HIGH POTASSIUM-INDUCED EPILEPTIFORM BURSTING IN RAT HIPPOCAMPAL SLICES

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ABSTRACT: Hippocampal slices from adult Wistar rats were used to assess the presence and frequency of epileptiform bursting (EB) induced by high potassium levels in the bathing medium. In vitro hippocampal slices were stabilized initially in 3.5 mM K^+ artificial cerebrospinal fluid for one hour. All slices were checked for evoked responses in region CA1. The bath K^+ was then increased to 8.0 mM. Spontaneous epileptiform bursting was checked and recorded from regions CA1 and CA3. A branching logic design was used to define bursting, non-bursting and non-responsive slices. At the end of the 6-7 h experiment, all slices were checked, again, for evoked responses in CA1. Of 76 slices, 70 were responsive at the beginning and 68 at the end. Exposure to 8.0 mM K^+ produced EB in both regions CA1 (38/68) and CA3 (35/68). Average time to first EB in CA3 was 23 min. Bursting frequencies in CA1 and CA3 were highly correlated (r^2=0.95, df=62). These results suggest that EB in region CA1 is as reliable as that in CA3. Consequently, in screening large numbers of slices for EB, recording from CA1 is sufficient. *(JUMMEC 1997 2(2): 95-98)*

KEYWORDS: Electrophysiology, hippocampal slices, *in vitro*, potassium, rat, seizures

Introduction

High potassium levels (above 6 mM) in artificial cerebrospinal fluid (ACSF) are known to induce spontaneous epileptiform bursting (EB) in rat hippocampal slices in vitro (1,2). The mechanism by which these bursts are generated is not fully understood (2); however, it may be caused by a rise in the intracellular chloride level and/or alterations in the potassium pump mechanism (1).

An extensive study by Korn *et al.* (1) of spontaneous EB in the rat hippocampal slice indicated that bursts seemed to originate from region CA3. This same study showed that bursts were most intense and frequent at the boundary between CA3b-CA3c, but were relatively small and infrequent in region CA1.

The purpose of this study was to assess the presence and frequency of spontaneous EB activity in the rat hippocampal slice, induced by raising the potassium level in the bathing medium to 8.0 mM.

Materials and Methods

Adult male Wistar rats, 250-480 gm, were decapitated and their brains were rapidly removed and rinsed with ACSF. The hippocampi were isolated and immediately cut perpendicularly to their long axis with a McIlwain tissue chopper into 0.4 mm thick slices (Figure 1). Only slices from the middle third of each hippocampus were used in this study. Slices were placed in oxygenated ACSF for 2-5 min, then transferred to an interface chamber (3). Slices were mounted on a nylon mesh and superfused with oxygenated (95% O_2/5% CO_2) ACSF at a rate of 0.4 ml/min. The composition of the ACSF (in mM) was: NaCl 130, KCl 3.5, NaH_2PO_4 1.25, $NaHCO_3$ 24, $CaCl_2$ 1.5, MgSO4 1.5 and glucose 10 (1). Temperature in the chamber was maintained at 34 ± 0.5°C. A humidified gas mixture of 95% O_2 and 5% CO_2 was circulated over the slices in the chamber.

Slices were bathed in the low potassium (3.5 mM) ACSF for one hour to stabilize, after which their viability was checked electrophysiologically, as described by Shields *et al.* (4). A bipolar tungsten electrode was placed in the Schaffer collateral pathway (stratum radiatum) for orthodromic stimulation of the CA1 region pyramidal neurons. A glass recording electrode, filled with ACSF, that had an impedance of 2-5 megohms was placed in the cell body layer (stratum pyramidale) for recording of postsynaptic population field potentials. All recordings of evoked potential responses were made using stimuli 0.1 msec in duration and a voltage level equal to twice threshold. A "Grass S44" stimulator with a "SIU7"

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isolation unit was used for stimulation. Records were taken using a high-impedance headstage, an x100 AC amplifier, and a "Gould - Model 1425" digital oscilloscope. Single or averaged responses were transferred to an XT-type personal computer via serial link, stored on disk, and later plotted for analysis.

After initial stabilization, all slices were checked for evoked responses in region CA1. Those not showing responses of 1 mV or greater in amplitude were discarded. The active slices were also checked in the same manner at the end of each experiment, approximately 7 h from time of placement in the chamber.

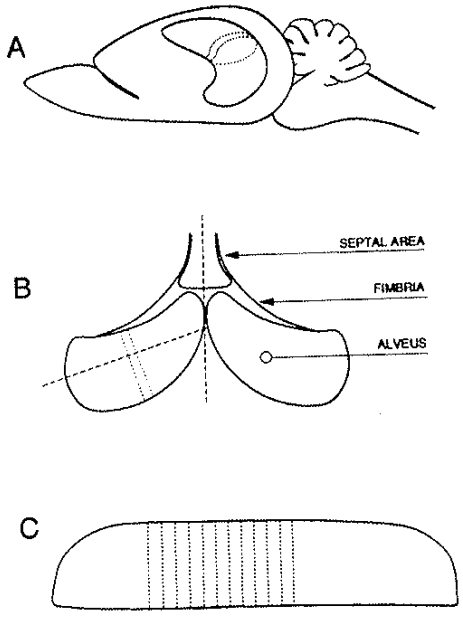

Figure 1. A schematic presentation of the preparation of the rat hippocampal slices: (A) position of hippocampus in the brain. (B) angle of cutting. (C) only the middle third of hippocampus was used in preparation of slices.

In preliminary experiments using 8.5 mM K^+ ACSF, EB rarely lasted long enough for an adequate survey of all slices. For this reason we reduced the K^+ level in the ACSF to 8.0 mM. In this medium EB was sustained for 3-5 h. The high potassium ACSF was introduced 165 min from the time slices were placed in the chamber. This period was used for slice viability checking. Immediately after the change to high potassium ACSF, slices were checked in sequence for the presence of spontaneous EB in region CA3. A branching logic plan was used to check each slice for: (a) presence or absence of spontaneous bursting in region CA3; (b) presence or absence of spontaneous bursting in region CA1; (c) burst frequency in CA3; and (d) burst frequency in region CA1. Due to equipment limitations it was not possible to measure burst frequency simultaneously in both regions CA1 and CA3. Burst frequency was measured only if the bursts were of sufficient amplitude to reliably trigger the "Gould" digital oscilloscope. Bursts of smaller amplitude were noted, and selected bursts captured for analysis, but adequate frequency measurements could not be made.

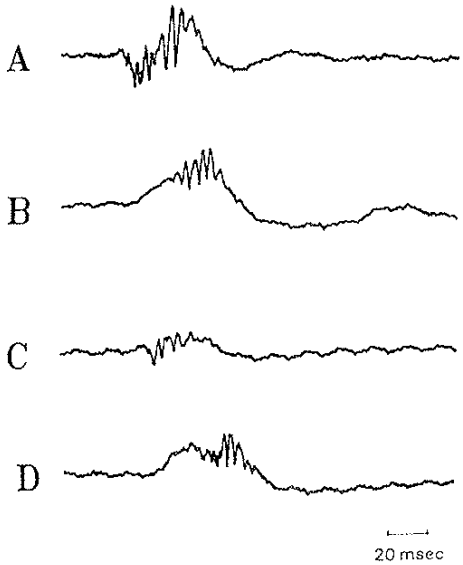

Figure 2. Records of spontaneous epileptiform bursts in CA1 (A and C) and CA3 (B and D) regions of two rat hippocampal slices in 8 mM K+ ACSF. Records A and B were from one slice, C and D from another slice. In A and B, the burst in CA1 is larger than the burst in CA3. In C and D the burst in CA3 is larger than in CA1. Within any one slice, the relative sizes of bursts in CA1 and CA3 tended to be similar.

Results

Of the 76 total slices used, 70 were found viable at the beginning of the experiment. At the end of the experiment, 68 slices were still active; showing evoked responses greater than 1 mV in amplitude in region CA1. The data presented here were taken from only the 68 slices that remained viable throughout the experiment. Almost all population responses showed single spikes when slices were bathed in the 3.5 mM K^+ ACSF. Multi-spike responses were consistently obtained at the end of the experiment as slices were then bathed in the 8.0 mM K^+ ACSF.

Sequential checking for spontaneous activity in regions CA1 and CA3 during the initial viability testing revealed no detectable bursting under low K^+ ACSF bathing conditions. The average time for the first epileptiform burst to appear in region CA3 after changing to 8.0 mM K^+ ACSF was 23 min from the time the high K^+ ACSF reached the chamber. Bursting during the first

15 minutes after the appearance of the first burst was weak, irregular and infrequent. We, therefore, decided to allow slices to stabilize their bursting patterns by waiting for about 25 min after the first burst appeared before assessing and recording burst activity.

Burst amplitude, even after stabilization, was quite variable. Within the same slice, bursts from CA1 could be larger or smaller than bursts from CA3 (Figure 2). As our primary goal was to survey a substantial number of slices, we did not explore these amplitude variations in detail.

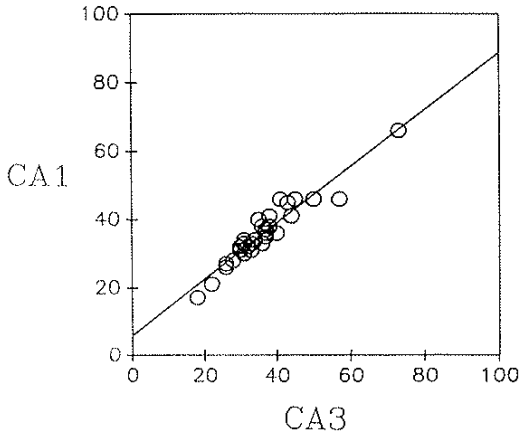

Figure 3. Correlation between spontaneous burst frequencies in CA1 and CA3 regions of the same hippocampal slice

As shown in Table 1, only 68 % of the slices surveyed showed EP activity. Even though the number of slices that had EP activity in region CA1 was identical to the number that had EP activity in region CA3 (46/68), they were not necessarily the same slices. Only 31 slices had bursts large enough for adequate rate measurement in both regions CA1 and CA3.

Data on frequency of EB in CA1 and CA3 are summarized in Table 2. The mean bursting frequencies in regions CA1 and CA3 were the same for those slices from which counts and records of bursts were made. The range of burst frequencies observed was substantial: from 20 to over 70/min. Nevertheless, burst frequencies recorded from CA1 and CA3 regions of the same slice were highly correlated (r^2=0.95), as shown in Figure 3.

Discussion

Korn *et al.* (1) reported that the average number of bursts/min resulting from raising the potassium level from 3.5 mM to 8.5 mM was 58±3 (standard deviation of the mean) in region CA3 (n=64). Dingledine and Korn (5) reported a rate of bursting of 1/sec (60/min) under similar experimental conditions. Traynelis and Dingledine (6) reported a rate of 47.5±0.8 (N=490), also with 8.5 mM K^+. Our results show a lower count/min in both regions CA1 and CA3 (Table 2), at least in part because we used an 8.0 mM rather than 8.5 mM potassium medium. Rutecki *et al.* (2) recorded increases in intensity and frequency of spontaneous bursting as the level of potassium in the ACSF was increased from 5.0 mM to 10.0 mM; from this data a change from 8.5 mM to 8.0 mM K^+ would be expected to reduce the burst frequency by about 6/min.

Table 1. Distribution of epileptiform bursting activity in regions CA1 and CA3 of the rat hippocampus

Region	CA1	CA3
Total No. of active slices	68	68
No. of slices with bursting activity*	46	46
% of total slices	68	68
No. of slices with counted bursts**	38	35
% of total slices	56	51
% of bursting slices	83	76
No. of slices with counted bursts in both CA1 + CA3	31	31
% of total slices	46	46
% of bursting slices	67	67

* Whether with counted bursts or not.

** Bursts that were large enough for accurate counting.

Table 2. Frequency of epileptiform bursts in regions CA1 and CA3 of the rat hippocampus

Region	CA1	CA3
No. of slices with counted bursts	38	35
Av. No. of bursts/min	33 ± 1.5 (n = 38)	35 ± 1.7 (n = 35)
No. of slices with counted bursts in both CA1 + CA3	31	31
Av. No. of bursts/min	36 ± 1.6 (n = 31)	36 ± 1.8 (n = 31)

Our results differ from those reported by Korn *et al.* (1), regarding the presence and frequency of spontaneous bursting activity in region CA1. Our finding of a level of EB in region CA1 equal to that present in CA3 may be attributable to the fact that our hippocampal slices were cut perpendicularly to the longitudinal axis; as well as our use of slices only from the middle third of each hippocampus. Anderson *et al.* (7) showed that the Schaffer collaterals and the

mossy fibers in the hippocampus are oriented in a direction nearly transverse to the longitudinal axis of the hippocampal formation. Our method of slicing the hippocampus and our choice of slices may have improved the chances for the connections between regions CA1 and CA3 to remain intact, in at least 46% of the slices used. We do not have an explanation for the fact that nearly 1/3 of the slices we tested showed no detectable spontaneous bursting activity. We did not observe the prolonged electrographic discharges in CA1 described by Traynelis and Dingledine (6). The findings presented in this study facilitate the detection of measurable spontaneous bursting activity in hippocampal slices. Because of the high correlation between activity levels in region CA1 and region CA3, checking for EB activity in region CA1 may be sufficient; i.e., if EB is present in region CA1 then it can be assumed to also be present in region CA3.

It is appropriate to note that the technique and equipment used in this study allow for diverse utilization, for several purposes, involving the biological properties of different tissues.

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