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Regulation of extracellular calcium in the hippocampus *in vivo* during epileptiform activity – role of astrocytes

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Abstract

Astrocytes have been suggested to regulate the extracellular calcium concentration ($[Ca^{2+}]_o$), but this has not been thoroughly investigated. Adult, male Sprague-Dawley rats were used to record changes in $[Ca^{2+}]_o$ in the hippocampus during epileptiform activity. Maximal decreases in $[Ca^{2+}]_o$ in CA1 were measured in the pyramidal cell layer during 20 Hz, 20 sec stimulus trains to the contralateral CA3 region. Maximal decreases in $[Ca^{2+}]_o$ in the dentate gyrus were measured when maximal dentate activation had appeared - irrespective of the location, frequency or duration of the stimulation. Maximal decreases were 36% greater in the dentate gyrus than in CA1. During prolonged discharges, $[Ca^{2+}]_o$ recovered partially towards the baseline in both hippocampal regions. To investigate the role of astrocytes, local injections of fluorocitrate (FC), a metabolic toxin selectively taken up by astrocytes, were used. FC (0.1, 0.25 or 0.5 mM FC), but not vehicle (2 l), caused a small, but significant decrease in the maximal changes in CA1, but an increase in the dentate gyrus. The results suggest that maximal decreases in $[Ca^{2+}]_o$ occur in the hippocampus in response to burst firing of neurons and that astrocytes play a minimal role in the regulation of $[Ca^{2+}]_o$ during epileptiform activity.

Keywords

calcium; astrocytes; fluorocitrate; hippocampus; seizures

1. Introduction

Although epilepsy is a common neurological problem, the mechanisms involved in initiation and termination of seizure discharges are poorly understood. In general, mechanisms underlying seizure initiation are divided into three main categories – 1) a decrease in inhibition, 2) an increase in excitation and 3) changes in the levels of critical extracellular ion concentrations. Changes in each of these mechanisms could lower the seizure threshold and result in increased, or augmented, synchronization of neurons. The extracellular ions that have been studied in most detail are potassium and calcium and both have been found to change during seizure discharges and to alter neuronal excitability.

Both potassium and calcium levels in the extracellular space change in response to neuronal activity. Decreases in extracellular calcium ($[Ca^{2+}]_o$) have been recorded in the absence of synaptic activity in hippocampal slices from rats (Konnerth and Heinemann, 1983; Jones and

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Heinemann, 1987). These data suggest that calcium will move into both pre- and postsynaptic elements in response to electrical activity. In anesthetized rats, decreases in $[Ca^{2+}]_o$ have been recorded in response to stimulus trains and seizure activity in the cortex (Heinemann et al, 1977; Heinemann and Louvel, 1983) and hippocampus (Krnjevic et al, 1982a, 1982b; Somjen and Giacchino, 1985). In the CA1 area of the hippocampus, when lower stimulus intensities or lower frequency stimulation was used, the extracellular potassium ($[K^+]_o$) would rise, but there was a submaximal decrease in $[Ca^{2+}]_o$, unless the neurons fired in bursts (Krnjevic et al, 1982b). In the dentate gyrus of urethane-anesthetized rats (Somjen and Giacchino, 1985), the $[Ca^{2+}]_o$ decreases with stimulation, or the onset of epileptiform activity, but it appears that the maximal decrease requires maximal dentate activation. Maximal dentate activation is characterized by bursts of large amplitude population spikes (15-40 mV) in the dentate gyrus that represent synchronous firing of the entire hippocampal-parahippocampal circuit (Stringer and Lothman, 1992).

The role of astrocytes in the regulation of the extracellular environment has been debated for a number of years. In some brain regions, there is evidence that glia can buffer the extracellular potassium (Kofuji and Newman, 2004). During seizures in the hippocampus, local application of a relatively specific glial toxin causes a significant, but small, decrease in the rate of clearance of potassium from the extracellular space (Xiong and Stringer, 1999). The role of glia in the regulation of extracellular calcium has not been examined during seizure activity, but has been examined during spreading depression. During spreading depression in anesthetized animals, $[Ca^{2+}]_o$ falls. As the spreading depression resolves, there was a fairly prompt return of $[Ca^{2+}]_o$ towards the baseline and then an overshoot in the recovery to levels higher than normal (Krnjevic et al, 1982b; Lian and Stringer, 2004). Local application of the selective glial toxins fluorocitrate or fluoroacetate resulted in loss of the prompt recovery in the $[Ca^{2+}]_o$ levels and a lengthening of the time that the $[Ca^{2+}]_o$ was at low levels (Lian and Stringer, 2004). These results suggest that astrocytes in the region play an active role in the recovery of $[Ca^{2+}]_o$ during spreading depression. Whether astrocytes contribute to the regulation of $[Ca^{2+}]_o$ during seizures is not known.

2. Methods

Adult male Sprague-Dawley rats (150-280 g) were anesthetized with urethane (1.2-1.5 g/kg ip) and placed in a stereotaxic frame. Body temperature was maintained with a water-recirculating heating pad. A concentric bipolar electrode was placed in the CA3 region of the left hippocampus at an angle of 5° (AP, -3 from bregma: ML, 3.5: depth 2.5-3.0 mm) for stimulation of the contralateral CA1 region. Another stimulating electrode, fashioned by twisting 0.01 in Teflon-coated stainless steel wire, was placed in the right angular bundle (AP, -8 from bregma: ML, 4.4: depth 2.5-3.5 mm) for stimulation of the ipsilateral dentate gyrus. A recording electrode was placed in CA1 or dentate gyrus (lateral 1.2-1.8 mm) on the right side, in the same AP plane as the CA3 stimulating electrode. The animals were grounded through a subcutaneous Ag/AgCl wire in the scapular region. Stimulus trains with a range of frequencies (5-100 Hz, 400-800 A, 0.3-ms biphasic pulses) and durations (1-30 sec) were administered to the CA3 region or the angular bundle while recording in CA1 or the dentate gyrus, respectively. Stimulus trains were given at least 10 minutes apart. Two or three stimulus trains at each frequency and duration were used to elicit extracellular field potentials in each recording location.

Double-barrel capillary glass was pulled to make ion-sensitive microelectrodes with a tip of 60-100 m along the widest side (Stringer and Lothman, 1989). One barrel of the double-barreled electrode was silanized with 15% tri-*N*-butylchlorosilane (Pfaltz & Bauer, Inc, Waterbury Ct) in chloroform, and the tip was filled with Fluka 21048 calcium cocktail or WPI Calcium IE200. The electrode was then backfilled with 0.1 M $CaCl_2$. The reference barrel was filled with 2 M

NaCl. The electrodes were calibrated before each experiment in a series of standard solutions (0.25-1.25 mM calcium concentrations and constant potassium, sodium, magnesium and chloride concentrations). The ion-sensitive microelectrodes had a response of 26-29 mV for a 10-fold change in calcium concentration. The extracellular field activity and ion signals were led to an Axoprobe 1A differential amplifier where the extracellular field response was subtracted to give the ionic changes. The negative capacitance compensation was set so that none of the responses were blunted. The responses were amplified for display on a chart recorder and digitized for storage.

Local application of fluorocitrate was used to inhibit astrocytic function (Clarke et al, 1970; Fonnum et al, 1997; Hassel et al, 1992; Largo et al, 1996; Paulsen et al, 1987; Swanson and Graham, 1994). Fluorocitrate is selectively taken up by astrocytes and is a suicide substrate for aconitase (Clarke et al, 1970). The fluorocitrate solution was prepared (Paulsen et al, 1987) by dissolving the barium salt (Sigma Chemical Co, St. Louis MO) in 0.1 M HCl and then precipitating with 0.1 M Na₂SO₄. The solution was then buffered with 0.1 M Na₂HPO₄ and centrifuged at 1000 X g for 5 min. The supernatant containing the fluorocitrate was diluted with 0.9% NaCl to the final concentration. Vehicle solution was prepared in exactly the same way, but without addition of the fluorocitrate. Injection was carried out by placing a 2 inch length of hypo-tubing (Small Parts Inc, outer diameter 0.012 in) into the groove between the two barrels of the ion-sensitive electrode giving a distance of 0.5-0.7 mm between the tip of the ion-sensitive electrode and the end of the injection tubing. The injection tubing was attached to a microliter syringe with PE10 tubing. The syringe and tubing were filled with the fluorocitrate (0.1, 0.25 or 0.5 mM) or vehicle before placement of the electrode into the brain. After baseline recordings were obtained, 2 μ l of the fluorocitrate solution, or vehicle, was injected over 2 minutes. Using these methods it was not possible to detect transient changes due to the injection itself. The first recordings were obtained 10 minutes after the injection of fluorocitrate or vehicle.

These doses of fluorocitrate were based on previous studies that have shown a decrease in ATP levels without any evidence of neuronal or glial loss after local injection of 0.3 nmol (Lian and Stringer, 2004) and a decrease in glutamine synthesis, with preservation of neuronal function, after local injection of 1 nmol (Hassel et al, 1992). The fluorocitrate requires 20-30 minutes to alter the metabolism within the glial cells and then the effect will last for several hours, depending on the dose (Xiong and Stringer, 1999). Animals were monitored for at least 3 hours after injection. At the conclusion of all experiments electrode placements were confirmed histologically. The animals were perfused through the heart with 10% buffered formalin. The brains were then subsequently equilibrated in 30% sucrose/10% buffered formalin. Sections were cut on a sliding microtome and stained with Cresyl violet.

The effects of fluorocitrate (FC) were determined by comparing the maximal changes in $[Ca^{2+}]_o$ response to each stimulus train before and after administration of FC or vehicle with a paired t-test. Significance was set at $p < 0.05$.

3. Results

3.1 Characteristics of $[Ca^{2+}]_o$ changes in the hippocampus

A range of stimulus frequencies (5, 10, 20, 50 and 100 Hz) with a fixed stimulus duration (10 sec) were tested while recording in either CA1 (n=7) or the dentate gyrus (n=7). A range of stimulus durations (2, 5, 10, 20, 30 sec) was then tested using the 20 Hz frequency stimulation. The stimulus amplitude was fixed for each animal at the intensity that elicited 80-90% of the maximal evoked population spike. With stimulation of the contralateral CA3 region, the maximal changes in extracellular calcium ($[Ca^{2+}]_o$) in CA1 were obtained with a stimulus frequency of 20 Hz (Figure 1A). Using the 20 Hz stimulus frequency, maximal changes in

$[Ca^{2+}]_o$ were obtained with at least 20 sec of stimulation (Figure 1B). In five animals, a laminar map was completed – using 100 μ m steps from the pyramidal cell layer in CA1 through the hippocampal fissure. In agreement with Krnjevic et al (1982a,1982b), maximal changes in $[Ca^{2+}]_o$ were recorded in the cell body layer of CA1. Using the maximal changes in CA1 for comparison, the maximal changes in $[Ca^{2+}]_o$ in stratum radiatum and stratum lacunosum-moleculare were $72 \pm 7\%$ and $67 \pm 9\%$, respectively (mean of 5 animals). Identification of the recording location was confirmed by observation of the response evoked from both the ipsilateral angular bundle and contralateral CA3 stimulation, as well as the depth from the CA1 cell layer.

The peak change in $[Ca^{2+}]_o$ occurred in the first 10-20 seconds of the stimulation or afterdischarge. With repeated elicitations, the seizures in these experiments will lengthen (Stringer and Lothman 1989,1992). To determine whether this change in seizure duration will affect the measurements of maximal $[Ca^{2+}]_o$, the change in $[Ca^{2+}]_o$ was compared to the total seizure duration (from beginning of population spike bursts during the stimulus train to the end of the afterdischarge) using data from 33 seizures elicited in 9 animals. There was no relationship between total seizure duration and the maximal change in $[Ca^{2+}]_o$ (Figure 1C). When recording in CA1, the $[Ca^{2+}]_o$ did not change with the onset of maximal dentate activation, which can be detected in CA1 by the appearance of small up-going spikes. With prolonged afterdischarges in CA1, the $[Ca^{2+}]_o$ either remained steady at the lower level or recovered partially towards the baseline and remained at that level (Figure 2).

With stimulation of the contralateral CA3 region or ipsilateral angular bundle, the maximal changes in $[Ca^{2+}]_o$ in the dentate gyrus were recorded with stimulus frequencies of 10 (100% of maximum change) and 20 Hz (83% of maximum) when maximal dentate activation was not initiated. These changes in $[Ca^{2+}]_o$ were in the range of 0.02-0.05 mM. There was no difference in the change in $[Ca^{2+}]_o$ in the dentate gyrus with CA3 versus angular bundle stimulation. With the appearance of maximal dentate activation (15-40 mV population spikes) there was a secondary drop in $[Ca^{2+}]_o$ irrespective of the location, frequency or duration of the stimulation (Figure 3). To separate the effect of the stimulus train from the appearance of maximal dentate activation, in 3 animals, an afterdischarge was initiated with 50 Hz / 5 sec stimulus trains to the contralateral CA3 region. Occasionally, maximal dentate activation would appear several seconds after the stimulus had stopped. One example is shown in Figure 3B. While there was a small decrease in $[Ca^{2+}]_o$ with the stimulus train and/or early afterdischarge, the largest decrease in $[Ca^{2+}]_o$ was measured with the onset of maximal dentate activation.

A laminar analysis (n=5) showed that, as in CA1, the maximal changes in $[Ca^{2+}]_o$ were in the granule cell layer of the dentate gyrus. This is in agreement with previous studies using anti- or orthodromic stimulation in hippocampal slices (Mody and Heinemann, 1986). The maximal change in $[Ca^{2+}]_o$ was significantly larger in the dentate gyrus ($36.0 \pm 8.2\%$ greater change, 0.102 ± 0.002 mM in CA1 compared to 0.139 ± 0.007 mM in the dentate gyrus) than in CA1. The maximal change in $[Ca^{2+}]_o$ in the dendrites of the dentate gyrus (just above the granule cell layer where the evoked EPSP is negative-going) was $45 \pm 5\%$ of the maximal changes in the granule cell layer (mean of 5 animals). Identification of the recording location was confirmed by observation of the response evoked from both the ipsilateral angular bundle and contralateral CA3 stimulation, as well as the depth from the CA1 cell layer. To test whether the $[Ca^{2+}]_o$ remained low during prolonged discharges in the dentate gyrus, extra long stimulus trains were used while recording in the dentate gyrus (Figure 3C). There was an initial decrease in $[Ca^{2+}]_o$ with the stimulus train, but there was a secondary decrease in $[Ca^{2+}]_o$ with the appearance of maximal dentate activation. There was some recovery of the $[Ca^{2+}]_o$ towards baseline even in the presence of continued neuronal activity.

3.2 Effect of local application of fluorocitrate on $[Ca^{2+}]_o$ changes

Inhibition of glial function by local application of fluorocitrate (FC) had a small, but statistically significant, effect on the changes in $[Ca^{2+}]_o$ in both CA1 and the dentate gyrus (Figure 4). To determine the effects of FC, recordings were obtained in the cell layer of either CA1 (n=6) or the dentate gyrus (n=5-8) and stimulus trains that elicit maximal changes in $[Ca^{2+}]_o$ were used (20 Hz for 10 or 20 sec). When recording in the dentate gyrus, only stimulus trains that initiated maximal dentate activation were used for analysis. Stimulus trains were administered every 10 minutes to the contralateral CA3 region. The changes in $[Ca^{2+}]_o$ during 3 stimulus trains were averaged and then vehicle or FC (2 l of solution) was administered. The changes in $[Ca^{2+}]_o$ during 3 stimulus trains given at least 20 minutes after the FC were then averaged and compared to the results before administration of FC (or vehicle) using a paired t-test.

Administration of vehicle had no effect on the changes in $[Ca^{2+}]_o$ during the stimulus trains in either CA1 or the dentate gyrus. Administration of FC, in the doses used in these experiments, had no apparent effect on baseline levels of $[Ca^{2+}]_o$, but it is possible that transient changes due to the injection were missed. In CA1, administration of 2 l of either 0.1 or 0.5 mM FC caused a small, but consistent, decrease in the change in $[Ca^{2+}]_o$ during the stimulus train. The mean values for the change in $[Ca^{2+}]_o$ are quite close, but because the paired t-test analyzes the change in $[Ca^{2+}]_o$, this decrease reached statistical significance. In the dentate gyrus, administration of 2 l of 0.1 mM FC had no effect on the change in $[Ca^{2+}]_o$, but injection of 0.25 mM FC caused a small, but consistent, increase in the change in $[Ca^{2+}]_o$ during maximal dentate activation. After injection of FC in either CA1 or the dentate gyrus, there was no change in the overall shape of the $[Ca^{2+}]_o$ during or after the stimulus train (Figure 5). As in the control animals, the maximal changes in $[Ca^{2+}]_o$ occurred relatively early in the discharge and then the level of $[Ca^{2+}]_o$ was maintained or there was a slight return towards baseline levels during the afterdischarge (Figure 5 shows an example from the dentate gyrus).

4. Discussion

In summary, stimulus trains of 10-20 Hz produce the most reliable changes in $[Ca^{2+}]_o$ in CA1 and the dentate gyrus and the largest changes in $[Ca^{2+}]_o$ are recorded in the cell layers. Maximal changes in $[Ca^{2+}]_o$ in the dentate gyrus require the initiation of maximal dentate activation. This suggests that synchronous burst firing of neurons, to recruit all of the neurons into the discharge, is needed to see the maximal changes in the extracellular calcium. Local administration of the selective glial toxin, fluorocitrate, caused a significant decrease in the change in $[Ca^{2+}]_o$ in CA1, but a significant increase in the dentate gyrus. Although these changes reached statistical significance, the changes were quite small. This suggests that astrocytes play a minor role in the regulation of extracellular calcium in the hippocampus during epileptiform activity. This is in contrast to the large changes in the regulation of $[Ca^{2+}]_o$ during spreading depression in the cortex after administration of fluorocitrate (Lian and Stringer, 2004).

With the onset of neuronal activity, intracellular levels of calcium increase in neurons after action potential firing (Bezzi and Volterra, 2001; Segal, 1993). If the neurons are stimulated close together, the calcium levels internally will be unable to decline to baseline between action potentials. Whether there is an upper limit to the intracellular calcium levels in neurons is not known. A recent study in cortical pyramidal cells with a low affinity calcium indicator suggests that there is no upper limit (Abel et al, 2004). Elevations in internal calcium in astrocytes have been found with synaptic transmission (Bezzi and Volterra, 2001) or application of glutamate (Pasti et al, 1997). In an elegant series of experiments by McCarthy and colleagues, it was demonstrated that intracellular calcium levels in astrocytes in hippocampal slices will increase in response to synaptic stimulation (Porter and McCarthy, 1996). The source of the increased intracellular calcium could either be movement from the outside of the cell or release from

internal stores. There was no increase in intracellular calcium after single stimulation, but trains (50 Hz, 2 sec) were very effective. These same stimulus parameters have been shown to increase intracellular calcium in hippocampal neurons in situ (Regehr et al, 1989; Regehr and Tank, 1990). The evidence suggests that neurotransmitter released during synaptic activity binds to and activates receptors on the adjacent glial membranes. Alternatively, increases in extracellular potassium have been proposed to induce influx of calcium into astrocytes (Duffy and MacVicar, 1996; Latour et al, 2001). However, with prolonged neuronal activity, the $[Ca^{2+}]_o$ does not continue to decrease, but reaches a plateau level, or actually recovers towards baseline, in both CA1 and the dentate gyrus. This raises the question – if, during intense neuronal activity the intracellular calcium continues to rise but the extracellular calcium does not continue to fall, then is calcium being pumped into the extracellular space by glial cells?

A continued decrease in $[Ca^{2+}]_o$ during prolonged seizure activity could be masked by shrinkage of the extracellular space. Iontophoretic application of excitatory amino acids in hippocampal slices causes an initial decrease in $[Ca^{2+}]_o$ followed by an increase (Arens et al, 1992). This secondary increase is postulated to be due to shrinkage of the extracellular space and activation of calcium exchangers. At sites of intense neuronal activity, a reduction in extracellular space of more than 30% has been measured (Lux et al, 1986). Thus, during a seizure, this reduction in extracellular space would be predicted to increase the concentrations of extracellular ions, or could mask a continued decrease. During spreading depression there is also a significant shrinkage of the extracellular space (Hansen and Olsen, 1980; Mazel et al, 2002), which corresponds temporally with the increase in $[Ca^{2+}]_o$ (Lian and Stringer, 2004). However, after administration of the glial toxin fluorocitrate, there was a loss of this increase in $[Ca^{2+}]_o$ suggesting that the increase was due to an active process in astrocytes.

Therefore, while a change in extracellular space is certainly occurring, it is probably not the only process contributing to the changes in $[Ca^{2+}]_o$. Other processes, active or passive, may be contributing to regulation of the $[Ca^{2+}]_o$. If there was active extrusion of calcium from astrocytes to limit the decrease in concentration in the extracellular space, then one would predict that local administration of fluorocitrate would result in a larger maximal decrease during the seizure discharge. This was not the case. Passive movement of calcium out of cells is unlikely because it would require reversal of the electrochemical gradient for calcium, which in turn would require excessively high internal calcium levels. Therefore, it is possible that an active pumping of calcium out of neurons during intense or prolonged neuronal activity may contribute to the limits in changes to the calcium concentration in the extracellular space.

It was somewhat surprising that the maximal changes in extracellular calcium were higher in the dentate gyrus than in CA1. However, due to differences in the anatomy and physiology (Johnston and Amaral, 2003), the responses to various stimulus frequencies are quite distinct between CA1 and the dentate gyrus. For example, CA1 cannot produce a population spike in response to each stimulus when the frequency is 50 or 100 Hz, but the dentate gyrus can. This probably explains the decline in $[Ca^{2+}]_o$ changes at the higher frequencies. In addition, during epileptiform activity in CA1, the population spikes are usually 5-10 mV, while in the dentate gyrus the population spike amplitude can reach 40 mV. There are also larger changes in extracellular potassium in the dentate gyrus with seizure activity (Xiong and Stringer, 1999), so we hypothesize that the difference in calcium between CA1 and the dentate gyrus is most likely due to the difference in bursting characteristics in these regions.

Although the effects of FC on the change in $[Ca^{2+}]_o$ reached statistical significance, the effects were quite small, particularly in the CA1 region. If cell swelling is reducing the extracellular space and limiting the decreases in $[Ca^{2+}]_o$, then it is possible that the metabolic toxin affects cell swelling resulting in the small change. We had hypothesized that, if astrocytes are actively maintaining, and limiting, the $[Ca^{2+}]_o$, then local administration of FC would increase the

maximal change. This only occurred to a small extent in the dentate gyrus and not at all in CA1. Although glial cells in the dentate gyrus have not been specifically studied, glial cells in the hippocampus are reported to vary, with subpopulations of astrocytes reported (Matthias et al, 2003). Glial contributions to ionic regulation have also been shown to differ between CA1 and CA3. Specifically, with neuronal activity the extracellular pH shows biphasic changes in CA1, but only acidification in the dentate gyrus (Xiong and Stringer, 2000). Metabolic activity in astrocytes appears to contribute to regulation of acidification, but not alkalization in CA1 (Stringer and Aribi, 2003). It is possible that the differences in CA1 and dentate gyrus in this study are due to different sensitivities of the glial cells to the fluorocitrate. Because of the limitations in the use of fluorocitrate, it was not possible to increase the dose in CA1 to determine whether there was simply a shift in the dose response curve to fluorocitrate. Given these differences between CA1 and the dentate gyrus one could hypothesize that the involvement of glia in the regulation of extracellular calcium is dependent on location. Whether this is due to a specific subtype of glial cells is not known.

There are a couple of technical limitations to this study. The first is the responsiveness of the calcium-sensitive electrodes. These double-barrel electrodes do not have the optimal speed of response (Fedirko et al, 2006) that one would like to be able to investigate rates of change in extracellular calcium. Therefore, it is only possible to compare relative changes in calcium. The second limitation to this study is the relative selectivity of fluorocitrate. At low doses, fluorocitrate is only taken up by glial cells, but at higher concentrations it can be taken up by neurons. The doses used in the present experiment are low compared to doses in previous studies (Xiong and Stringer, 1999). The lowest dose used in the present experiments is the same dose used in a previous study (Stringer and Aribi, 2003). This dose (2 l of 0.1 mM) had a significant, and more obvious, effect on the movement of hydrogen ions. Therefore, the doses used in the present study should be sufficient to alter glial metabolism. Higher doses might have produced a larger response, but run the risk of altering neuronal metabolism – making interpretation of the results quite difficult.

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Figure 1.

Effects of stimulus parameters and discharge duration on the changes in extracellular calcium in CA1. Maximal changes in $[\text{Ca}^{2+}]_o$ in CA1 in response to a range of stimulus frequencies, with the duration set at 10 sec, are presented in A ($n=7$). The effect of stimulus duration, using 20 Hz as the set frequency, on the changes in $[\text{Ca}^{2+}]_o$ are presented in B ($n=7$). Finally, to determine whether the maximal change in $[\text{Ca}^{2+}]_o$ depends on the duration of the epileptiform discharge, the changes in $[\text{Ca}^{2+}]_o$ during 33 seizures in CA1 were graphed as a function of the total seizure duration (data from 9 animals).

Figure 2.

Examples of changes in $[Ca^{2+}]_0$ in CA1 during and after 20 Hz, 10 sec stimulus trains to the contralateral CA3 region. Examples from the same animal of a short (A) and long (B) afterdischarge are shown. The maximal change in $[Ca^{2+}]_0$ and the end of the discharge are indicated on each recording. For both stimulus trains, the stimulus was set at 700 A.

Figure 3.

Examples of changes in $[Ca^{2+}]_o$ in the dentate gyrus from different animals. In A, the response to a 20 Hz (700 A) stimulus train to the contralateral CA3 is presented. The onset of maximal dentate activation is labeled (dashed arrow) and the stimulation was stopped 3 seconds after the appearance of maximal dentate activation. In B, the contralateral CA3 region was stimulated with a 5 sec train at 50 Hz (500 A) to initiate an epileptiform discharge in the dentate gyrus. The appearance of the large amplitude population spikes marks the onset of maximal dentate activation about 15 sec after the end of the stimulus train. In C, recordings from the cell layer in the dentate gyrus are shown before, during and after a 60 sec, 20 Hz stimulus train (700 A) to the ipsilateral angular bundle. The onset and termination of maximal dentate activation are marked with the arrows. Note that the $[Ca^{2+}]_o$ recovers somewhat towards baseline even in the presence of highly synchronized neuronal activity.

Figure 4.

Effects of local administration of fluorocitrate on $[Ca^{2+}]_o$ in CA1 and the dentate gyrus. Maximal changes in $[Ca^{2+}]_o$ were measured before and after local administration of 2 μ l of fluorocitrate (FC; concentration is indicated under the bars) or vehicle into the CA1 (A) or dentate gyrus (B) cell layers. Injection of vehicle had no effect on the maximal changes in $[Ca^{2+}]_o$. Fluorocitrate caused a small, but consistent, decrease in the maximal changes in $[Ca^{2+}]_o$ in CA1, but an increase in the dentate gyrus. Number of animals in each group is indicated above the bars. Error bars are standard error of the means. Statistical comparison was done with a paired t-test using the values before and after injection in individual animals.

After FC injection

Figure 5.

Changes in $[Ca^{2+}]_o$ during a prolonged discharge in the dentate gyrus after injection of FC. The $[Ca^{2+}]_o$ and extracellular DC recording from the cell layer in the dentate gyrus are shown before, during and after a 10 sec, 20 Hz stimulus train (400 A) to the contralateral CA3 region. The onset and termination of maximal dentate activation are marked with the dashed arrows. This stimulus train was administered 1 hour after injection of 2 μ l of 0.1 mM FC.