Spatiotemporal Distribution of Intracellular Calcium Transients During Epileptiform Activity in Guinea Pig Hippocampal Slices

B. Albowitz, P. König, and U. Kuhnt. Spatiotemporal distribution of intracellular calcium transients during epileptiform activity in guinea pig hippocampal slices. J. Neurophysiol. 77: 491–501, 1997. Calcium ions are known to play an important role in epileptogenesis. Although there is clear evidence for increased neuronal calcium influx during epileptiform potentials, direct measurements of the corresponding intracellular calcium transients are rare and the origin of calcium influx is not known. Therefore the spatial and temporal distribution of intracellular calcium transients during epileptiform activity in guinea pig hippocampal slices was monitored with the use of the indicator Calcium-Green and a fast optical recording method. Two models of epilepsy (bicuculline and low Mg\(^{2+}\)) were compared. In both models, single epileptiform events were evoked by electrical stimulation of the Schaffer collaterals in CA1 or of stratum pyramidale in area CA3. Intracellular calcium transients during epileptiform activity were \(~5\) times larger than during control stimulation. Calcium transients during epileptiform activity were present across at least the entire CA1 area, whereas presynaptic calcium transients from stimulated fibers were only seen at a distance up to \(1\) mm from the stimulation site. DL-2-amino-5-phosphonovaleric acid (APV), a specific antagonist of the N-methyl-d-aspartate (NMDA) receptor, abolished low-Mg\(^{2+}\) epileptiform activity and reduced bicuculline-induced epileptiform activity; it reduced calcium transients following stimulation of CA1 by only \(29\%\) (bicuculline) and \(38\%\) (low Mg\(^{2+}\)). For comparison, calcium transients during control stimulation were \(78\%\) (bicuculline) and \(69\%\) (low Mg\(^{2+}\)) smaller than epileptiform calcium transients. At a distance from the stimulation site, calcium transients and their NMDA-receptor-dependent components were largest in stratum pyramidale in the bicuculline model and in stratum oriens in the low-Mg\(^{2+}\) model. In both models, minimal onset latencies of calcium influx shifted with increasing distance to the stimulation electrode from stratum radiatum to stratum oriens. APV reduced the extent of spread of calcium transients in the low-Mg\(^{2+}\) model. In the bicuculline model, the spatial extent of spread of epileptiform calcium transients was not affected by application of APV; however, the mean velocity of spread was reduced from \(0.20\) to \(0.12\) m/s. In conclusion, the large size of calcium transients and of their NMDA-receptor-dependent components in stratum pyramidale or stratum oriens as well as shortest onset latencies of calcium transients at these sites suggest an important role of cell somata, basal dendrites, and possibly local circuit excitatory interactions for the generation and spread of epileptiform activity.

INTRODUCTION

Calcium ions play an important role in the generation of epileptiform activity. Epileptiform potentials are suppressed by organic calcium channel blockers (e.g., Speckmann et al. 1990; Straub et al. 1990, 1994) and reduced by N-methyl-d-aspartate receptor (NMDAR) antagonists (Dingledine et al. 1990). Thus it is thought that calcium currents through both voltage-activated calcium channels and NMDAR-dependent channels are strongly enhanced during epileptic activity, leading to further cell depolarization and to an increase of the intracellular calcium concentration. Accordingly, the extracellular calcium concentration decreases during epileptiform activity (Benninger et al. 1980; Hamon and Heinemann 1986). The concomitant seizure-related increase of bound intracellular calcium has been demonstrated histochemically (Griffiths et al. 1982; Kuhnt et al. 1983). In living tissue, an increase of the free intracellular calcium concentration during epileptiform activity has been shown in invertebrate neurons (Sugaya et al. 1987) with the use of ion-sensitive microelectrodes.

Intracellular transients of free calcium are best monitored by optical measurements with fluorescent calcium indicators. With the use of this method, a transient increase of the concentration of free intracellular calcium ions during epileptiform potentials has been demonstrated in primary cortical cell cultures (Murphy et al. 1992; Robinson et al. 1993) and in acute hippocampal slices (Sinha et al. 1995; van der Linden et al. 1993). The latter investigations on hippocampal slices focused either on single neurons (van der Linden et al. 1993) or measured calcium transients at only few selected sites (Sinha et al. 1995), such that the spatial distribution of calcium transients along the axis of pyramidal cells and across the hippocampal slice is not known.

Therefore we investigated the spatial distribution of intracellular calcium transients during epileptiform activity in acute hippocampal slices across the entire CA1 area. We used the indicator Calcium-Green and optical recordings with good spatial (60 \(\mu\)m) and temporal (0.4 ms) resolution.

Two major questions were addressed in this investigation. First, does evoked intracellular calcium influx differ in various regions of the hippocampus, e.g., in stratum pyramidale (SP) versus stratum radiatum (SR) and stratum oriens (SO)? This was achieved by analyzing the spatiotemporal distribution of evoked calcium transients during control and epileptiform activity. Second, how important is the calcium influx through NMDAR-dependent channels in the models of epilepsy compared, and how are NMDAR-dependent calcium components spatially distributed? For this purpose, NMDARs were blocked by DL-2-amino-5-phosphonovaleric acid (APV) during epileptiform activity and the evoked calcium transients were analyzed.

The spatiotemporal distribution of epileptiform calcium transients was compared for two different models of epilepsy: 1) the bicuculline model (reduction of GABAergic inhibitory input) and 2) the low-Mg\(^{2+}\) model (increased...
excitation by increasing transmitter release and NMDAR-dependent currents).

In the present study, the magnitude, the duration, and the spatial distribution of calcium transients were significantly increased during epileptiform activity. Maximal calcium transients were found in SP. In both models of epilepsy, increased calcium influx was only partially mediated by NMDARs even though their activation was crucial for the generation of epileptiform activity in the low-Mg$^{2+}$ model.

Some of the results have been published in abstract form (Albowitz and Kuhnt 1994).

**Methods**

**Preparation**

Adult guinea pigs (400-600 g) were killed by a blow to the neck and then decapitated. After the skull was removed, the brain was placed in ice-cold oxygenated medium. Transverse hippocampal slices 350 μm thick were cut in ice-cold oxygenated medium on a rotorslicer. Slices were kept in oxygenated medium in a storage chamber at a temperature of 28°C. After ≥2 h, slices were stained in a separate compartment of the storage chamber with the use of 50 μg Calcium-Green-1 acetoxymethylester (Molecular Probes, Eugene, OR) dissolved in 15 μl aceton, 5 μl C-1-methyl-1-glycerine (Schuff-Werner et al. 1988), with pluronic F127 (12.5%), and 1 μl perfusion medium (the final concentration of Calcium-Green in the staining solution was 35–40 μM). The dye solution was incubated at 30°C for ≥1 h before slices are stained, allowing for the evaporation of the aceton. Slices were stained for 3 h, washed shortly in the storage chamber, and then transferred to the recording chamber where they were superfused for another hour before optical recording was started.

**Recording chamber, electrical recording, and stimulation**

Recordings were made in a superfusion chamber at a temperature of 32 ± 0.5°C. The chamber was perfused at a rate of 5 ml/h (chamber volume ~ 0.5 ml) with a standard medium containing (in mM) 124 NaCl, 5 KCl, 1.25 NaH$_2$PO$_4$, 2 MgSO$_4$, 2 CaCl$_2$, 26 NaHCO$_3$, and 10 glucose. The medium was gassed with 95% O$_2$/5% CO$_2$, giving a pH of 7.4. The humidified O$_2$/CO$_2$ mixture was continuously blown over the surface of the medium in the recording chamber. Extracellular field potentials were recorded with glass pipettes filled with 3 M NaCl (5–10 MΩ) placed in SP or SR ~1 mm from the stimulation site in CA1. Stimulation electrodes (tungsten in glass, 50–200 kΩ, tip diameter < 30 μm) were inserted in SR of area CA1 and SP of area CA3 (Fig. 1A). These two stimulation sites were chosen for the following reason. Stimulation of CA1 allows a comparison of control and epileptiform activity at the same site in CA1. In contrast, because stimulation of SP in CA3 induced no discernable activity in CA1 during control conditions, the distribution and spread of epileptiform activity in CA1 can be investigated without the contamination by primary suprathreshold activation.

Calcium influx was investigated and compared in two models of epilepsy. Epileptiform potentials were either elicited by adding bicuculline methiodide to (20 μM) or by removing Mg$^{2+}$ from the perfusion medium. In both models, epileptiform potentials were evoked by single-pulse stimulation (pulse width 40 μs) of CA1 or CA3.

During control conditions, different stimulation intensities were tested. Usually, the lowest intensity used was threshold to detect calcium transients with our optical recording setup (about threshold to elicit a detectable field potential). Then, 2 and 4 times the threshold stimulation strength was used. During epileptiform activity, the lowest of the three control stimulation intensities that reliably evoked epileptiform events at a constant latency was chosen. Thus stimulation intensity was the same when control and epileptiform activity were compared. This stimulus strength differed among slices and could correspond to threshold or to maximum of the control stimulation strength. Therefore the spatial extent of control responses shown together with epileptiform potentials may differ significantly (e.g., compare Figs. 2B and 3B). Stimuli were given at intervals of ~90 s.

APV (Sigma) was added to the medium, giving a final concentration of 100 μM. In three slices, isolated presynaptic calcium signals were obtained by adding kynurenic acid (3 mM), an excitatory amino acid receptor blocker, to the bath medium.

Calcium transients were measured in 22 slices. In 10 slices epileptiform activity was induced with bicuculline; in 12 slices it was induced in low Mg$^{2+}$.

**Optical recordings**

An inverted microscope (IM35, Zeiss) was mounted on an X/Y table below a rigid stand holding the recording chamber and micromanipulators. A xenon arc lamp (75 W) provided epi-illumination of the field; the objective also served as a condensor. The excitation light passed through a heat and band-pass filter (477 ± 20 nm) via a dichroic mirror (510 nm) and through the objective to the preparation; the emitted fluorescence light passed through the objective, the dichroic mirror (510 nm), and a long-pass filter (520 nm) to a 10 × 10 photodiode array (MD100-5, Centronic) that was placed in the real image plane of the microscope. According to the objective used (∼25 Zeiss Neofluar), the spatial resolution was 60 μm. Thus calcium transients were detected simultaneously from a total area of (600 μm)$^2$. To monitor epileptiform activity across the entire slice, the microscope with the attached photodiode array was moved below the stationary slice...
FIG. 2. Distribution of mean window amplitudes of calcium transients across area CA1 of a slice during bicuculline-induced epileptiform activity. A: schematic drawing of the slice with positions of stimulation electrodes. Calcium transients following stimulation were recorded from 10 partially overlapping positions of the photodiode array covering the area marked by the square. Thus, for each stimulation condition, 1,000 photodiode records were gained. From each record the mean window amplitude was determined. These values were used to construct the color-coded plots shown in B–D. B–D: distribution of mean window amplitudes during control conditions following stimulation of CA1 (B, left) and CA3 (B, right), during the application of bicuculline following stimulation of CA1 (C, left) and CA3 (C, right) and during the application of dl-2-amino-5-phosphonovaleric acid (APV) with stimulation of CA1 (D, left) and CA3 (D, right). E: field potentials recorded from the stationary recording electrode in SR. Calibration marks: 1 mV, 5 ms.
by use of the X/Y table (Fig. 1A). Adjacent positions of the photodiode array were monitored at intervals of ~90 s. Data were only considered for further analysis if the continuously recorded field potential from a stationary position was stable (onset latency, amplitude, shape) during the mapping procedure. The signals from each photodiode were current-to-voltage converted and amplified. The amplifiers incorporated an analog “sample-and-hold” circuit allowing the use of DC signals for amplification (modified from Grinvald et al. 1981). Signals were digitized by two digital-to-analog converters (DT 3382, Data Translations, multiplexer with 64 channels each, 250 kHz) and processed by a computer system (LSI 11/73, DEC). The system permitted a time resolution of 0.4 ms if all 100 channels were used. Single sweeps were recorded. After data acquisition, a photograph of each position of the slice was taken with a scheme of the photodiode array superimposed.

Data analysis

Signals were weakly smoothed (each data point was calculated as an average of its own value weighted 6/8 and the values of each neighboring point weighted 1/8 each). Dye bleaching, staining, and illumination irregularities were corrected off-line by expressing signals as relative fluorescence changes (dF/F), where F is the fluorescence light intensity of the stained slice during illumination without evoked neuronal activity and dF is the fluorescence change during evoked neuronal activity. An increase of fluorescence corresponds to an increase in the intracellular calcium concentration and is plotted upward in all records shown.

For each record, a baseline was calculated as the average amplitude of the 250 data points (100 ms) preceding the stimulus. The first point after the stimulus that was followed by ≥100 data points that were above baseline was defined as onset of calcium influx. For each photodiode record, a window of 150 ms was set from this onset point (Fig. 1B). From these window settings, onset latencies and mean window amplitudes were determined. The “mean window amplitude” is the average of the amplitude values from all data points within the window. Thus, with the use of mean window amplitude for comparison of different experimental situations, both magnitude and duration of calcium influx determine the differences measured.

Statistical values are provided as means ± SE if not otherwise indicated. Averaged values are from all slices investigated and from 10 photodiodes representing the axis of pyramidal cells.

Histological procedures

The position of the photodiode array in respect to the slice and to the stimulation electrodes was determined photographically in situ after the recording procedure. Slices were then placed in fixative composed of formaldehyde (1%), glutaraldehyde (1.5%), sucrose (4%), and phosphate buffer (0.1 M) in distilled water. Frozen sections 52 µm thick were cut parallel to the surface of the slices and stained with cresyl violet. Shrinkage of slices due to fixation was compensated by correlating the electrode positions on the photographs taken in situ and the lesions induced by the stimulation electrodes in the histological sections. Only those experiments in which no patches of damaged cells (swollen or pyknotic) in area CA1 could be detected were considered for further analysis.

FIG. 4. Presynaptic activity following stimulation of CA1 with 3 mM kynurenic acid. A: schematic drawing of the slice, indicating the positions of the photodiode array and of the stimulation and recording electrodes in CA1. One row of photodiodes representing the longitudinal axis of pyramidal neurons is marked in black. B: values of mean window amplitudes of calcium transients in SP plotted against distance to the stimulation site in CA1 under control conditions (●) and with 3 mM kynurenic acid (○). C: field potential recordings during control conditions and with 3 mM kynurenic acid are superimposed. Calibration signal: 1 mV, 5 ms. Arrow: time point of stimulus. D: optically recorded calcium transients (from the photodiode marked with a black circle and arrow in A) during control conditions and with 3 mM kynurenic acid are superimposed. Arrow: time point of stimulus.

RESULTS

Intracellular calcium transients during control and epileptiform activity

In slices stained with Calcium-Green, the fluorescent light intensity increased after electrical stimulation of CA1 or CA3, corresponding to an increase of the intracellular free calcium concentration. This increase was transient. After control stimulation of CA3, the calcium increase was limited to a small area close to the stimulation electrode. With control stimulation in SR of CA1, calcium transients were observed along the longitudinal axis of pyramidal neurons and for <3 mm parallel to SP (Figs. 2B and 3B).

These fluorescence signals represent both presynaptic calcium transients in stimulated fibers and postsynaptic (and possibly glia) calcium signals. To compare the magnitude and spatial extent of presynaptic calcium influx, kynurenic acid was added to the bath medium (3 mM). Presynaptic calcium signals from stimulated fibers were restricted to a small area (<1 mm with all stimulation intensities tested) near the stimulation site (Fig. 4). Because axonal calcium influx is small (Meves and Vogel 1973), presynaptic cal-

FIG. 3. Distribution of mean window amplitudes of calcium transients across area CA1 of a slice during low-Mg 2+-induced epileptiform potentials. A: schematic drawing of the slice with positions of stimulation electrodes. Calcium transients following stimulation were recorded from 10 partially overlapping positions of the photodiode array covering the area marked by the square. Color-coded plots were constructed as described for Fig. 2. B–D: distribution of mean window amplitudes during control conditions following stimulation of CA1 (B, left) and CA3 (B, right), during perfusion with low-Mg 2+-medium following stimulation of CA1 (C, left) and CA3 (C, right), and during application of APV with stimulation of CA1 (D, left) and CA3 (D, right). E: field potentials recorded from the stationary recording electrode in SP. Calibration marks: 1 mV, 5 ms.
calcium transients originate most likely from calcium influx into terminals.

Stimulation of CA1 or CA3 in 20 \( \mu \text{M} \) bicuculline or in low-Mg\(^{2+} \) perfusion medium induced epileptiform activity defined by the following criteria: 1) field potentials were of long duration (> 150 ms), 2) the magnitude of the response was independent from stimulation strength once initiated, and 3) the second pulse of a double stimulus (separated by 50 ms) evoked no discernable response. During epileptiform potentials, intracellular calcium transients were significantly increased across the entire part of the slice recorded (Figs. 2A and 3A), independent of the site of the induction in CA1 and CA3 (Figs. 2C and 3C). At a distance of 500 \( \mu \text{m} \) from the stimulation site, during the first 150 ms after onset of calcium influx, free intracellular calcium evoked by stimulation of CA1 increased to 467 \( \pm 6\% \) of the control value for bicuculline-induced epileptiform potentials (Figs. 5C and 6B) and to 345 \( \pm 10\% \) during epileptiform activity in low-Mg\(^{2+} \) medium (Figs. 5F and 6F). A similar comparison of control and epileptiform activity elicited by stimulation of CA3 is not provided, because the spatial extent of the corresponding control signal was small and varied among slices. Intracellular calcium transients during evoked epileptiform activity were longer lasting and reached peak amplitudes later than during control activity (Fig. 5, C and F) and did not decline to baseline during the optical recording period (280 ms).

The spatial extent of calcium transients following stimulation of CA1 and CA3 in both the control and epileptiform condition was comparable with the extent of the respective voltage transients measured in slices stained with RH795 (Albowitz and Kuhn, 1991). Simultaneous electrical recordings of evoked field potentials from SP or SR in area CA1 revealed population spikes or evoked field potentials. These changed after the addition of bicuculline or in low-Mg\(^{2+} \) medium and became epileptiform (Figs. 2E and 3E).

**Effect of the NMDAR antagonist APV on calcium transients during epileptiform activity**

According to the criteria for epileptiform activity stated above, adding 100 \( \mu \text{M} \) APV abolished low-Mg\(^{2+} \) but only reduced bicuculline-induced epileptiform activity. This is most evident from the changes of field potentials (Figs. 2E and 3E). Intracellular calcium transients decreased in both models (Figs. 2D and 3D). In the low-Mg\(^{2+} \) model, calcium transients were reduced to 62.4 \( \pm 0.6\% \) after stimulation of CA1 (Figs. 5F and 6F) and to 20.9 \( \pm 0.4\% \) after stimulation of CA3. During control stimulation of CA1, calcium transients were 31.0 \( \pm 0.8\% \) compared with epileptiform activity. Thus, even though APV abolished low-Mg\(^{2+} \) epileptiform activity, calcium influx (and field potentials) were still larger than during control conditions. During bicuculline-induced epileptiform activity, calcium transients were reduced to 71.2 \( \pm 0.1\% \) after stimulation of CA1 and to 78.4 \( \pm 0.6\% \) after stimulation of CA3. During control stimulation of CA1, calcium transients were 22.3 \( \pm 0.3\% \) compared with epileptiform activity.

**Distribution of intracellular calcium transients along the axis of pyramidal neurons**

Figures 5 and 6 show the distribution of calcium transients during control and evoked epileptiform activity along the longitudinal axis of pyramidal neurons at a distance of \( \sim 500 \mu \text{m} \) from the stimulation site in CA1 (Figs. 5A and 6A). For evoked control activity, maximal calcium transients were located 50 \( \mu \text{m} \) above SP in SR. Calcium transients during evoked epileptiform activity in both models both before and after addition of APV were maximal in SP (Fig. 6, B and F). Onset latencies (Fig. 6, D and H) were minimal between 250 and 300 \( \mu \text{m} \) above SP in SR, which corresponds to the distance of the stimulation site from SP.

By subtracting traces of evoked calcium transients during the application of APV from traces of epileptiform calcium transients without APV, the NMDAR-dependent components of calcium transients during epileptiform activity were isolated (Figs. 5, D and G, and 6, C and G). In both models, NMDAR-dependent calcium transients were largest in SP. Earliest onset latencies of NMDAR-dependent calcium components were later than onset latencies of calcium components during both the control condition and epileptiform activity (Fig. 6, E and I).

**Relative distribution of intracellular calcium transients along the axis of pyramidal neurons at different distances (parallel to SP) from the stimulation site**

Mean window amplitude profiles as shown in Fig. 6 were normalized to the mean window amplitude maximum (100\%), and profiles from comparable distances from the stimulation site were averaged among slices (Fig. 7, B and C). Close to the stimulation site in CA1 (300–1,300 \( \mu \text{m} \), Fig. 7, B and C, left), maximal calcium transients of control activity were located 100 \( \mu \text{m} \) above SP in SR.

During epileptiform activity, maximal calcium transients in both models were located at SP. Also, NMDAR-dependent calcium components were located in SP. At a larger distance from the stimulation site (2,300–3,300 \( \mu \text{m} \), Fig. 7, B and C, right), where no presynaptic activity from stimulated fibers or postsynaptic control activity could be monitored, calcium transients of epileptiform activity and their NMDAR-dependent components were maximal in SP in the bicuculline model and in SO in the low-Mg\(^{2+} \) model.

Latency profiles as shown in Fig. 6, D–I, were corrected to the minimum of onset latencies (0 ms), and profiles from comparable distances from the stimulation site were averaged among slices (Fig. 7, D and E). Close to the stimulation site in CA1 (300–1,300 \( \mu \text{m} \), Fig. 7, D and E, left), onset latencies of calcium transients during both control and epileptiform activity in both models were minimal 200 \( \mu \text{m} \) above SP in SR, which corresponds to the location of the stimulation electrode in SR. At a larger distance from the stimulation site (2,300–3,300 \( \mu \text{m} \), Fig. 7, D and E, right), where no presynaptic activity from stimulated fibers or control activity was detected, onset latencies of calcium transients during epileptiform activity in both models were minimal in SO (Fig. 7, D and E). Latencies of NMDAR-dependent calcium components, because of their low signal-to-noise ratio, were too variable to provide a statistically significant profile after averaging.

**Spread of intracellular calcium transients across the slice**

Figure 8 shows the distribution of mean window amplitude (Fig. 8, B and D) and onset latencies (Fig. 8, C and
E) of calcium transients along SP following stimulation of CA1. Lines of linear regression were calculated through plots of latency against distance along a section of the slice ranging from ~500 μm from the CA1 stimulation site (toward the subicular side) to the border of recording near the subiculum (Fig. 8, C and E). Velocities of spread were calculated from slopes of regression lines.

Horizontal spread of calcium transients was larger for epileptiform activity compared with control recordings (Fig. 8, B and D). This was particularly evident for activity following stimulation of CA3: whereas calcium transients during the control condition were restricted to a small area near the stimulation electrode, epileptiform calcium transients spread along the entire slice (Figs. 2 and 3).

The addition of APV reduced the spread of calcium transients during low-Mg$$^{2+}$$ (Fig. 8D) but not during bicuculline-induced epileptiform activity (Fig. 8B).

In both models, the addition of APV reduced the velocity of spread (Fig. 8, C and E). In the bicuculline model, for all slices and both stimulation sites ($N = 20$), the velocity of spread was $0.204 \pm 0.036$ m/s before and $0.120 \pm 0.014$ m/s after the addition of APV. The difference was significant at $P < 0.01$ (paired t-test). In the low-Mg$$^{2+}$$ model, the velocity of spread was also reduced by the addition of APV; however, because the distance of spread was significantly smaller after the addition of APV, a statistical analysis could not be performed. In the presented example (Fig. 8E), the velocity of spread was 0.195 m/s before and 0.156 m/s after the addition of APV.

**DISCUSSION**

Interpretation of fluorescent transients recorded with Calcium-Green-1 AM

Calcium-Green-1 AM is taken up by cells and cleaved by intracellular esterases to the cell-impermeant and calcium-sensitive form. Thus the calcium-sensitive indicator is trapped within the cell. Remaining extracellular Calcium-Green-1 AM is partly washed out and in any case not fluorescent. The recorded fluorescent signals represent an increase in the intracellular concentration of free calcium ions. We cannot exclude loading of the calcium indicator into glia cells. Indicator signals most likely consist of calcium transients from both neurons and glia.

Ideally, the calcium-sensitive indicator should be distributed equally throughout the cytosol; however, the acectoxy-methylene form of some indicators is known to compartmentalize in organelles (Blatter and Wier 1990). This fraction of the indicator is fluorescent, but does not contribute to the cytoplasmatic calcium signal. When estimating changes of cytoplasmatic calcium by comparing fluorescence changes in different experimental situations, this should not matter provided that the calcium indicator in both the cytosol and organelles is distributed equally throughout the cell. There were no major differences in background fluorescence along the axis of pyramidal cells. Thus, at least down to the spatial resolution of the photodiodes (60 μm), the dye was equally distributed. The dye concentration in somatic and dendritic areas was comparable.
FIG. 6. Values of mean window amplitudes and onset latencies of calcium transients along the longitudinal axis of pyramidal neurons. A: schematic drawing of the slice, indicating the positions of the photodiode array, and of the stimulation and recording electrodes in CA1. One row of photodiodes representing the longitudinal axis of pyramidal neurons, is marked in black. B–I: values of mean window amplitudes (B, C, F, and G) and onset latencies (D, E, H, and I) of calcium transients from records of photodiodes marked in A for bicuculline-induced (B–E) and low-Mg\textsuperscript{2+}-induced (F–I) epileptiform activity. B, D, F, and H: values from records of calcium transients following stimulation of CA1 during control condition (○), in epileptogenic medium (●), and with 100 μM APV (▲). C, E, G, and I: values from traces representing NMDA-receptor-dependent components of calcium transients during epileptiform activity (△). These traces were gained by subtraction of records of calcium transients with APV from records of calcium transients with epileptogenic medium only.

The stimulus-evoked increase of the intracellular calcium concentration was transient; however, the initial slopes were less steep and the rate of decay was slower than that of voltage signals (Albowitz and Kuhnt 1991; Hess and Kuhnt 1992; Sinha et al. 1995). The reason for these differences might be of both methodological and physiological nature. Fast voltage-sensitive dyes respond to changes of membrane potential in the submicrosecond range (Salzberg et al. 1993), whereas calcium indicators

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FIG. 7. Relative distribution of mean window amplitudes of intracellular calcium transients along the longitudinal axis of pyramidal neurons. A: schematic drawing of the slice with position of the stimulation electrode in CA1. Amplitude profiles from the areas marked black in the respective drawing were compared. B and C: amplitude values were normalized to amplitude maximum (% of Max.) and averaged among slices (N = 10). Mean and SE values are shown. D and E: latency values were normalized to latency minimum (ms from Min.) and averaged among slices (N = 10). Mean and SE values are shown. Profiles close to the stimulation site (300–1300 μm) and at a distance (2300–3300 μm) were compared. Profiles are from calcium transients following stimulation of CA1 during control conditions (○) and in epileptogenic medium (●), and of NMDA-receptor-dependent epileptiform calcium components (▲). The distribution for bicuculline-induced (B and D) and low-Mg\textsuperscript{2+}-induced (C and E) epileptiform activity is compared.
INTRACELLULAR CALCIUM TRANSIENTS IN EPILEPTIFORM ACTIVITY

**FIG. 8.** Distribution of amplitudes and onset latencies of calcium transients along SP. **A:** schematic drawing of the slice indicating the positions of the photodiode array, and of the stimulation and recording electrodes. Photodiodes representing SP are marked in black. **B–E:** values of mean window amplitudes (B and D) and onset latencies (C and E) of calcium transients in SP plotted against distance to the respective stimulation site in CA1 for bicuculline-induced (B and C) and low-Mg$^{2+}$-induced (D and E) epileptiform activity. Position of the respective stimulation site is marked by vertical 0 lines. In plots of onset latencies (C and E), lines of linear regression were calculated through data points from 500 μm from the stimulation site toward the subiculum (see text). Slopes of regression lines indicate velocity of spread of calcium transients.

are subject to reaction kinetics and might have slower response times. The association speed of the indicator with calcium ions depends (among other factors) on the concentration of both calcium ions and indicator. Both values can only be estimated and local differences of the calcium concentration cannot be excluded. However, the dissociation rate constants provided by Eberhard and Erne (1991) allow an approximate calculation of the dissociation time to half-value to 3.5 ms. Thus, even though the actual rising phase of calcium transients might be slightly faster than the indicator signals, the difference cannot account for the much slower decay of calcium transients observed, exceeding the voltage signal by >100 ms. Extrusion and/or intracellular buffering of calcium seems to be slower than the repolarization of the membrane (Blaustein 1988).

**Increase of free intracellular calcium during epileptiform activity**

During evoked epileptiform activity, fluorescence signals recorded with Calcium-Green were up to 5 times larger than during control stimulation in the two models of epilepsy studied (Figs. 5 and 6). Because the increase of fluorescence is not linearly related to the concentration of free calcium, and is relatively smaller at high concentrations of calcium (Haugland 1992), our observed increases of fluorescence correspond to a calcium increase of at least 5 times compared with control stimulation. Only relative values are provided, because ratio imaging is not possible with Calcium-Green. Furthermore, with the present conditions, quantitative values may be misleading because of averaging neuronal elements from different neurons in a column of tissue.

**Effect of the NMDAR antagonist APV on bicuculline- and low-Mg$^{2+}$-induced enhancement of intracellular calcium**

APV reduced calcium transients during both bicuculline- and low-Mg$^{2+}$-induced epileptiform activity. In bicuculline-induced epileptiform activity, the APV-sensitive component of epileptiform calcium influx was relatively small (Fig. 2). Similarly, field and intracellular epileptiform potentials were only partially reduced by APV (Dingledine et al. 1986, 1990). The small APV-sensitive component of calcium influx may not even be exclusively mediated by NMDAR-activated channels, because the additional NMDAR-mediated depolarization may open more voltage-dependent calcium channels either directly or by a polysynaptic excitatory
effect (Dingledine 1983). The reduction of the decreases of the extracellular calcium concentration by APV during epileptic activity in hippocampal slices was comparable with the APV effect seen here (Köhr and Heinemann 1989). From that work and our results it is, however, not possible to determine to what degree the observed decrease of calcium is caused by a decrease of synchronization among neurons (i.e., a polysynaptic effect) and/or a direct effect of APV on the single neuron.

According to the definition provided, epileptiform activity was abolished by application of APV in the low-Mg$^{2+}$/Ca$^{2+}$ model (Fig. 3); however, the response was still larger than during the control condition, probably because of increased transmitter release due to the low extracellular Mg$^{2+}$ concentration.

APV reduced the extent of spread of calcium transients in the low-Mg$^{2+}$ (Fig. 3) but not in the bicuculline (Fig. 2) model. However, APV reduced the velocity of spread of bicuculline-induced epileptiform activity significantly (Fig. 8). This might be attributed to an APV-dependent decrease of excitatory synaptic strength, such that spike thresholds were reached later, thereby reducing the degree of synchronization, which then reduces the speed of polysynthetically mediated spread.

**Spread of intracellular calcium transients during epileptiform activity**

Close to the CA1 stimulation electrode, onset latencies of calcium transients during epileptiform activity were minimal in SR, near the termination of Schaffer collaterals (Ishizaka et al. 1990). Presumably this is the site of synaptic contacts initiating epileptiform activity. With increasing distance from the stimulation electrode (parallel to SP) minimal onset latencies shifted toward SO, indicating initiation of epileptiform activity at basal dendrites (Fig. 7). Also, at least in the low-Mg$^{2+}$ model, NMDAR-dependent calcium components were largest in SO and, as discussed above, the block of NMDARs by application of APV reduced the velocity of spread of epileptiform activity. Thus it is likely that local circuit excitatory interactions in SO (Finch and Babb 1981; Knowles and Schwartzkroin 1981; Radpour and Thomson 1991; Thomson and Radpour 1991), which are partially mediated by NMDARs (Radpour and Thomson 1991; Thomson and Radpour 1991), mediate spread at a distance from the stimulation electrode where neither presynaptic signals from stimulated fibers nor postsynaptic control activity was observed. A role of SO for the propagation of epileptiform activity has been previously suggested on the basis of optical recordings of voltage transients during aminopyridine-induced epileptiform activity (Albowitz and Kuhnt 1991). Local excitatory circuits in SO might be the main route of propagation for epileptiform activity in the hippocampus. This pathway could be of particular significance for spontaneous epileptiform activity, which at present, because of technical reasons, cannot be studied with our system.

In summary, during epileptiform activity, large intracellular calcium transients are found predominantly in stratum pyramidale and stratum oriens. Calcium transients were only partially mediated by NMDAR-dependent channels. The distribution of calcium transients suggests a strong involvement of L-type calcium channels near stratum pyramidale and of local circuit excitatory interactions in stratum oriens.

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