Pan, Enhui and Janet L. Stringer. Role of potassium and calcium in the generation of cellular bursts in the dentate gyrus. J. Neurophysiol. 77: 2293–2299, 1997. Epileptiform activity, which appears to be endogenous, has been recorded in the granule cells of the dentate gyrus before the onset of synchronized seizure activity and has been termed cellular bursts. It has been postulated that an increase in input to the dentate gyrus causes a local increase in extracellular K⁺ concentration ([K⁺]₀) and a decrease in [Ca²⁺]₀, that results in this cellular bursting. The first test of this hypothesis is to determine whether the cellular bursts appear in ionic conditions that occur in vivo before the onset of synchronized epileptic activity. This hypothesis was tested in vitro by varying the ionic concentrations in the perfusing solution and recording changes in the granule cells of the dentate gyrus. Intra- and extracellular recordings were made in the dentate gyri of hippocampal slices prepared from anesthetized adult Sprague-Dawley rats. Increasing the extracellular potassium or decreasing the extracellular calcium of the perfusing solution caused three forms of spontaneous activity to appear: depolarizing potentials, action potentials, and cellular bursts. Increasing potassium or decreasing calcium also caused the granule cells to depolarize and reduced their input resistance. No synchronized extracellular field activity was detected. Simultaneously increasing potassium and decreasing calcium caused cellular bursts to appear at concentrations recorded in vivo before the onset of synchronized reverberatory seizure activity.

INTRODUCTION

Although the membrane properties and seizure capabilities of the CA1 (Church 1992; Harkins and Armstrong 1992; Mason 1993) and CA3 (Anderson et al. 1990; Straub et al. 1990; Traynelis and Dingledine 1989) pyramidal cells of the hippocampus have been well studied, less is known about the granule cells of the dentate gyrus. In vivo, the dentate gyrus has been shown to sustain epileptiform activity consisting of bursts of large-amplitude population spikes. This epileptiform activity can be initiated by stimulus trains to the angular bundle (Somjen et al. 1985; Stringer et al. 1989), CA3 region (Stringer et al. 1989), and amygdala (Stringer et al. 1991). The initiation of these granule cell paroxysms is an important step in the propagation of seizures into and through the hippocampus (Stringer and Lothman 1992a,b; Stringer et al. 1989). Recently, prolonged field bursts in the dentate gyrus in vitro have been produced that appear quite similar to the synchronized activity recorded in vivo (Pan and Stringer 1996; Patrylo et al. 1994; Schweitzer et al. 1992). These prolonged bursts of large-amplitude population spikes are produced by raising the extracellular potassium concentration ([K⁺]₀) to 10–12 mM and lowering the [Ca²⁺]₀ to 0.5 mM (or by raising the potassium to 8 mM and using 0 added calcium). These field bursts in the dentate gyrus are not dependent on synaptic transmission and appear to be synchronized by nonsynaptic mechanisms.

To investigate the cellular changes that occur before and during the prolonged field events, intracellular recording from the granule cells was carried out during perfusion of hippocampal slices in 8 mM [K⁺]₀ and zero added calcium in vitro (Pan and Stringer 1996). Interestingly, spontaneous activity occurs in the granule cells before the onset of the prolonged field bursts. The spontaneous activity, which can appear in the absence of synaptic transmission, consists of small depolarizing potentials, action potentials, and bursts of action potentials on a depolarizing envelope. This activity continues to occur between prolonged extracellular bursts and is not associated with any extracellular field activity. The frequency of the cellular bursts is sensitive to membrane potential, suggesting that the bursts are generated endogenously within the granule cells (Pan and Stringer 1996).

On the basis of these data a hypothesis can be proposed about the function of the dentate gyrus in the propagation of epileptiform activity into and through the hippocampal formation. Under normal conditions, the dentate gyrus has a very high threshold for the onset of seizure discharges (Fricke and Prince 1984; Stringer et al. 1989). When an epileptogenic insult to the brain occurs, input to the dentate gyrus increases, resulting in a local increase in [K⁺]₀, and a decrease in [Ca²⁺]₀ (Krnjevic et al. 1980; Lux et al. 1986; Stringer and Lothman 1989). The changes in the extracellular environment initiate endogenous bursting properties latent in the granule cells, resulting in cellular bursts. Then, with the contribution of nonsynaptic mechanisms (Dudek et al. 1986), these bursts synchronize to produce the prolonged field discharges, which then propagate into and through the hippocampus. The first test of this hypothesis is to determine whether the cellular bursts appear in ionic conditions that occur in vivo before the onset of synchronized epileptic activity. This hypothesis was tested in this study by varying the ionic concentrations in the perfusing solution and recording changes in the granule cells of the dentate gyrus.

METHODS

Hippocampal slices were prepared by conventional methods (Stringer and Lothman 1988) from 60 adult Sprague-Dawley rats (Sasco, St. Louis, MO, 150–250 g) of both sexes. Principles of laboratory animal care (National Institutes of Health publication No. 86–23, revised 1985) were followed, as well as the specific principles approved by the Council of the American Physiological Society. Every effort was made to reduce animal suffering and to reduce the number of animals used. After the rats were anesthetized (ketamine 25 mg/kg, xylazine 5 mg/kg, acepromazine 0.8 mg/kg...
ip), the brains were removed and the hippocampus was dissected away from surrounding tissues. Hippocampal slices (450–500 μm) were cut with the use of a vibrating tissue slicer (Vibratome) and slices from the middle third of the hippocampus were placed in an interface-type chamber. Slices were continuously perfused by artificial cerebrospinal fluid (ACSF) at 33°C under a stream of humidified 95% O₂-5% CO₂. Composition of the ACSF was (in mM) 127 NaCl, 2 KC₁, 1.5 MgSO₄, 1.1 KH₂PO₄, 26 NaHCO₃, 2 CaCl₂, and 10 glucose. All solutions were bubbled constantly with 95% O₂-5% CO₂. Slices were allowed to equilibrate for 1–1.5 h before electrophysiological recording was begun. The concentration of potassium was altered by changing the amount of KCI without changing the other ions. The concentration of calcium was altered by changing the amount of CaCl₂ without altering the concentrations of the other ions.

Recording electrodes were made of microfilament capillary thin-walled glass (A-M Systems, 0.9 mm ID, 1.2 mm OD) pulled on a micropipette puller (P-87, Sutter Instruments). Intracellular electrodes were filled with 4 M potassium acetate and had impedances between 60 and 100 MΩ (tested with 20-nA current pulses). Extracellular electrodes were filled with 2 M NaCl and had impedances between 4 and 10 MΩ. Bipolar stimulating electrodes consisted of twisted Teflon-coated tungsten wire (0.002 in. diam). One stimulating electrode was placed in the molecular layer of the dentate gyrus to activate perforant path fibers and a second stimulating electrode was placed in the hilar region. Two recording electrodes were placed in the granule cell layer of the dentate gyrus, one for intracellular recording and one for extracellular recording.

A slice was considered adequate for intracellular recording if a single perforant path stimulus was able to elicit a population spike of ≥10 mV in the granule cell layer of the dentate gyrus. A dentate granule cell was considered suitable for inclusion in the study if the resting membrane potential was at least −65 mV and current injection or perforant path stimulation could evoke an overshooting action potential. During intracellular recording, the bridge balance was checked regularly. Single stimulation of the perforant path or hilar region consisted of a square-wave pulse of 0.1–0.2 ms in duration and 20–80 μA in intensity. Signals were amplified (Axon probe 1A, Axon Instruments), monitored on an oscilloscope (Tektronics 2212), recorded on a chart recorder (Astro-Med Dash IV), and stored digitally by computer for off-line processing. The results from intracellular recordings from 49 granule cells are presented here.

The absolute membrane potential was determined after entering the cell and again when removing the electrode from the cell. Changes in the membrane potential were continuously monitored. The membrane resistance was determined from responses to rectangular current pulses of ±0.2 or 0.4 nA, passed through the intracellular electrode. Statistical comparisons were made with the use of t-tests, with significance taken as P < 0.05.

Results

Spontaneous activity recorded from the granule cells in altered [K⁺]₀ and [Ca²⁺]₀.

To determine which ion is responsible for the initiation of the cellular activity, the response to increasing [K⁺]₀ alone or decreasing [Ca²⁺]₀ alone was tested. Several types of spontaneous activity were recorded intracellularly in 8 mM [K⁺]₀ and low [Ca²⁺]₀, (both 0.8 mM and 0 added calcium) solutions: depolarizing potentials, action potentials, and bursts (Fig. 1). All three types of activity were recorded in cells that were held for an adequate length of time (n = 16, 5 in 0.8 mM [Ca²⁺]₀, 6 in 0 added calcium, 5 in 8 mM [K⁺]₀). No activity was detected by the extracellular recording electrode at any time during these experiments.

Depolarizing potentials were recorded (Fig. 1A) after 10–15 min in the high-[K⁺]₀ or low-[Ca²⁺]₀ solution. The potentials ranged from 5 to 15 mV in amplitude and from 10 to 15 ms in duration. There was some variability within a single cell (Fig. 1A, top), but most of the depolarizing potentials recorded in a single cell were of the same amplitude and duration (Fig. 1A, bottom). After 15–30 min in the test solutions, spontaneous action potentials appeared in all cells (Fig. 1B). Five to 15 min after the appearance of the spontaneous action potentials, bursts of action potentials began to appear, which were termed cellular bursts (Fig. 1C).

When the slice was perfused in the altered ionic solutions for longer periods, the bursts gradually lengthened (Fig. 1D). After 45–60 min, the burst duration “stabilized” and the mean duration was determined. For this measurement, a single burst was defined as a group of action potentials that was clearly separated from other action potentials by ≥60 ms of baseline DC potential. The durations of 10 consecutive bursts were measured to determine the mean duration of the bursts for that cell. The mean duration was 188 ± 86 (SD) ms (n = 6) in zero added calcium, 55 ± 14 ms (n = 7) in 0.8 mM [Ca²⁺]₀, and 69 ± 20 ms (n = 10) in 8 mM [K⁺]₀. The burst duration in zero added calcium was significantly different from the duration in the other ionic conditions (nonparametric analysis of variance).

When the shapes of the spontaneous activity recorded in 8 mM [K⁺]₀ and low [Ca²⁺]₀ solutions were compared, it was noted that the base of the depolarizing potentials and action potentials was broader in low [Ca²⁺]₀ than in 8 mM [K⁺]₀. The widths of the spontaneous action potentials were measured at a distance from the baseline corresponding to one sixth of the height of the action potential. In normal ACSF, this width was 15.5 ± 2.6 (SE) ms (n = 5). In 8 mM [K⁺]₀, the width of the action potential was 6.0 ± 0.3 ms (n = 5), which was significantly different from the controls (grouped t-test, P < 0.05). In contrast, the width of the action potentials in zero added calcium (10.5 ± 0.6 ms, n = 6) was not significantly different from that in the controls (P > 0.1). The shape of the cellular bursts was also different. When the slice was equilibrated in 8 mM [K⁺]₀, each action potential (after the 1st) appeared to arise from the repolarization phase of the previous action potential (Fig. 1C, top). When the slice was equilibrated in low [Ca²⁺]₀, the bursts had a more characteristic pattern with a depolarizing envelope and action potentials on top of this depolarizing potential (Fig. 1C, bottom).

Role of [K⁺]₀ and [Ca²⁺]₀ in the onset of cellular bursts in the dentate gyrus

A total of 20 cells was used to determine the ionic concentration necessary for the onset of the cellular bursts. Granule cells were impaled while the slice was equilibrated in ACSF with 3.1 mM potassium and 2 mM calcium. If the cell was judged suitable for inclusion in the study, then the perfusing solution was changed to one containing either increased [K⁺]₀ or decreased [Ca²⁺]₀. Cells were allowed ≥30 min to equilibrate in each new solution. Seven cells were held while the [K⁺]₀ was increased from 3 to 8 mM in increments
of 1 mM. None of the cells had spontaneous bursting in \([K^+]_o\), up to 6 mM. Two of seven (27%) cells had cellular bursts in 7 mM, and the remaining five cells (100%) had bursts in 8 mM (Fig. 2A). Thirteen cells were held while the \([Ca^{2+}]_o\) was lowered from 2.0 to 0.8 mM in three steps. There was no bursting in 2.0, 1.5, or 1.2 mM \([Ca^{2+}]_o\), but 5 of 13 (38%) cells had cellular bursts in 1.0 mM \([Ca^{2+}]_o\). The remaining eight cells began bursting in 0.8 mM \([Ca^{2+}]_o\). (Fig. 2B). To confirm that the spontaneous activity was not due to damage to the membrane, four cells (2 from each group) were switched back to ACSF. In each case, the spontaneous activity ceased and the membrane potential returned to its baseline value.

To more closely mimic the in vivo situation, we next tested the effect of changing both \([K^+]_o\), and \([Ca^{2+}]_o\), on the appearance of the cellular bursts. To do this, the \([Ca^{2+}]_o\), was decreased to 1.5 mM (or 1.2 or 1.0 mM) and then a granule cell was impaled. After a stable recording was achieved (n = 15), the \([K^+]_o\) was increased from 3 to 7 mM in 1-mM steps and the \([K^+]_o\), at which the cellular bursts appeared was determined (Fig. 3A). The same procedure was repeated on a second group of cells (n = 14), but this time the \([K^+]_o\) was fixed and the \([Ca^{2+}]_o\), was varied. The \([Ca^{2+}]_o\), at which each cell began bursting was determined (Fig. 3B). The results indicate that combining an increase in \([K^+]_o\), and a decrease in \([Ca^{2+}]_o\), produces cellular bursting in the granule cells with less drastic changes than if either ion concentration was changed alone. For example, in 5 mM \([K^+]_o\), and 1.2 mM \([Ca^{2+}]_o\), 60–70% of the neurons were bursting.

**Effect of \([K^+]_o\), and \([Ca^{2+}]_o\), on the cellular properties of the granule cells**

During the above experiments it was noted that the membrane potential and input resistance of the granule cells changed when the concentration of the extracellular ions was changed. To study these changes systematically and to determine which properties may be related to the onset of the cellular bursts, the cellular properties in normal ACSF were compared with those in 8 mM \([K^+]_o\), or 0.8 mM \([Ca^{2+}]_o\). These are the levels of \([K^+]_o\), and \([Ca^{2+}]_o\), that are sufficient to produce cellular bursting when only one ion is changed (Fig. 2). An additional group of cells was recorded in zero added calcium.

Switching to either 8 mM \([K^+]_o\), (n = 4), 0.8 mM \([Ca^{2+}]_o\), (n = 5), or zero added \([Ca^{2+}]_o\), (n = 6) from normal ACSF caused a significant depolarization of the membrane potential and decrease in the input resistance (Table 1). It took 15–20 min for the cell to stabilize at the new values. Interestingly, when the input resistance in the 8 mM \([K^+]_o\), and the 0.8 mM \([Ca^{2+}]_o\), groups were compared, they were significantly different. The input resistance in low calcium was lower than that measured in 8 mM \([K^+]_o\). In zero added calcium, the input resistance of the granule cells was not significantly different from cells in 0.8 mM \([Ca^{2+}]_o\).

In contrast to the effect of \([Ca^{2+}]_o\), on input resistance, \([K^+]_o\) appears to have a greater effect on the membrane potential (Table 1). Intracellular recording was established in normal ACSF and then the perfusing solution was switched to one with an altered potassium or calcium con-
below the threshold for bursting. These comparisons suggest that the onset of the spontaneous activity in the present experiments was not simply due to depolarization of the membrane. To further test this hypothesis, neurons equilibrated in 8 mM $[K^+]_o (n = 6)$ were hyperpolarized back to $-84$ to $-86$ mV. This hyperpolarization slowed the rate of firing, but did not block the spontaneous activity. Together these data show that the spontaneous activity reported here is not simply a result of the depolarization induced by the ionic changes.

**DISCUSSION**

One hypothesis about the role of the dentate gyrus in the propagation of seizure activity into the hippocampus suggests that the onset of cellular bursts in the granule cells is a precursor to the onset of synchronized activity. It is postulated that an increase in activity coming into the dentate gyrus increases $[K^+]_o$ and decreases $[Ca^{2+}]_o$ (Krnjevic et al. 1980; Lux et al. 1986; Stringer and Lothman 1989; Stringer et al. 1989) sufficiently to initiate bursting in the granule cells. This role of $[K^+]_o$ and $[Ca^{2+}]_o$ in the onset of cellular bursts was examined in this study. Increasing $[K^+]_o$, or decreasing $[Ca^{2+}]_o$, caused spontaneous activity that took three forms: depolarizing potentials, action potentials, and the cellular bursts. At no time was any extracellular field activity recorded, indicating that this spontaneous activity was not synchronized. Changing both ions together caused cellular bursts to appear in $[K^+]_o$ and $[Ca^{2+}]_o$ that appear in vivo before the onset of synchronized reverberatory seizure activity (Stringer and Lothman 1989; Stringer et al. 1989). Therefore these experiments support the hypothesis that changes in $[K^+]_o$ and $[Ca^{2+}]_o$ are sufficient to initiate bursting in the granule cells. Although there is no evidence that the appearance of the cellular bursts is causally linked to the appearance of the synchronized bursts, the data presented here are consistent with the hypothesis that the cellular bursts are a necessary precursor to the onset of the synchronized activity.

These experiments show changes in both input resistance and membrane potential associated with the change in ionic environment. But, are these changes necessary for the onset of the bursts? Repolarization of the neuron (in high $[K^+]_o$) back to baseline did not block the cellular bursts. This suggests that depolarization alone is not sufficient to produce the cellular bursts. In addition, levels of $[Ca^{2+}]_o$ that initiated the cellular bursts did not depolarize the membrane to as great an extent relative to the membrane depolarization produced by increased $[K^+]_o$. (Table 1). The same argument holds for the input resistance. Lowering the extracellular $[Ca^{2+}]_o$ to 0.8 mM (sufficient to produce cellular bursts) lowered the input resistance more than raising the extracellular $[Ca^{2+}]_o$, to 8 mM (Table 1), suggesting that a simple decrease in input resistance is not initiating the cellular bursts. The data here, plus some previously published data (Pan and Stringer 1996), indicate that the effects of $[K^+]_o$ and $[Ca^{2+}]_o$ on the membrane potential are not additive. From the present study, the membrane potential in 8 mM $[K^+]_o$ had a mean of $-60$ mV and in zero added calcium a mean of $-68$ mV. When the cells were equilibrated in both 8 mM $[K^+]_o$, and zero added calcium the mean membrane potential was $-60$ mV (Pan and Stringer 1996).
Although calcium is not as directly involved in setting the membrane potential as potassium, others have recorded depolarization of cells in low calcium (Agopyan and Avoli 1988; Chai and Webb 1992; Jefferys and Haas 1982; Palant et al. 1989) and a number of mechanisms have been proposed for this effect (Hille 1992). In the present experiments, the membrane potential was not significantly altered until the [Ca\(^{2+}\)]\(_o\) was dropped to <1.2 mM. This suggests that the mechanisms proposed for [Ca\(^{2+}\)], regulation of the membrane potential are not active until the [Ca\(^{2+}\)] falls to <1.2 mM, which is the estimated level of free calcium in the extracellular fluid of the brain (Ames et al. 1964; Heinemann et al. 1992; Lux et al. 1986; Morris 1981). These estimates of the extracellular calcium may be slightly high. It has been suggested that buffering by bicarbonate and phosphate reduce the free calcium concentration by ~25% (Heinemann et al. 1992). This would mean that the free calcium concentration at which the membrane potential is affected is ~0.9 mM.

An increase in [K\(^+\)], may have an important role in regulating neuronal excitability independently of the effect on the membrane potential (Hablitz and Lundervold 1981; Jensen et al. 1994; Traynelis and Dingledine 1989). Increasing the [K\(^+\)], has been proposed to decrease the potassium driving force, decrease chloride-mediated \(\gamma\)-aminobutyric acid inhibitory potentials, and decrease potassium-mediated after-hyperpolarizations (Jensen et al. 1994; Traynelis and Dingledine 1989). Increasing the [K\(^+\)], will also produce glial swelling and a change in the extracellular space (Traynelis and Dingledine 1989). Decreasing extracellular calcium also would be predicted to increase neuronal excitability independently of an effect on the membrane potential (Heinemann et al. 1992). Decreasing [Ca\(^{2+}\)], may 1) reduce inhibition by blocking transmitter release from spontaneously active inhibitory interneurons, 2) depress the after-hyperpolarization through a decrease in the calcium-activated potassium conductance (Hotson and Prince 1980), 3) increase excitability through an effect on surface charge density (Hille 1992), or 4) decrease the ability of the sodium-potassium pump to clear the extracellular potassium, thus leading to an increased [K\(^-\)]\(_o\) (Yaari et al. 1986). Thus both [K\(^+\)], and [Ca\(^{2+}\)], can have direct and indirect effects on the neurons that may contribute to the generation of the bursting. Changes in both ions may also change the shape of the spontaneous activity. In low [Ca\(^{2+}\)], the change in shape of the spontaneous activity is consistent with a reduction in the calcium-activated potassium current resulting in a slower repolarization after an action potential. Conversely, in increased [K\(^+\)], the increased rate of repolarization may be a result of an increase in current through potassium channels that mediate repolarization.

Data have shown that a key element in the generation of pacemaker activity is a voltage-sensitive calcium conductance and an associated slower potassium conductance mediated by intracellular calcium (for review see Wong and Schwartzkroin 1982). Granule cells of the dentate gyrus

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**TABLE 1. Effect of 8 mM [K\(^+\)], or 0.8 mM [Ca\(^{2+}\)], on the cellular properties of dentate granule cells**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Input Resistance, M(\Omega)</th>
<th>Membrane Potential, mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>55.0 ± 2.2</td>
<td>-84.0 ± 1.0</td>
</tr>
<tr>
<td>0.8 mM [Ca(^{2+})](_o)</td>
<td>33.0 ± 2.0*</td>
<td>-73.2 ± 1.0*</td>
</tr>
<tr>
<td>0 Added [Ca(^{2+})]</td>
<td>29.3 ± 6.1*</td>
<td>-68.0 ± 4.9*</td>
</tr>
<tr>
<td>8 mM [K(^+)]</td>
<td>41.3 ± 1.3*</td>
<td>-60.8 ± 2.2*</td>
</tr>
</tbody>
</table>

\(n = \) number of cells in each group. For both input resistance and membrane potential, the [K\(^-\)]\(_o\) group was significantly different from both [Ca\(^{2+}\)]\(_o\) groups. The 2 [Ca\(^{2+}\)]\(_o\) groups were not significantly different from each other. * Significant difference compared with control (\(P < 0.05\)).
contain a variety of voltage-sensitive calcium channels (Elliott and Johnston 1994) that may contribute to a type of pacemaker activity. However, it is clear that simply decreasing the \([\text{Ca}^{2+}]_o\) can produce spontaneous bursting in the dentate gyrus (present experiments) and in CA1 in vitro (Haas and Jefferys 1984; Heinemann et al. 1992; Stringer and Lothman 1988). This effect of decreasing the \([\text{Ca}^{2+}]_o\) is probably due more to the general effects of calcium on excitability (Hille 1992) than to an effect on calcium channels that underlie endogenous pacemaker activity.

Compared with the pyramidal cells in CA1 and CA3, there appears to be something unique about the granule cells of the dentate gyrus in that they readily produce cellular bursts, but do not as readily produce synchronized field bursts. This is in contrast to the pyramidal cells, where extracellular field bursts are commonly recorded simultaneously with the intracellularly recorded neuronal burst (Agopyan and Avoli 1988; Haas and Jefferys 1984; Konnerth et al. 1986; Traynelis and Dingledine 1989). High potassium concentrations alone can induce synchronized bursting in CA3-CA2 and prolonged seizure-like discharges in CA1 that can be detected with extracellular electrodes (Jensen and Yaari 1988; Rutecki et al. 1985; Traynelis and Dingledine 1988, 1989). The relative role of potassium and calcium on the synchronization of the epileptiform activity in the dentate gyrus remains to be determined.

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