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Changes in $[Ca^{2+}]_o$ and $[K^+]_o$ during repetitive electrical stimulation and during pentetrazol induced seizure activity in the sensorimotor cortex of cats

Uwe Heinemann and Jacques Louvel*

Abteilung Neurophysiologie, Max Planck Institut für Psychiatrie, Kraepelinstraße 2, D-8000 München 40, Federal Republic of Germany

Abstract. Changes in $[Ca^{2+}]_o$ and $[K^+]_o$ were measured in the sensorimotor cortex of cats during repetitive electrical stimulation and during pentetrazol induced epileptiform activity. Repetitive stimulation of the thalamic ventrobasal complex (VB) or of the cortical surface (CS) caused decreases in $[Ca^{2+}]_o$ by up to 0.45 mM and increases in $[K^+]_o$ by up to 7 mM. Maximum reductions of $[Ca^{2+}]_o \Delta[Ca^{2+}]_o$ were found in depths of 100 to 300 µm below cortical surface, while rises in $[K^+]_o$ were largest in depths of 600 to 1000 µm dependent on stimulation site. At depths below 700–900 µm increases in $[K^+]_o$ were often accompanied by rises in $[Ca^{2+}]_o$ of about 0.2 mM. Pentetrazol (PTZ) when injected at doses of 25 to 40 mg/kg body weight induced spontaneous seizure activity, which was in about 40 % preceeded by a slight fall of baseline $[Ca^+]_o$. Repetitive stimulation and spontaneous seizures resulted in $\Delta[Ca^{2+}]_o$ of up to 0.6 mM, whereas rises in $[K^+]_o$ remained limited to a 'ceiling level' of about 10 mM. After PTZ application, peak $\Delta[Ca^{2+}]_o$ were found at the same recording sites, but, in contrast to normal cortex, decreases in $[Ca^{2+}]_o$ were observed in all cortical layers. The enhanced Ca^{2+}-signals after PTZ application and the observed reductions of $[Ca^{2+}]_o$ before seizure onset suggest that PTZ utilizes Ca^{2+}-dependent mechanisms to initiate seizure activity.

Key words: $[K^+]_o$ – $[Ca^{2+}]_o$ – Electrical stimulation – Pentetrazol – Epilepsy – Sensorimotor cortex

Introduction

Neuronal hyperactivity induced by repetitive electrical stimulation or convulsants is associated with decreases in $[Ca^{2+}]_o$ and rises in $[K^+]_o$ (Heinemann et al. 1977, 1978, 1982; Somjen 1980; Dietzel et al. 1982; Lux and Heinemann 1982). Here we compare laminar variations of changes in $[Ca^{2+}]_o$ in normal cortex and during PTZ induced seizures to those in $[K^+]_o$. Both in normal and PTZ treated animals rises in $[K^+]_o$ are largest in depths of about 1000 µm below cortical surface, whereas reductions in $[Ca^{2+}]_o$ are greatest in depths of 100–300 µm. This discrepancy points to the existence of postsynaptic Ca^{2+} conductances in apical dendrites and neurones of upper cortical layers, as has been suggested also by other experiments (Heinemann and Pumain 1980, 1981). We further show that PTZ has no effect on the laminar distribution of rises in $[K^+]_o$ and their maximal amplitudes. In contrast, decreases in $[Ca^{2+}]_o$ were enlarged, more widespread in the cortex and $[Ca^{2+}]_o$ tended even to decrease before actual onset of seizure activity. These observations suggest that PTZ utilizes Ca^{2+} dependent mechanisms for initiation of seizure activity.

Offprint requests to: U. Heinemann at the above address

* Present address: INSERM U97, Centre Paul Broca, 2ter, rue d'Alesia, F-75014 Paris, France

Methods

The experiments were performed under N_2O/halothane anaesthesia on 22 adult cats (2.5 to 4.5 kg), prepared and supervised as previously described (Heinemann and Lux 1975). For stimulation of the ventrobasal complex (VB) two concentric stimulating electrodes were inserted into the thalamus at Horsley Clarke coordinates of ant. 9.5, lat. 6.5 and vert. 11, as well as at ant. 9.0, lat. 4.5 and vert. 10. The correct position of the stimulating electrodes was controlled by monitoring the evoked responses at the cortical surface. The exposed sensorimotor cortex was steadied by a perspex pressure plate with a central hole (3 mm in diameter) at the border of which chlorided silverball EEG- and cortical surface (CS) stimulating electrodes were located. Stimulus trains were delivered to these or VB electrodes for 5 to 10 s at 5 to 50 Hz with pulses of 0.1 or 0.2 ms and intensities of 0.1 to 1.2 mA.

Ion selective K^+- and Ca^{2+}-reference electrodes were manufactured according to the method of Lux and Neher (1973). K^+-selective microelectrodes (K-ISM) were prepared with Corning 477113 ion exchanger resin and Ca^{2+}-selective microelectrodes (Ca-ISM) with a Ca^{2+}-selective cocktail (Oehme et al. 1976; Ammann et al. 1979). Ca^{2+}-ISM and K^+-ISM with minimum responses of 25 mV to a tenfold change in Ca^{2+}-concentration and of 45 mV to a tenfold change in K^+-concentration were accepted. Since the activity coefficients were kept constant, responses of such electrodes could be directly converted into concentration changes. For simultaneous measurements of $[K^+]_o$ and $[Ca^{2+}]_o$, electrodes were glued together at tip intervals of 50–200 µm. DC field potentials (fp) and EEG were recorded against a remote Ag/AgCl electrode located in the contralateral temporal muscle.

When PTZ effects on stimulus induced changes in $[K^+]_o$ and $[Ca^{2+}]_o$ were studied (8 experiments) PTZ was applied at doses of 10–40 mg/kg bodyweight (b.w.) at intervals of 40–80 mn. This allowed for recovery from PTZ effects. Thus control measurements could be performed at different recording depths immediately before and at various times after drug application. In 7 further experiments changes in $[K^+]_o$ and $[Ca^{2+}]_o$ were studied during PTZ induced seizures. In these

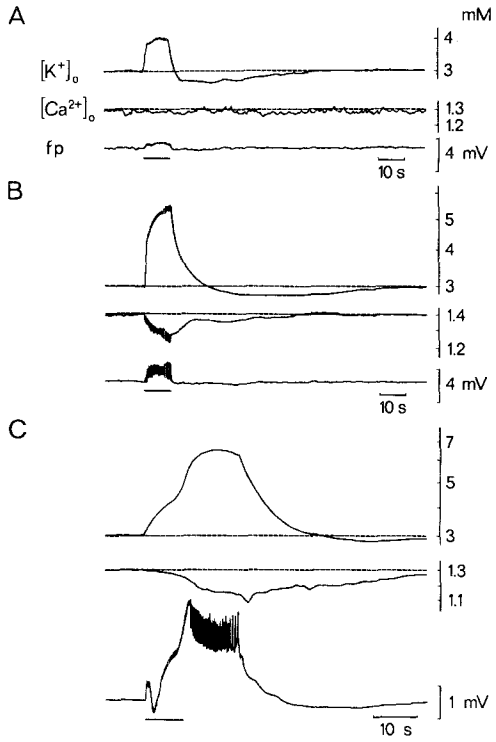

Fig. 1A—C. Relationship between rises in $[K^+]_o$, decreases in $[Ca^{2+}]_o$ and field potentials (*fp*). Recordings in **A** and **B** from the same experiment; in **C** from a different one. Recording depth 300 µm. (**A**) VB-stimulation 10 Hz, 0.3 mA. (**B**) VB-stimulation 20 Hz, 0.7 mA. (**C**) VB-stimulation 15 Hz, 0.5 mA. The recording was at a distance of 3 mm from area of maximal evoked potentials. Horizontal bars indicate duration of stimulus trains

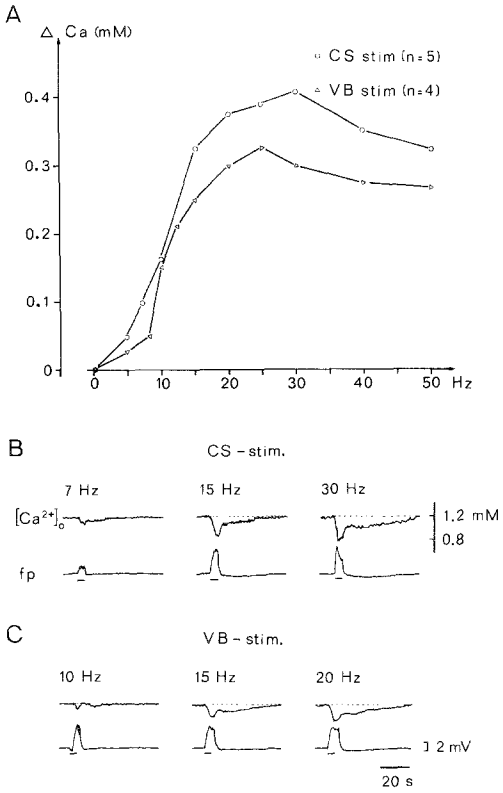

Fig. 2A—C. Relationship between stimulus frequency and amplitude of $[Ca^{2+}]_o$. (**A**) Plot of average reductions in $[Ca^{2+}]_o$ vs. stimulus frequency. Stimulating sites were cortical surface (*CS*) and the thalamic ventrobasal complex (*VB*). (**B**) Specimen recording of changes in $[Ca^{2+}]_o$ and fp evoked by CS stimulations of different frequency. 0.1 ms pulses and 0.6 mA. Stimulus duration indicated by horizontal bars. (**C**) Specimen recordings of $[Ca^{2+}]_o$ and fp elicited by VB-stimulations of different frequencies with 0.1 ms pulses and an intensity of 1 mA

experiments PTZ was initially applied with doses of 25–40 mg/kg. Subsequently and at intervals of 10–20 mn, PTZ was applied at doses of 10 to 20 mg/kg b.w. to induce further seizures.

In 5 of these 7 experiments laminar profiles of stimulus induced changes in $[Ca^{2+}]_o$ were studied before application of PTZ. In further 7 experiments only stimulus induced changes in $[K^+]_o$ and $[Ca^{2+}]_o$ were investigated.

Results

Stimulus-induced changes in $[K^+]_o$ and $[Ca^{2+}]_o$

Baseline $[K^+]_o$ and $[Ca^{2+}]_o$ were 2.9 and 1.2 mM respectively. Repetitive stimulation of CS or VB resulted in rises of $[K^+]_o$ ($\Delta[K^+]_o$) and in decreases of $[Ca^{2+}]_o$ ($\Delta[Ca^{2+}]_o$). Whereas rises in $[K^+]_o$ were present in all cortical layers with a maximum at depths of 600–800 µm for VB-stimulation and at 1000 µm for CS stimulation (Cordingley and Somjen 1978; Heinemann et al. 1979; Dietzel et al. 1980) decreases in $[Ca^{2+}]_o$ were observed regularly only at depths of 100–600 µm with a maximum at 100–300 µm (Heinemann et al. 1981). In normal cortex rises in $[K^+]_o$ were limited to a 'ceiling level' of roughly 10 mM (Moody et al. 1974; Heinemann and Lux 1977). $[Ca^{2+}]_o$ decreased by up to 0.45 mM. A limit in the decrease of $[Ca^{2+}]_o$ could not be defined since decreases in $[Ca^{2+}]_o$ could summate to some extent when repeatedly evoked. At depths of more than 600 µm, stimulation of VB or CS often resulted in increases of $[Ca^{2+}]_o$ to higher than normal levels (Figs. 3 and 4).

The relationship between intensity of stimulation and $\Delta[Ca^{2+}]_o$ is illustrated in Figs. 1 and 2. $\Delta[K^+]_o$ by more than 1 mM were regularly associated with decreases in $[Ca^{2+}]_o$. Stimulation with intermediate intensities often evoked diphasic changes in $[Ca^{2+}]_o$ and $[K^+]_o$ (see Fig. 1C) characterized by an initial slow and then accelerated change in both concentrations. Diphasic changes were observed in 60% of VB stimulations. They were also induced by CS stimulation when the recordings were obtained at some distance (> 2 mm) from the stimulating electrodes.

Increases in $[K^+]_o$ to near 10 mM were accompanied by reductions in $[Ca^{2+}]_o$ of up to 0.45 mM. However, a level of 0.85 mM was not underceded during repetitive stimulation and subsequent epileptiform afterdischarges. The average $\Delta[Ca^{2+}]_o$ measured at recording depths of 100–300 µm associated with $\Delta[K^+]_o$ of 5–7 mM was 0.33 ± 0.09 mM (M.V. ± S.D., $n = 29$) for CS stimulation and 0.34 ± 0.11 mM ($n = 34$) for VB stimulation.

Variations in stimulus induced changes in $[Ca^{2+}]_o$

Stimulus induced reductions in $[Ca^{2+}]_o$ did not vary by more than 10% at a given recording site, provided, that intervals between stimuli were larger than 5 mn and that the cortex did not pulsate (but see Somjen 1980).

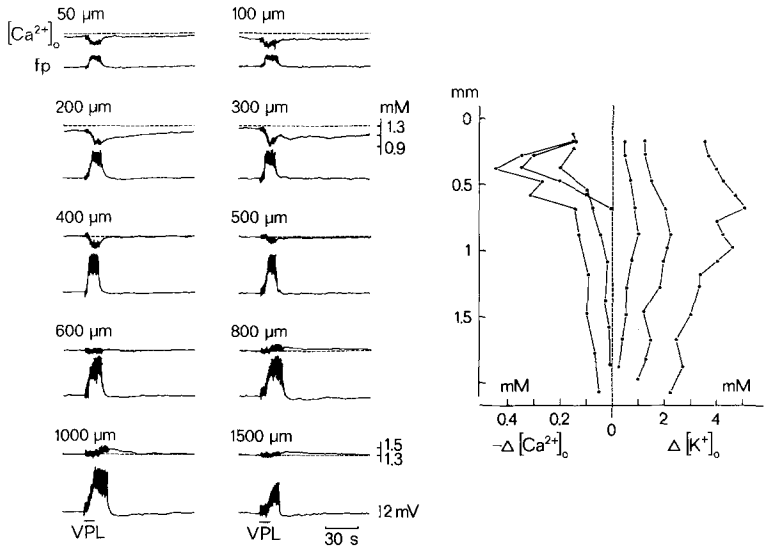

Fig. 3
Laminar variations of VB-induced changes in $[Ca^{2+}]_o$ and $[K^+]_o$. *Left:* VPL-stimulation 20 Hz, 0.9 mA, 10 s. *Right:* VPL-stimulation with 10 Hz, 0.6 mA, 20 Hz, 0.6 ma and 20 Hz, 1.2 mA. The three laminar profiles were obtained in the same experiment. The middle intensity was applied.first, then the largest and then the weakes

When stimulations were repeated at intervals of less than 2 mn, reductions in $[Ca^{2+}]_o$ in response to the second stimulation were depressed in comparison to $\Delta[Ca^{2+}]_o$ evoked by the first stimulus train. This depression was paralleled by a diminution of stimulus induced $\Delta[K^+]_o$ (Heinemann and Lux 1975; Heinemann and Gutnick 1979). Two minutes after end of stimulation and/or of eventually evoked selfsustained epileptiform afterdischarges, amplitudes of stimulus induced $\Delta[Ca^{2+}]_o$ and $\Delta[K^+]_o$ were normal. Since at this time baseline $[Ca^{2+}]_o$ had not regularly recovered to prestimulus levels, a deeper level of $[Ca^{2+}]_o$ was sometimes obtained during the second stimulation. This summation of Ca^{2+}-signals was restricted to recording depths of 150 to 400 µm. While the minimum level of $[Ca^{2+}]_o$ during a single stimulus train was 0.85 mM, the minimum level obtained during repeated stimulation was 0.75 mM.

Recovery of $[Ca^{2+}]_o$

Often and particularly during intense stimulation recovery of $[Ca^{2+}]_o$ started already during stimulation and/or during selfsustained epileptiform afterdischarges. This partial recovery of $[Ca^{2+}]_o$ by up to 50% of the initial decrease was mirrored to some extent in fp- and $[K^+]_o$-recordings (Heinemann and Lux 1975, 1977).

The recovery of $[Ca^{2+}]_o$ was different from that of $[K^+]_o$. After end of stimulation and seizure activity $[K^+]_o$ regularly decayed to subnormal levels and then returned slowly to normal baseline within 30–180 s (Heinemann and Lux 1975; Vern et al. 1977). $[Ca^{2+}]_o$ recovery had at least two phases: it initially rose within 3–8 s to a new plateau level of about 0.1–0.2 mM below baseline, from which a secondary fall in $[Ca^{2+}]_o$ by about 0.1 mM developed in 37% of recordings. The secondary minimum was reached about 5–30 s after end of stimulation. Then $[Ca^{2+}]_o$ slowly returned to resting activity within another 30–240 s. In total, the recovery of $[Ca^{2+}]_o$ could last up to 5 mn.

Changes in $[Ca^{2+}]_o$ in relation to recording depth

Besides on stimulus parameters Ca^{2+}-signals were prominently dependent on the recording depth. When advancing the electrode assembly into the cortex decreases in $[Ca^{2+}]_o$ became smaller and recovery of $[Ca^{2+}]_o$ became more similar to that of $[K^+]_o$ (Fig. 3). Thus, $[Ca^{2+}]_o$ rapidly recovered from lowered levels, increased to above baseline and then slowly returned to normal at recording depths of 400–600 µm. At depths below 600–900 µm, $[Ca^{2+}]_o$ did not necessarily decrease during stimulation of CS and VB and instead rose by between 0.1 and 0.3 mM, provided the recording was performed during the first 10–40 stimulations and provided that stimulus frequencies below 40 Hz were used. More stimulations lead to a known increase in excitability and seizure susceptibility, with prolonged after-discharges (Mares et al. 1980) and to alterations of Ca^{2+}-signals. Under this condition $[Ca^{2+}]_o$ initially decreased also at depths below 700 µm, before it increased 3–5 s after onset of stimulation to levels above baseline. The increase in $[Ca^{2+}]_o$ overlasted the stimulation by 30–180 s.

The development of initial decreases in $[Ca^{2+}]_o$ in deeper cortical layers is illustrated in Fig. 3. VB stimulus induced changes in $[Ca^{2+}]_o$ at different depths were compared with $\Delta[K^+]_o$ at different depths. The three laminar profiles were obtained in the same experiment with different stimulus parameters. Whereas in the first profile only increased in $[Ca^{2+}]_o$ were observed at depths of 600 µm and more it was found in later laminar tracks that VB-stimulation evoked transient decreases also in deeper layers followed by rises in $[Ca^{2+}]_o$ to levels above normal. The rises in $[Ca^{2+}]_o$ had their maximum at about the end of ictal activity. Reductions in $[Ca^{2+}]_o$ became apparent in deeper cortical layers also when stimulations were repeated at a frequency of more than one stimulation every 3 mn. While the first stimulation in such stimulation sequences elicited an increase in $[Ca^{2+}]_o$ later stimulations evoked diphasic responses with an initial decreases.

Effects of pentetrazol on baseline concentrations of extracullar free calcium and potassium

Pentetrazol applied at doses of 10–40 mg/kg b.w. did not affect baseline $[K^+]_o$. With doses of 15–40 mg $[Ca^{2+}]_o$ started to decrease within 5–25 s after PTZ injection (Heinemann et al. 1977, 1978). These $\Delta[Ca^{2+}]_o$ were particularly frequent at recording depths of 100–500 µm and their occurrence was dose dependent. Thus 'sponataneous'

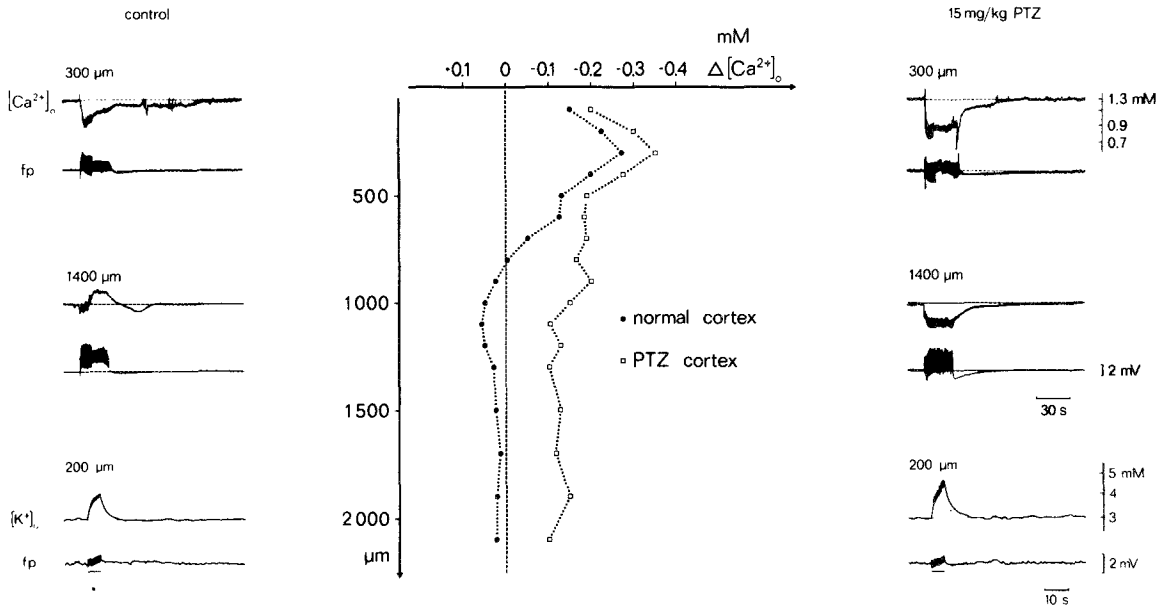

Fig. 4. Effects of subconvulsive doses of PTZ on CS stimulus-induced changes in $[Ca^{2+}]_o$, $[K^+]_o$ and fp. *Left* control, *middle* average laminar profiles of CS-induced $[Ca^{2+}]_o$ before (●) and after PTZ application (□), $n = 5$. Numbers refer to depth. *Right:* After 15 mg/kg PTZ. Calibrations on the right apply also to traces on the left

$\Delta[Ca^{2+}]_o$ by in average 0.15 mM were elicited in 9% of cases with 15 mg/kg. Baseline $[Ca^{2+}]_o$ decayed before seizure onset in 37% and 43% with 25 mg/kg and 45 mg/kg b.w., respecitively. When PTZ injections were repeated and a state was reached in which spontaneous seizures occurred at intervals of 20–60 s, $[Ca^{2+}]_o$ was regularly lowered also during interictal periods by up to 0.4 mM. These reductions in baseline $[Ca^{2+}]_o$ were still largest in depths of 100–300 µm. Baseline $[Ca^{2+}]_o$ was lowered in about 90% in the upper 1000 µm, and in 40% in all cortical layers.

Effects of pentetrazol on stimulus induced changes in $[Ca^{2+}]_o$ and $[K^+]_o$

A dose of below 25 mg/kg b.w. PTZ did not elicit seizure activity, even if injections were repeated every 40 mn. Thus it was possible to investigate the effects of PTZ in subictal doses on stimulus induced changes in $[Ca^{2+}]_o$ and $[K^+]_o$. Stimulus induced reductions in $[Ca^{2+}]_o$ were regularly larger after intravenous injection of subictal doses of PTZ as shown in Fig. 4. In this particular experiment application of 15 mg/kg b.w. enhanced the $[Ca^{2+}]_o$ decreases by roughly 30%. In deeper cortical layers, where control stimulations caused rises in $[Ca^{2+}]_o$, the same stimuli elicited decreases in $[Ca^{2+}]_o$ after PTZ application (see Fig. 4).

Stimulus induced $\Delta[K^+]_o$ were also enhanced. When analysing the effects of 15 mg/kg PTZ injections on the first 4–7 stimulus induced $\Delta[K^+]_o$ and $\Delta[Ca^{2+}]_o$ of 7 different experiments it was found that amplitudes of $\Delta[Ca^{2+}]_o$ and $\Delta[K^+]_o$ were in average enhanced by 27% and 22%, respectively. The reductions in $[Ca^{2+}]_o$ were 0.22 ± 0.08 mM before and 0.3 ± 0.11 (m.V. ± SD.; $n = 27$) after PTZ application. The mean rise of $[K^+]_o$ before PTZ application was 2.74 ± 1.39 mM and 3.5 ± 1.5 mM (m.V. ± S.D.; $n = 27$) after PTZ. PTZ did however not enhance $\Delta[K^+]_o$, when intensive CS or VB stimulation prior to PTZ application increased $[K^+]_o$ already to ceiling levels near 10 mM. In contrast, accompanying reductions in $[Ca^{2+}]_o$ could still be augmented by PTZ, and $[Ca^{2+}]_o$ could decrease to levels of 0.6 mM. The augmenting effect of PTZ on reductions in $[Ca^{2+}]_o$ is also expressed in the average laminar profile of Fig. 4 constructed from 7 experiments. Whereas under control conditions $[Ca^{2+}]_o$ reductions were present only in upper cortical layers they were after PTZ application present in all cortical layers.

The laminar profile of $\Delta[K^+]_o$ (not shown) revealed that in the presence of PTZ in general peak increases in $[K^+]_o$ were largest at 600–1000 µm, similar as in normal cortex (Dietzel et al. 1980). This compares to the effect of PTZ on $\Delta[K^+]_o$ in the visual cortex (Lux 1974).

Changes in extracellular free calcium and potassium concentration during 'spontaneous' seizure discharge

Injection of 25 mg/kg b.w. PTZ induced 'spontaneous' seizures in about 80% of cases. Seizure activity began 20–80 s after end of PTZ-administration. Often the first seizures were preceded by a fall in $[Ca^{2+}]_o$ (Heinemann et al. 1977, 1978), whereas $[K^+]_o$ remained unaltered. The first seizure was generally characterized by clonic activity in field potential recordings. These ictal events lasted between 8 and 12 s. During such seizures $[K^+]_o$ rose by 2 to 4 mM and $[Ca^{2+}]_o$ decreased by 0.2 to 0.4 mM.

Longerlasting seizures with repeated ictal episodes were induced by 40 mg/kg b.w. or when PTZ was repeatedly injected at time intervals of less than 30 mn. These seizures where characterized by 3–10 ictal episodes each lasting between 20 and 50 s. Ictal episodes were interrupted by pauses of 20–40 s. The duration of interictal pauses increased towards the end of a seizure period. In EEG and f.p. recordings of seizure activity a few spike like components were followed by an episode during which lower amplitude voltage deflections occurred with a frequency of 8–20/s. This tonic phase lasted for up to 15 s. It was followed by a period of clonic activity characterized by larger amplitude voltage changes which occurred at a frequency of 2–5/s. Each ictal

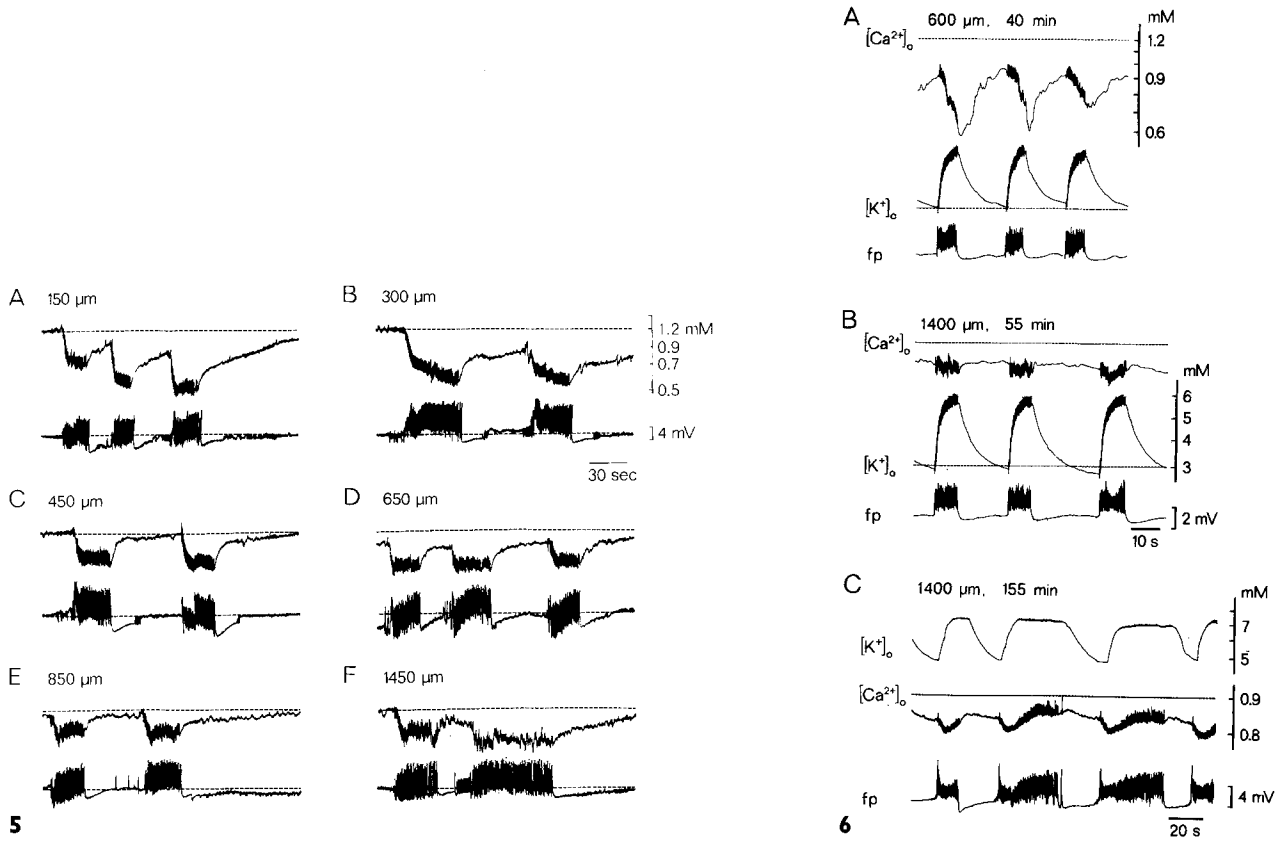

Fig. 5A—F. Laminar profile of PTZ (30 mg/kg) induced seizures. PTZ injected every 45 mn. Calibrations in **B** apply also to other recordings. Numbers refer to recording depth below cortical surface

Fig. 6A—C. Changes in $[Ca^{2+}]_o$ and $[K^+]_o$ during PTZ induced status epilepticus. Recordings in A and B from the same, in C from a different experiment. Calibration in A also valid for B, calibrations in B also valid for A

episode within a seizure period was accompanied by a rise in $[K^+]_o$ and by a fall in $[Ca^{2+}]_o$. $\Delta[Ca^{2+}]_o$ and $\Delta[K^+]_o$ could be as large as 0.6 mM and 6 mM, respectively. However in general ionic fluctuations were smaller. Reductions in $[Ca^{2+}]_o$ averaged 0.41 ± 0.1 mM ($n = 37$) and increases in $[K^+]_o$ were 3.7 ± 1.2 mM ($n = 31$) at 300 µm below cortical surface. At 1000 µm the respective values were 0.15 ± 0.08 mM and 4.8 ± 1.4 mM ($n = 23, 27$). The $\Delta[Ca^{2+}]_o$ appeared also to be dependent on levels from which the fall in $[Ca^{2+}]_o$ started. With decreasing preseizure levels $\Delta[Ca^{2+}]_o$ became smaller (Fig. 5). $[K^+]_o$ recovered after each ictal episode to near baseline levels and often even undershot resting activity. Thus 43% of $\Delta[K^+]_o$ started from a decreased preictal level.

$[Ca^{2+}]_o$ only rarely recovered to normal activity after an ictal episode within a paroxysmal period. Therefore 70% of ictal episodes within a paroxysmal period started from lower than normal levels and Ca^{2+}-signals summated as shown in Fig. 5. During such paroxysmal periods $[Ca^{2+}]_o$ could fall to levels of 0.4 mM. $\Delta[Ca^{2+}]_o$ were largest in upper cortical layers. However, decreases in $[Ca^{2+}]_o$ were in 90% of experiments present in all cortical layers. The amount of recovery of $[Ca^{2+}]_o$ during interictal pauses was also depth dependent. A complete recovery of $[Ca^{2+}]_o$ to baseline levels during interictal episodes was observed in 15% at recording sites of 100 to 1000 µm and in 40% at recording sites of 1000 µm and more below cortical surface.

Repeated administration of PTZ at time intervalls of 20—30 mn induced longlasting seizure activity for up to 3 h, which reflected a status epilepticus.

In this condition one ictal episode followed the other. Durations of single ictal episodes varied between 20 s and 80 s (Fig. 6), and of interictal pauses between 15 and 30 s. Baseline $[Ca^{2+}]_o$ was lowered in all cortical layers. About 1—2 h after onset of the status also $[K^+]_o$ baseline levels were elevated by 1 to 2 mM (Fig. 6). The $[K^+]_o$ baseline increase was more likely to occur in deeper cortical layers where decay times of $[K^+]_o$ after ictal episodes were usually slower than at sites of maximum rises in $[K^+]_o$.

Ictal episodes were regularly accompanied by increases in $[K^+]_o$ by up to 7 mM and by reductions in $[Ca^{2+}]_o$ by up to 0.7 mM to a level of about 0.4 mM. However, whereas $\Delta[K^+]_o$ closely followed seizure activity, reductions in $[Ca^{2+}]_o$ began in 3 out of 5 cases 2—9 s before onset of ictal episodes. Recovery of $[Ca^{2+}]_o$ started 5—18 s before the end of an ictal episode. Thus the time course of changes in $[Ca^{2+}]_o$ appeared to be shifted against the time course of ictal episodes.

Discussion

Changes in extracellular free calcium

The present experiments show that increases in $[K^+]_o$ and reductions in $[Ca^{2+}]_o$ regularly accompany seizure activity.

However, whereas elevations in $[K^+]_o$ are observed in all cortical layers and under all conditions, reductions in $[Ca^{2+}]_o$ occur in normal cortex only at recording depths of 100–600 µm (Heinemann et al. 1981), while in deeper cortical layers $[Ca^{2+}]_o$ increases to levels above normal. These rises result very likely from a stimulus induced shrinkage of the size of extracellular space (ES) as a consequence of water movements from the extracellular into the intracellular space. Indeed, Dietzel et al. (1980, 1982) have shown that comparable stimulations induce ES-reductions of about 30% in middle cortical layers. This should lead to an increase in concentration of particles, which remain in the extracellular space. In case of resting $[Ca^{2+}]_o$ an increase from 1.2 to 1.6 mM is expected for a 30% reductionof ES-size, if no Ca^{2+} leaves the ES. Since the rise in $[Ca^{2+}]_o$ in depths of 1000 µm is maximally 0.3 mM a net loss of extracellular Ca^{2+} can be inferred. Even when correcting for ES-space size changes extracellular Ca^{2+}-loss remains largest in upper cortical layers. Interestingly repeated stimulation results intially in increases of $[Ca^{2+}]_o$ and later in decreases. This is explained by the observation that stimulus induced reductions in ES-size do only summate to a certain degree (Heinemann and Dietzel, unpublished).

Changes in ES-size are well comparable in freshly prepared and frequently stimulated cortex as well as in PTZ-treated preparations (Dietzel et al., in preparation). Thus enhancement of reductions in $[Ca^{2+}]_o$ reflect changes in neuronal excitability.

Laminar profiles of changes in extracellular free calcium and potassium concentration

The discrepancy of laminar profiles of changes in $[Ca^{2+}]_o$ and $[K^+]_o$ was present under all experimental conditions. Whereas the largest reductions in $[Ca^{2+}]_o$ occurred in a depth of 300 µm below cortical surface, rises in $[K^+]_o$ appeared to be maximal at recording depths of 600–1200 µm. In this respect the cerebral cortex is different from other structures such as hippocampus (Benninger et al. 1980) and cerebellum (Nicholson et al. 1978; Nicholson 1980) where stimulus induced rises in $[K^+]_o$ and reductions in $[Ca^{2+}]_o$ are largest at about the same site. The relatively large increases in $[K^+]_o$ in depths of 600–800 µm during VB stimulation correspond to the main projection area of thalamocortical fibers (Hassler and Muhs-Clement 1964; White 1979). That $[K^+]_o$ is also largest in these layers during CS stimulation is probably due to a direct activation of cells in these layers via depolarisation of dendrites and due to excitatory recruitment of a relatively large neuronal population in the thalamic relay nucleic (Gutnick et al. 1979; Heinemann and Gutnick 1979) which projects into middle cortical layers. Thus a good part of extracellular K^+ may be presynaptically released, as suggested by effects of Mn^{2+} on stimulus induced changes of $[K^+]_o$ in the cerebellum, which are reduced by only 40 to 60% in spite of a complete blockade of synaptic transmission (Nicholson et al. 1978).

Reductions in $[Ca^{2+}]_o$ can be ascribed to various mechanisms: Ca^{2+} enters presynaptic terminals where it is required for transmitter release (Katz and Miledi 1969). In addition Ca^{2+} may flow into postsynaptic elements through voltage dependent selective or relatively unselective cation conductances activated for example by excitatory amino acid transmitters. The observation that Ca^{2+}-signals are not largest in the layer of greatest synaptic input and of largest rises in $[K^+]_o$ points to a considerable contribution of postsynaptic Ca^{2+}-entry to reductions in $[Ca^{2+}]_o$ during repetitive stimulation of CS and VB and during pentetrazol induced seizure activity. This conclusion is further supported by observations on effects of putative excitatory amino acid transmitters and their various related agonists (Heinemann and Pumain 1980, 1981; Pumain and Heinemann 1982; Heinemann and Lux 1983) on $[Ca^{2+}]_o$, which decreased by up to 90% with maximal reductions at about those sites where also stimulus induced $\Delta[Ca^{2+}]_o$ were greatest. Since $[Ca^{2+}]_o$ reductions were blocked by GABA (see also Dunlap and Fischbach (1978) for a similar effect on dorsal root ganglion cells) and by Ca^{2+}-antagonists whereas simultaneously measured amino acid induced reductions of $[Na^+]_o$ by up to 25 mM were not affected by these manipulations (Heinemann and Lux 1983) it is likely that most of the Ca^{2+} leaves the extracellular space through selective channels. The conclusion that Ca^{2+}-conductances are located in postsynaptic elements is further supported by experiments in rat neocortex, where it was found that the relatively large sinks for Ca^{2+} in upper cortical layers decreased in efficacy after lesions of the pyramidal tract with subsequent retrograde degeneration of pyramidal tract cells (Pumain and Heinemann 1982). Further support for this conclusion is derived from chronic stimulation experiments which revealed Ca^{2+} deposits in apical dendrites after prolonged electrical stimulation (Agnew et al. 1979). Apical dendrites in neocortex might therefore compare to dendrites in other structures of the central nervous system (Schwartzkroin and Slawski 1977; Llinas and Sugimori 1980) and possess large Ca^{2+}-conductances.

Effects of pentetrazole on changes in $[Ca^{2+}]_o$ and $[K^+]_o$

The mechanisms by which pentetrazol induces seizures are yet unclear. Previous findings in various preparations suggested that PTZ impairs postsynaptic gabaergic inhibition (Wilson and Escueta 1974; Mac Donald and Barker 1977; Pellmar and Wilson 1977; Antoniadis et al. 1980; but see Macon and King 1979). Analysis of PTZ effects in mollusc neurones, where PTZ induces also paroxysmal depolarisations (Speckmann and Caspers 1973), revealed that PTZ in addition has a blocking action on repolarizing K^+-conductances (Klee et al. 1973; David et al. 1974). Our own studies on the effects of PTZ on hippocampal neurones in the 'in vitro' slice preparation confirmed both effects (Louvel and Heinemann 1981). In invertebrate preparations (Klee et al. 1973; David et al. 1974) it has been shown that PTZ accentuated an inward going rectification, suggesting that the membrane effects of PTZ result in a relatively facilitated activation of inward currents. These observations indicate that loss of inhibitory control and impairment of K^+-conductances can support activation of Ca^{2+}-conductances. This would compare to effects of penicillin on one hand (Schwartzkroin and Prince 1980; Schwindt and Crill 1980a) and of Ba^{2+} and tetramethylammonium on motoneurones (Wong and Prince 1979; Schwindt and Crill 1980b) and on hippocampal pyramidal cells (Schwartzkroin and Pedley 1979; Hotson 1982) on the other. Similarly facilitated activation of Ca^{2+} inward currents may account for the increased Ca^{2+}-signals observed after PTZ application.

The often noted spontaneous decreases in $[Ca^{2+}]_o$ before seizure onset are very likely the result of an enhanced synaptic activity prior to onset of seizure activity. A blocking action of PTZ on K^+-conductances will both pre- and postsynaptically

increase Ca^{2+}-entry. Presynaptically this will lead to a facilitated and increased transmitter release. Postsynaptic activation of intrinsic Ca^{2+}-conductances will serve to amplify EPSP's. In addition, PTZ has in molluscan neurones the capability to mobilize intracellular Ca^{2+} from various storage sites (Sugaya et al. 1982). Since in some preparations incresed $[Ca^{2+}]_i$ can activate depolarizing conductances which in turn facilitates Ca^{2+} entry, Ca^{2+} loss preceeding seizure activity may also result from such effects of PTZ (Hofmeier and Lux 1982; Lux and Heinemann 1982; Zanotto and Heinemann 1983).

Other epileptogenic drugs had similar actions on stimulus-induced changes in $[Ca^{2+}]_o$. Increased Ca^{2+}-signals were observed in chronic epileptic foci (Heinemann et al. 1981) as well as in drug induced acute epilepsies (Galvan et al. 1982; Heinemann et al. 1982; Louvel et al. 1982). These included epilepsies where seizure activity is induced by impairment of postsynaptic inhibition (penicillin, picrotoxin, cobalt, for review see Woodbury 1981) as well as epilepsies where seizure activity in initiated as a result of blocking effects on K^+-conductances [Oenanthotoxin (Louvel et al. 1982), 4-aminopyridine (Galvan et al. 1982)]. These findings suggest that activation of pre- and postsynaptic Ca^{2+}-conductances forms a common link in epileptogenesis.

Changes in $[Ca^{2+}]_o$ and $[K^+]_o$ and seizure generation

It has already previously been described (Lux 1973; Moody et al. 1974) that $[K^+]_o$ accumulation is not a critical factor for initiation of seizure activity since ictal episodes usually start from normal or even lowered $[K^+]_o$ levels. This finding is confirmed in the present paper. Although $[Ca^{2+}]_o$ could slightly decrese before onset of epileptiform activity, this lowering alone is not essential for initiation of seizure discharge because often ictal episodes started from a normal level in $[Ca^{2+}]_o$. This does not exclude however, that increases in $[K^+]_o$ and reductions in $[Ca^{2+}]_o$ can support the development of seizure discharge by their various excitability increasing effects (Heinemann and Lux 1983). Such effects would be particularly strong during the status epilepticus, where baseline $[K^+]_o$ is often elevated and $[Ca^{2+}]_o$ is decreased by 0.4 mM. Indeed, such effects of increased $[K^+]_o$ and lowered $[Ca^{2+}]_o$ on development, spread and synchronisation of seizure activity have been demonstrated in experiments on 'in vitro' hippocampal slice preparation, where lowering of $[Ca^{2+}]_o$ to 0.2 mM in the presence of 5 mM K^+ induced spontaneous spreading epileptiform activity (Jefferys and Haas 1982; Heinemann and Lux 1983; Yaari et al. 1983; Konnerth et al. submitted for publication). Since $[K^+]_o$ released at one site is very likely spatially redistributed by a glia buffering mechanism (Orkand et al. 1966; Dietzel et al. 1980, 1982), $[K^+]_o$ can accumulate at sites where it is not yet released from neurones recruited into seizures. By an excitatory effect on neurones in these areas, increased $[K^+]_o$ may support spread of ictal activity. Evidence for such a role of elevated $[K^+]_o$ was found by demonstrating that iontophoretically elevated $[K^+]_o$ facilitates the local recruitment into seizure activity generated primarily at a distant area (Gutnick et al. 1979; Heinemann and Lux 1983; Heinemann et al. 1978; Heinemann and Gutnick 1979).

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