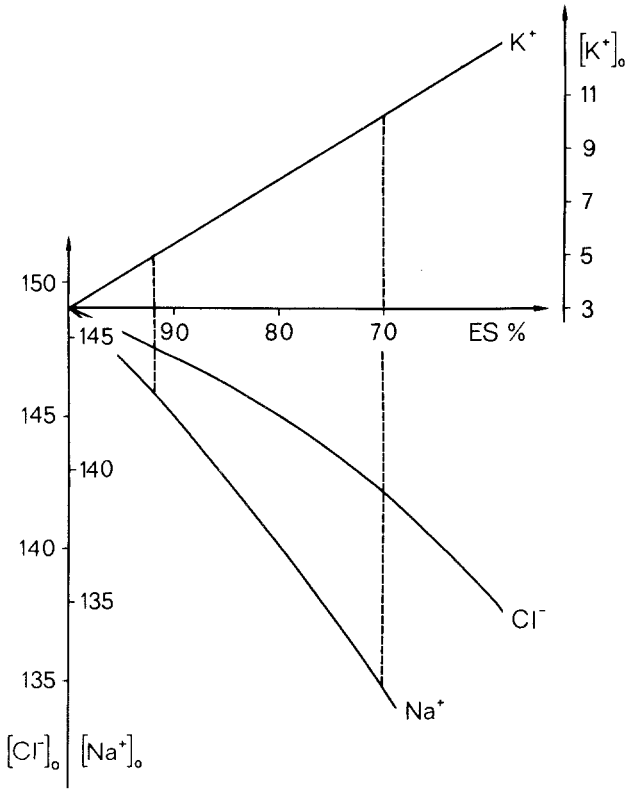

Fig. 10. Solution of Eqs. (1)–(9), explaining ES changes by Na^+ displacement (y between 0 and 13) across neuronal membranes without metabolic contributions (x = 0). Initial values and constants c_1–c_6 are the same as in Fig. 9. Note that $[Cl^-]_o$ and $[Na^+]_o$ values are always lower and $[K^+]_o$ values always larger than those seen in the experiments. Concentrations are calibrated in mM

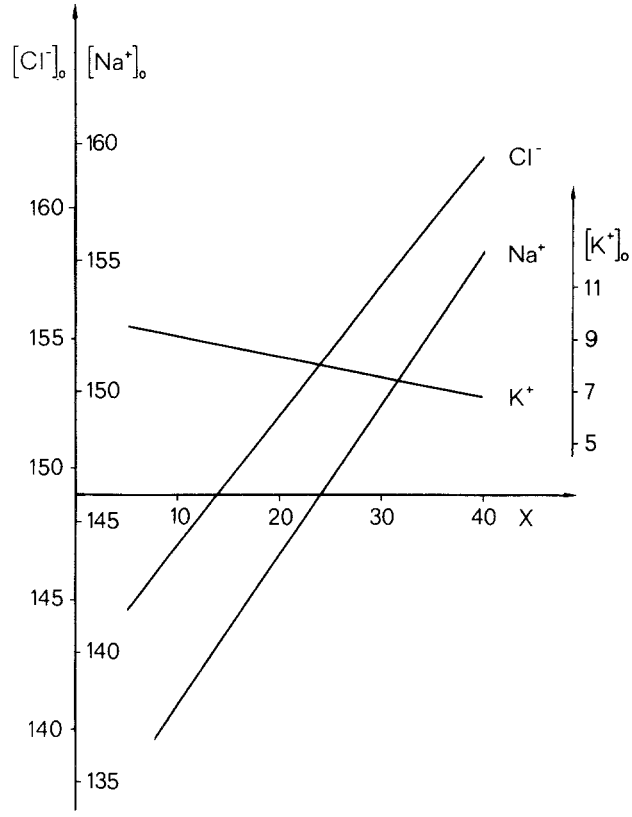

Fig. 11. Combination of an increase in osmolarity (x in mOsm) and of a Na^+ displacement to induce an ES reduction by 30% of its initial size (A_i = 0.2 A = 0.14, solution of Eqs. (1)–(9) with same initial values and constants as in Fig. 9; x varying in the reasonable range of increase in osmolarity). For all possible combinations $[K^+]_o$ is at least 2 mM higher than expected from the experimental data. Concentrations are calibrated in mM

caused by a combination of Na^+ flux into neurons and additon of particles into neurons and glial cells (z = x/2). In the possible range of an osmolarity increase (i.e., by 5–40 mOsm), $[K^+]_o$ values of 9–6 mM are predicted. This is in contrast to our data in which after termination of SADs, decreases in the size of the ES were recorded concomitant to undershoots of $[K^+]_o$ below the baseline value of 3 mM.

Another mechanism leading to a reduction of the size of the ES is described by an extension of the glial buffering hypothesis (Dietzel et al. 1980). According to the concept of glial buffering, inhomogeneous $[K^+]_o$ elevations, as occur during SADs, induce transmembrane K^+ currents through extended neuronal or coupled glial networks (Orkand et al. 1966; Trachtenberg and Pollen 1970; Somjen 1975; Gardner-Medwin et al. 1981). If a quantity of w K-ions crosses the membranes, this current is accompanied by a compensating extracellular current carried by 0.4 w Na- and 0.6 w Cl-ions according to their transport numbers in the extracellular fluid. As w K- and 0.6 w Cl-ions leave the site of maximal $[K^+]_o$ and only the 0.4 w Na-ions compensate for this loss, a local decrease in the number of extracellular particles occurs at that site. According to intraglial transport numbers we may expect an intracellular current carried by 0.04 w Cl-ions and 0.96 w K-ions, leading to a small increase in the number of intracellular particles (Dietzel et al. 1980, Fig. 6). The resultant water flux will equilibrate transmembrane osmolarity differences, thus reducing the size of the ES. However, a net loss of particles may occur at the site of maximal K^+ entry into cells. Thus an increase in the number of particles should occur at deep and superficial cortical layers, where the K-ions are supposed to leave the cells, resulting in a transcortical osmotic gradient. As the distances for diffusional equilibration may amount to several hundred microns, half times of recovery may be in the range of more than 10 s when a water diffusion coefficient of about 2.6×10^{-5} cm^2 s^{-1} (House 1974) and standard diffusion

equations (Crank 1956) are employed. However, such decreases in osmolarity, at the site of maximal decrease in the size of the ES, were not observed in the present investigation. To estimate whether a combination of metabolically induced osmolarity increase and glial buffering can explain our results, the consequences of glial buffering were incorporated into Eqs. (1)–(9). As described above a transmembrane displacement of w K-ions leads to a local depletion of 0.96 w K-ions at the site of maximal decrease of the ES. This is concomitant with a local loss of 0.56 w Cl-ions. Thus the local K^+ and Cl^- content is changed such that c_1 changes into $c_1' = c_1 - 0.96$ w and c_2 into $c_2' = c_2 - 0.56$ w. During a seizure of 30 s duration a total release of K^+ of up to 20 mM can be expected (Dietzel et al. 1980). As shown in Fig. 12, reasonable parameter constellations permit the description of the observed ionic changes concomitant with the expected reductions of the size of the ES. Thus, we can assume that the amount of local changes in the sizes of the ES and of $[Na^+]_o$, $[K^+]_o$ and $[Cl^-]_o$, which occur after stimulus induced self-sustained neuronal afterdischarges, can be explained as a consequence of glial buffering. Small increases in osmolarity may contribute to cell swelling and $[K^+]_o$ undershoots after SADs, but they are much too small to account fully for these phenomena.

Additionally, Eqs. (1)–(9) provide predictions of the accompanying intracellular $[K^+]$ and $[Cl^-]$-changes.

If the ES reduction is induced by NaCl influx into neurons (Fig. 10), an increase in intracellular $[Cl^-]$ is expected in both, nerve and glia cells, whereas a slight increase in intraglial $[K^+]$ and a larger decrease in intraneuronal $[K^+]$ occurs.

If the reduction of the ES is exclusively due to an increase in intraneuronal osmolarity we expect a decrease in $[K^+]_n$, an increase in $[Cl^-]_n$, a small decrease in $[Cl^-]_g$ and an increase in $[K^+]_g$.

If glial buffering accounts for the major part of the change of the ES a strong decrease in $[K^+]_n$ and a slight decrease in $[K^+]_g$ is expected to occur concomitant to an increase in $[Cl^-]_n$ and a small decrease in $[Cl^-]_g$ at the site of K^+ entry into the cells (parameters used in Fig. 12 were applied).

The present measurements further support the suggestion that SADs and spreading depression (SD) are different phenomena. SDs are accompanied by dramatic decreases in $[Na^+]_o$ and $[Cl^-]_o$ by about 90 mM and by increases in $[K^+]_o$ by more than 30 mM (Kraig and Nicholson 1978). At the same time decreases in the size of the ES occur, as shown electronmicroscopically (van Harreveld and Khattab 1967), by concentration measurements of extracellular marker ions (Phillips and Nicholson 1979; Hansen and Olsen 1980) and suggested by impedance measurements (i.e., Freygang and Landau 1955). At first glance these ionic and ES changes may exhibit some similarity to the phenomena observed during SADs. Besides quantitative differences, however, $[Cl^-]_o$ increases and $[Na^+]_o$ overshoots, which occur during SADs are not observed in SDs, suggesting, that large anion-channels (Phillips and Nicholson 1979) obviously do not open during SADs. The principle difference between both phenomena is clarified by looking for a solution of Eqs. (1)–(9), which comes near to the data of Kraig and Nicholson (1978). Huge $[NaCl]_o$ decreases combined with $[K^+]_o$ increases occur if an ES decrease is due to NaCl entry into the cells (Fig. 10). Assuming that the ES decrease is exclusively due to NaCl entry into depolarized neurons (x, z = 0, w = 0) and that Donnan equilibration determines KCl-distribution across both, neuronal and glial membranes, our model predicts that an ES reduction by 83% would be accompanied by a $[Cl^-]_o$ decrease of 80 mM, a $[Na^+]_o$ decrease by 109 mM and a $[K^+]_o$ increase by 31 mM (using the same parameters as in Fig. 10).

If Donnan equilibration takes place across glial membranes this may have an interesting consequence: Since the $[K^+]_o$ rise increases the $[K] \cdot [Cl]$

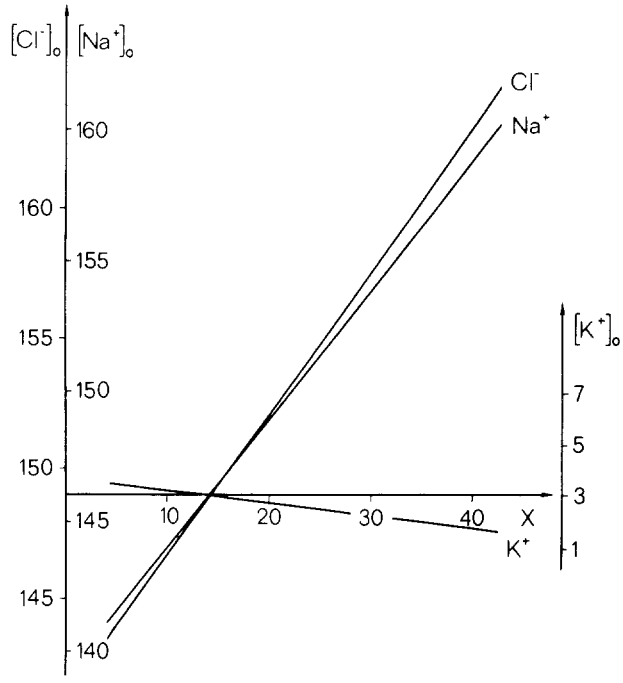

Fig. 12. Incorporation of the consequences of glial buffering into Eqs. (1)–(9). Solutions for an ES reduction by 30% ($A_i = 0.2$ A = 0.14), w = 13 mM, z = x/2, and x varying between 5 and 50 mOsm. All initial values and constants except c_1' and c_2' are identical to those used in Figs. 9–11. Concentrations are calibrated in mM

product this will lead to an intraglial increase in $[K^+]_g$ by about 18 mM in the special case considered. There will also be a predicted rise in $[Cl^-]_n$ by 35 mM, which is principally in accordance with the $[Cl^-]_n$ accumulation observed by van Harreveld and Schade (1959). Assuming, additionally, a possible local decrease in osmolarity (Kraig and Nicholson 1978), similarly large $[NaCl]_o$ decreases and $[K^+]_o$ increases as calculated above may be assumed to occur with considerably smaller ES changes. Although we did not attempt to find parameter constellations, which exactly yield the results of Kraig and Nicholson (1978), these estimates suggest that in some improved version of the model the ionic and ES changes during SD can be described predominantly by NaCl fluxes into depolarized neurons. The ionic changes accompanying SADs, however, could so far not be described without incorporation of the effects of glial-buffering into the calculations.

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References

Ames A, Sakanoue M, Endo S (1964) Na, K, Ca, Mg, and Cl concentrations in choroid plexus fluid and cisternal fluid compared with plasma ultrafiltrate. J Neurophysiol 27: 672–681

Bührle Ph, Sonnhof U (1981) Intracellular activities of K^+, Na^+, Ca^{2+}, and Cl^- in motoneurons and glial cells of the frog spinal cord. Pflügers Arch [Suppl] 389: R24

Crank J (1956) The mathematics of diffusion. Oxford University Press, London

Deisz RA, Lux HD (1978) Intracellular chloride concentration and postsynaptic inhibition in crayfish stretch receptor neurons. Arzneim Forsch 28: 870–871

Dietzel I, Heinemann U, Hofmeier G, Lux HD (1980) Transient changes in the size of the extracellular space in the sensorimotor cortex of cats in relation to stimulus induced changes in potassium concentration. Exp Brain Res 40: 432–439

Dietzel I, Heinemann U, Hofmeier G, Lux HD (1982) Changes in the extracellular volume in the cerebral cortex of cats in relation to stimulus induced epileptiform afterdischarges. In: Klee MR, Lux HD, Speckmann EJ (eds) Pharmacology and physiology of epileptogenic phenomena. Raven Press, New York (in press)

Freygang WH, Landau WM (1955) Some relations between resistivity and electrical activity in the cerebral cortex of the cat. J Cell Physiol 45: 377–392

Gardner-Medwin AR (1980) Membrane transport and solute migration affecting the brain cell microenvironment. In: Nicholson C (ed) Dynamics of the brain cell microenvironment. MIT Press, Cambridge (Neurosci Res Program Bull, vol 18, p 220)

Gardner-Medwin AR, Coles JA, Tsacopoulos M (1981) Clearance of extracellular potassium: Evidence for spatial buffering by glial cells in the retina of the drone. Brain Res 209: 452–457

Gutnick MJ, Heinemann U, Lux HD (1979) Stimulus-induced and seizure-related changes in extracellular potassium concentration in cat thalamus (VPL). Electroencephalogr Clin Neurophysiol 47: 329–344

Hand P, Greenberg J, Goochee C, Sylvestro A, Weller L, Reivich M (1979) A normal and developmentally altered cortical column: A laminar analysis of local glucose utilization with natural stimulation of a single receptor organ. Acta Neurol Scand [Suppl 72] 60: 46–47

Hansen AJ, Olsen CE (1980) Brain extracellular space during spreading depression and ischemia. Acta Physiol Scand 108: 355–365

Harreveld A van, Schadé JP (1959) Chloride movements in cerebral cortex after circulatory arrest and during spreading depression. J Cell Comp Physiol 54: 65–77

Harreveld A van, Khattab FI (1967) Changes in cortical extracellular space during spreading depression investigated with the electron microscope. J Neurophysiol 30: 911–929

Heinemann U, Konnerth A, Lux HD (1981) Stimulation induced changes in extracellular free calcium in normal and chronic alumina cream foci of cats. Brain Res 213: 246–250

Heinemann U, Lux HD (1975) Undershoots following stimulus induced rises of extracellular potassium concentration in cerebral cortex of cat. Brain Res 93: 63–76

Heinemann U, Lux HD (1977) Ceiling of stimulus induced rises in extracellular potassium concentration in the cerebral cortex of cats. Brain Res 120: 231–249

Heinemann U, Lux HD, Gutnick MJ (1977) Extracellular free calcium and potassium during paroxysmal activity in cerebral cortex of the cat. Exp Brain Res 27: 237–243

House CR (1974) Water transport in cells and tissues. Arnold, London

House CR (1980) cited by Nicholson C (1980)

Howse DC, Caronna JJ, Duffy TE, Plum F (1974) Cerebral energy metabolism, pH, and blood flow during seizures in the cat. Am J Physiol 227: 1444–1450

Janus J, Lehmenkühler A (1981) Changes of extracellular chloride, sodium and calcium activities during stimulus-induced DC-potential shifts in the CNS. Pflügers Arch [Suppl] 389: R18

King LJ, Carl JL, Lao L (1973) Carbohydrate metabolism in brain during convulsions and its modification by phenobarbitone. J Neurochem 20: 477–485

Kraig RP, Nicholson C (1978) Extracellular ionic variations during spreading depression. Neuroscience 3: 1045–1059

Lehmenkühler A, Zidek W, Caspers H (1982) Changes of extracellular Na^+ and Cl^- activity in the brain cortex during seizure discharges. In: Klee MR, Lux HD, Speckmann EJ (eds) Pharmacology and physiology of epileptogenic phenomena. Raven Press, New York (in press)

Lux HD (1974) Kinetics of extracellular potassium. Relation to epileptogenesis. Epilepsia 15: 375–393

Lux HD, Neher E (1973) The equilibration time course of $[K^+]_o$ in cat cortex. Exp Brain Res 17: 190–205

Moody W, Futamachi KJ, Prince DA (1974) Extracellular potassium activity during epileptogenesis. Exp Neurol 42: 248–263

Neher E, Lux HD (1973) Rapid changes of potassium concentration at the outer surface of exposed single neurons during membrane current flow. J Gen Physiol 61: 385–399

Nicholson C (1980) Dynamics of the brain cell microenvironment. MIT Press, Cambridge (Neurosci Res Program Bull, vol. 18)

Orkand RK, Nicholls JG, Kuffler SW (1966) Effect of nerve impulses on the membrane potential of glial cells in the central nervous system of amphibia. J Neurophysiol 29: 788–806

Phillips JM, Nicholson C (1978) Tetra-alkyl ammonium ions as probes of brain cell microenvironment. Soc Neurosci Abstr 4: 236

Phillips JM, Nicholson C (1979) Anion permeability in spreading depression investigated with ion-sensitive microelectrodes. Brain Res 173: 567–571

Siesjö BK (1978) Brain energy metabolism. Wiley, New York

Steiner RA, Oehme M, Ammann D, Simon W (1979) Neutral carrier sodium ion-selective microelectrode for intracellular studies. Anal Chem 51: 351–353

Somjen GG (1975) Electrophysiology of neuroglia. Ann Rev Physiol 37: 163–190

Sypert GW, Ward AA, Jr (1974) Changes in extracellular potassium activity during neocortical propagated seizures. Exp Neurol 45: 19–41

Trachtenberg MC, Pollen DA (1970) Neuroglia: Biophysical properties and physiologic function. Science 167: 1248–1253

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