pH Sensitivity of Non-Synaptic Field Bursts in the Dentate Gyrus

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Schweitzer, Jeffrey S., Haiwei Wang, Zhi-Qi Xiong, and Janet L. Stringer. pH sensitivity of non-synaptic field bursts in the dentate gyrus. J Neurophysiol 84: 927–933, 2000. Under conditions of low [Ca2+]o and high [K+]o, the rat dentate granule cell layer in vitro develops recurrent spontaneous prolonged field bursts that resemble an in vivo phenomenon called maximal dentate activation. To understand how pH changes in vivo might affect this phenomenon, the slices were exposed to different extracellular pH environments in vitro. The field bursts were highly sensitive to extracellular pH over the range 7.0–7.6 and were suppressed at low pH and enhanced at high pH. Granule cell resting membrane potential, action potentials, and postsynaptic potentials were not significantly altered by pH changes within the range that suppressed the bursts. The pH sensitivity of the bursts was not altered by pharmacologic blockade of N-methyl-D-aspartate (NMDA), non-NMDA, and GABAA receptors at concentrations of these agents sufficient to eliminate both spontaneous and evoked synaptic potentials. Gap junction function is known to be sensitive to pH, and agents that block gap junctions, including octanol, oleamide, and carbazoxolone, blocked the prolonged field bursts in a manner similar to low pH. Perfusion with gap junction blockers or acidic pH suppressed field bursts but did not block spontaneous firing of single and multiple units, including burst firing. These data suggest that the pH sensitivity of seizures and epileptiform phenomena in vivo may be mediated in large part through mechanisms other than suppression of NMDA-mediated or other excitatory synaptic transmission. Alterations in electrotonic coupling via gap junctions, affecting field synchronization, may be one such process.

INTRODUCTION

The occurrence of large changes in [Ca2+]o and [K+]o during seizures and seizure-like phenomena both in vitro and in vivo has been well documented (Hablitz and Lundervold 1981; Heinemann et al. 1977; Knjve et al. 1982; Pumain and Heinemann 1981; Somjen and Giacchino 1985). The significance of these changes, and particularly their role as either cause or effect of the associated electrophysiological excitatory activity, has been a matter of considerable debate. Changes in neuronal excitability resulting from such ionic changes must be considered in developing a complete model of seizure propagation (Jensen and Yaari 1994; Schweitzer and Williamson 1995; Schweitzer et al. 1992). Another extracellular ion, H+, is an important regulator of myriad physiological processes, and its relationship to epilepsy and epileptiform events has also been well recognized (Balestrino and Somjen 1988; Caspers and Speckmann 1972; Velisek et al. 1994), but the effects of [H+]o on mechanisms of propagation and synchronization other than classical synaptic transmission have not been fully characterized.

We have previously developed an in vitro model that in many respects resembles “maximal dentate activation,” a phenomenon described in the intact rat that is closely related to limbic seizures (Patrylo et al. 1994; Schweitzer et al. 1992; Stringer and Lothman 1989; Stringer et al. 1989). The in vitro model is based on alteration of the slice extracellular environment to approximate levels of [Ca2+]o and [K+]o measured during seizures in vivo. This manipulation by itself is capable of producing full-blown seizure-like events, and both extracellular and intracellular techniques have been applied to demonstrate that these events are independent of fast amino acid-mediated synaptic transmission (Pan and Stringer 1996; Schweitzer et al. 1992). Moreover, we have previously demonstrated that intense synaptic activity in the perforant path of this slice system is capable of modifying the extracellular ion environment sufficiently to create the conditions that support such nonsynaptic bursts in the dentate granule cell layer (Schweitzer and Williamson 1995).

Effects of [H+]o on seizure-like phenomena have sometimes been ascribed to modulation of N-methyl-D-aspartate (NMDA) channel activity, although this has not been directly demonstrated (Gottfried and Chesler 1994; Velisek et al. 1994; Yoneda et al. 1994). Excitatory synaptic transmission via the NMDA ligand-gated Ca2+ channel is sensitive to [H+]o (Taira et al. 1993; Tang et al. 1990), but to what extent NMDA-dependent synaptic transmission is involved in synchronization of the ictal seizure event is not known. Other pH-sensitive mechanisms might also participate in seizure synchronization. Among such mechanisms is electrotonic coupling via gap junctions (Connors et al. 1984; Deitmer and Rose 1996; Dremietzel and Spray 1993; Giaume and McCarthy 1996; Lee et al. 1995; Pappas et al. 1996; Venance et al. 1998), the role of which in burst synchronization has been examined using qualitative measures to alter intracellular pH in CA1 (Perez-Velazquez et al. 1994).

In this report we show, in quantitative fashion, the pH sensitivity of an in vitro model of maximal dentate activation. The results suggest that the pH sensitivity of these events is independent of synaptic transmission. These findings may provide clues to how pH alters seizure activity in vivo and may
provide information about the mechanisms of intercellular communication involved in these nonsynaptic seizures.

METHODS

Preparation of rat hippocampal slices

Rat hippocampal slices were prepared in standard fashion as previously described (Schweitzer et al. 1992). Briefly, adult male Sprague-Dawley rats (weights 100–350 g) were anesthetized with 150 mg/kg phenobarbital sodium and decapitated. The brain was rapidly removed and placed in artificial cerebrospinal fluid (ACSF, see following text) at 0–4°C. A block containing the hippocampus was cut and mounted on a vibratome slicer (Campden Instruments, Sileby, UK) where 500-μm slices were made. The hippocampus was removed from each slice by trimming with a sliver of razor blade. Slices were placed in an interface chamber at 34°C with a 95% O2-5% CO2 atmosphere in ACSF containing (in mM) 124 NaCl, 1.4 NaHPO4, 26 NaHCO3, 3.0 KCl, 1.3 MgSO4, 1.3 CaCl2, and 11 glucose. Chamber flow rate was 1.5–2.0 ml/min.

pH of test solutions was set by altering the concentration of NaHCO3, and adjustments in NaCl concentration were made to maintain constant Na+ concentration of the ACSF. For pH 7.3, NaHCO3 was 26 mM and NaCl 124 mM; for pH 7.0, NaHCO3 was 13 mM and NaCl 137 mM; and for pH 7.6, NaHCO3 was 39 mM and NaCl 111 mM. Osmolality of the resulting solutions was tested using an osmometer (Wescor) and remained unchanged with these pH adjustments. Test solutions were substituted for the control during continuous recording from the slices. Recordings were made 1–2 h after application of the test solutions. Maintenance of the adjusted pH level was confirmed by monitoring the pH of the effluent from the chamber.

Oleamide (Sigma) was nearly insoluble in water and was added to ACSF containing 10% fetal calf serum, as in the original description of its assay. Appropriate controls using fetal calf serum alone were performed for this set of experiments.

Recording and stimulation

Stimulating electrodes were made from insulated platinum-iridium wire twisted into a pair (75 μm). A Winston Electronics SC-100 isolation box was used; stimulus intensities ranged from 100 to 300 μA and duration of the stimulus pulse was typically 0.3 ms. Extracellular recording was performed with glass pipettes filled with 1 M NaCl and broken to a tip resistance of 5–10 MΩ. Intracellular recording was performed with sharp electrodes (resistance 50–80 MΩ) filled with 4 M potassium acetate. Unit and multunit recordings were made with double-barreled ion-sensitive electrodes. One barrel was silanized with 15% tri-N-butylchlorosilane (Alfrebro; Monroe, OH) and mounted on a vibratome slicer (Campden Instruments, Sileby, UK) where 500-μm slices were made. The hippocampus was removed from each slice by trimming with a sliver of razor blade. Slices were placed in an interface chamber at 34°C with a 95% O2-5% CO2 atmosphere in ACSF containing (in mM) 124 NaCl, 1.4 NaHPO4, 26 NaHCO3, 3.0 KCl, 1.3 MgSO4, 1.3 CaCl2, and 11 glucose. Chamber flow rate was 1.5–2.0 ml/min.

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RESULTS

Prolonged field bursts are sensitive to pHo

Extracellular pH was altered by changing the concentration of NaHCO3 added to the ACSF and adjusting the sodium appropriately. Increasing the pH from 7.3 to 7.6 increased the amplitude of the population spikes and the DC potential shift, while decreasing the pH from 7.3 to 7.0 decreased the population spike amplitude and DC shift (n = 18, Fig. 1, Table 1). In most instances, field bursts were nearly or totally suppressed at nominal pH 7.0 compared with pH 7.3. Changing slice perfusion bath using a similar protocol without altering the pH (“sham” procedure) had no significant effect on burst amplitude. Bursts evoked by antidromic stimulation at the hilus were similarly suppressed (not shown). The suppression of bursts by low pH was completely reversible after return to nominal pH 7.3 (Fig. 1A).

Measurement of pH near the center of the slices during perfusion with different test solutions revealed a small acid

# FIG. 1. pHo effects on prolonged field bursts in the dentate granule cell layer. A: field bursts appeared in artificial cerebrospinal fluid (ACSF) containing 0 mM [Ca2+]o and 9 mM [K+]o (pH 7.3). pH of the perfusing solution was then lowered to 7.0 with suppression of the field bursts. After 1 h at pH 7.0, the solution was returned to pH 7.3. Field bursts reappeared, often with a larger amplitude than previously. B: a different slice was first exposed to ACSF containing 0 mM [Ca2+]o and 9 mM [K+]o at pH 7.3 until field bursts appeared; the perfusing solution pH was then switched to pH 7.0 (top), back to 7.3 (middle), and finally to 7.6 (bottom).
Burst pH sensitivity is not a synaptic effect

Zero-added-Ca\(^{2+}\) ACSF eliminated all detectable evoked synaptic activity recorded both extracellularly and intracellularly in the dentate granule cell layer (Fig. 2). In normal ACSF, addition of 30 μM each of 6,7-dinitroquinoxaline-2,3-dione (DNQX), d,l-2-amino-5-phosphonopentanoate (AP-5), and bicuculline methiodide (BMI) also completely blocked evoked and spontaneous activity (Fig. 2). We did not observe spontaneous synaptic potentials in the dentate granule cell layer in our slices, but these did occur in CA1. This spontaneous activity was also eliminated either by deletion of calcium or by addition of the blockers.

In zero-added Ca\(^{2+}\), 9 mM K\(^+\) ACSF, spontaneous prolonged field bursts continued to occur after NMDA, non-NMDA, and GABA\(_A\) blockade at the doses that eliminated evoked and spontaneous activity (30 μM). Bursts in these conditions showed no difference in pH sensitivity compared with bursts occurring without the agonists (n = 5, Fig. 3) and the effects were similarly reversible. Therefore ligand-dependent NMDA, AMPA, and GABA\(_A\) receptor activation did not appear to be necessary for the appearance of the prolonged field bursts and their pH sensitivity did not depend on synaptic transmission via these transmitter systems.

Other synaptic properties not affected by the pharmacologic blockade might be sensitive to pH, including other putative transmitters or other receptor types. However, pH\(_o\) adjustment from 7.3 to 7.0 did not suppress evoked or spontaneous postsynaptic potentials recorded in normal [Ca\(^{2+}\)]\(_o\) and [K\(^+\)]\(_o\) ACSF either extracellularly or intracellularly (Fig. 4, n = 8). Thus even if a small amount of synaptic transmission were still occurring during the field bursts, it would be unlikely to account for the large changes in burst amplitude that occurred in this pH range. Granule cell resting membrane potential and action potential amplitude or frequency are known to be altered in the high [K\(^+\)]\(_o\), low [Ca\(^{2+}\)]\(_o\) environment (Pan and Stringer 1996), but the resting membrane potentials in granule cells of the dentate gyros and pyramidal cells in CA1 were not significantly affected by pH\(_o\) in the range 7.0–7.3. In granule cells of the dentate gyros, the resting membrane potential was 76.4 ± 1.5 (SD) mV in nominal pH 7.3 and 75.9 ± 2.5 mV in nominal pH 7.0 (n = 8). In pyramidal cells of CA1, the resting membrane potential was 66.7 ± 1.5 mM in nominal pH 7.3 and 67.5 ± 1.5 mM in pH 7.0 (n = 12).

Role of gap junctions in the effect of pH on field bursts

Since NMDA channel blockade by hydrogen ions does not appear to explain the pH sensitivity of the prolonged field bursts, the possible role of gap junctions, the patency of which is modulated by pH\(_o\), was tested. To determine if dentate granule cell field bursts are dependent on gap junction patency, slices in low [Ca\(^{2+}\)]\(_o\) plus receptor antagonists. A: field recordings of dentate granule cell layer response to single perforant path stimulation. B: intracellular recording from dentate granule cells during perforant path stimulation. In normal ACSF, an excitatory postsynaptic potential (EPSP) gives rise to an action potential; no EPSP is produced in the zero-Ca\(^{2+}\) ACSF (n = 5) or in the presence of the synaptic transmission blockers (n = 6). * stimulation artifact. C: spontaneous activity in CA1. Small spontaneous synaptic potentials were recorded in normal ACSF (top) but were absent in Ca\(^{2+}\)-free ACSF (n = 5) and in the presence of blockers (n = 6).
The effects of pH on epileptiform phenomena have sometimes been attributed to modulation of NMDA channel activity. Since NMDA-mediated and other fast amino acid-dependent synaptic transmission are not active in this model of epileptiform activity, modulation of NMDA channel activity by pH cannot explain the pH sensitivity of the field bursts. It could be argued that an undetected but still significant amount of transmitter release is occurring, perhaps by a calcium-independent mechanism, and that NMDA receptors might still be involved in synchronizing the field bursts. However, addition of NMDA, non-NMDA and GABA_A antagonists, which did block synaptic transmission, did not alter the pH sensitivity of the field bursts. Altogether, this suggests that the pH sensitivity of epileptiform events in vivo may be due in large part to effects other than NMDA receptor antagonism by hydrogen ions. Hydrogen ion concentration changes per se may also not be the sole agent of the effects shown here. In a CO_2-bicarbonate buffer system, pH alterations are accompanied by significant alterations in bicarbonate concentration such as those used to prepare the experimental solutions used here. Bicarbonate ion itself may have significant effects on both the passive and the active properties of hippocampal neurons (Grover et al. 1993; Perkins and Wong 1996) although the observation that the pH-dependent suppression of bursts occurred in the presence of GABA_A blockade makes it unlikely that this particular ligand-gated ion channel is involved in the effect. Because we could not test the effects of pH on synaptic potentials in zero-Ca_2+ conditions (where none were observed), it is possible that pH somehow alters the efficacy of the receptor antagonists or that sensitivity of synaptic potentials to pH is altered in low Ca_2+. However, both these possibilities would have to be true simultaneously to negate our conclusion that low pH burst suppression is a nonsynaptic phenomenon. We believe this scenario to be highly unlikely.

pH affects many physiological processes that could be involved in nonsynaptic synchronization, potentially including cell volume regulation (Hansson and Ronnback 1992; Plesnila et al. 1998), nonligand-dependent ion channel pa-
The second possibility, a pH effect on ion channel patency, is a unlikely explanation, though we have not ruled out the possibility that pHo alters water transport across the cell membrane, affecting the size of the extracellular space. The second possibility, a pH effect on ion channel patency, awaits testing by examining pH effects on specific ion channels. Our results favor the third hypothesis, that the pH changes affect the efficacy of intercellular communication via nonsynaptic signaling mechanisms such as calcium waves or gap junctions. Octanol has been used to block gap junctions in many systems (Bernardini et al. 1984; Charles et al. 1996; Fujita et al. 1998; Pappas et al. 1996; Venance et al. 1998) and is efficacious here, but its actions may be relatively nonspecific and in fact it may act at least partially via intracellular acidification also (Pappas et al. 1996). Oleamide is a recently described endogenous gap junction modulator, derived from the cerebrospinal fluid of sleep deprived cats (Boger et al. 1998). This substance blocks gap junctions in glial systems at low concentrations but does not affect glial calcium waves (Guan et al. 1997). Oleamide and carbenoxolone (a more traditional gap junction blocker) were effective in blocking the field bursts in our system. The continued appearance of unit activity, including burst behavior, during field blockade with low pH or gap junction blockers demonstrates that burst activity at the cellular and field levels are separable processes and suggests both of these manipulations act on burst synchronization.

pH measurements in the slice demonstrated a small pH gradient from the outside to the center, confirming the work of other authors (Walz 1989). This gradient may affect the pattern of burst propagation in the slice and similar gradients could affect seizure propagation properties in vivo. The pH measurements also demonstrated a small acid shift in the slice as a whole compared with the perfusing medium. We have not addressed directly the issue of whether pHo or pHi is the critical determining factor in affecting burst amplitude or which cellular element (neuronal, glial, or both) is involved. However, the relationship between pHo and pHi in similar systems at normal [Ca2+]o has been established and shows that relatively large changes in pHi are associated with relatively small ones in pHo (Mellergard et al. 1994a, b; Pappas et al. 1996; Siesjo et al. 1985). The pH ranges involved are within biologically meaningful limits. Recent work (Perez-Velazquez et al. 1994; Xiong et al. 2000) has suggested that pHi is the significant parameter. Since gap junctions are modulated by pHi, this would be consistent with the proposed role of gap junctions in burst synchronization.

The data in this study suggest that seizure propagation and synchronization “co-opt” existing pathways of intercellular communication under specific ionic and pH conditions. Such a hypothesis is attractive because it does not require novel machinery at the cellular or tissue levels to support seizure synchronization. This is consistent with the observations that seizures can occur not only in the context of epilepsy but also in normal cortex under the appropriate conditions as well as in acutely injured cortex. It fits well with other recent data on the role of pH, gap junctions, and epilepsy (de Curtis et al. 1998; Dermietzel and Spray 1993; Elisevich et al. 1997; Laxer et al. 1992; Lee et al. 1995; Valiante et al. 1995). The alteration of seizure-like activity by pH suggests a novel set of physiological changes that could explain lowered or raised seizure susceptibility in a number of systems independent of NMDA or other synaptic transmission. Changes in Na+- H+ transporters and electrogenic Na+-HCO3− pumps (Deitmer and Rose 1996; O’Connor et al. 1994; Pizzonia et al. 1996; Shrode and Putnam 1994), or altered composition, numbers, or function of gap junction channels could be involved (Elisevich et al. 1997; Giaume and McCarthy 1996). We hope that this train of investigation may stimulate interest in new targets for therapeutic intervention.
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