Extracellular pH Responses in CA1 and the Dentate Gyrus During Electrical Stimulation, Seizure Discharges, and Spreading Depression

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Xiong, Zhi-Qi and Janet L. Stringer. Extracellular pH responses in CA1 and the dentate gyrus during electrical stimulation, seizure discharges, and spreading depression. J Neurophysiol 83: 3519–3524, 2000. Since neuronal excitability is sensitive to changes in extracellular pH and there is regional diversity in the changes in extracellular pH during neuronal activity, we examined the activity-dependent extracellular pH changes in the CA1 region and the dentate gyrus. In vivo, in the CA1 region, recurrent epileptiform activity induced by stimulus trains, bicuculline, and kainic acid resulted in biphasic pH shifts, consisting of an initial extracellular alkalinization followed by a slower acidification. In vitro, stimulus trains also evoked biphasic pH shifts in the CA1 region. However, in CA1, seizure activity in vitro induced in the absence of synaptic transmission, by perfusing with 0 Ca²⁺/5 mM K⁺ medium, was only associated with extracellular acidification. In the dentate gyrus in vivo, seizure activity induced by stimulation to the angular bundle or by injection of either bicuculline or kainic acid was only associated with extracellular acidification. In vitro, stimulus trains evoked only acidification. In the dentate gyrus in vitro, recurrent epileptiform activity induced in the absence of synaptic transmission by perfusion with 0 Ca²⁺ /8 mM K⁺ medium was associated with extracellular acidification. To test whether glial cell depolarization plays a role in the regulation of the extracellular pH, slices were perfused with 1 mM barium. Barium increased the amplitude of the initial alkalinization in CA1 and caused the appearance of alkalinization in the dentate gyrus. In both CA1 and the dentate gyrus in vitro, spreading depression was associated with biphasic pH shifts. These results demonstrate that activity-dependent extracellular pH shifts differ between CA1 and dentate gyrus both in vivo and in vitro. The differences in pH fluctuations with neuronal activity might be a marker for the basis of the regional differences in seizure susceptibility between CA1 and the dentate gyrus.

INTRODUCTION

Seizure activity is accompanied by rapid changes in the ionic composition of the extracellular space, including rises in extracellular potassium concentration and changes in the extracellular pH. In contrast to the consistent increases in extracellular potassium during seizure activity, activity-dependent pH changes are notable for their regional diversity (Chesler and Kaila 1992; Deitmer and Rose 1996). In some structures, such as adult spinal cord (Sykova and Svoboda 1990) and optic nerve (Davis et al. 1987), electrical stimulation induces a predominant extracellular acidification. In other regions, neuronal activity is accompanied by an initial extracellular alkalinization, followed by a slower acidification. Such early alkaline shifts have been reported in the cerebellum (Chesler and Chan 1988; Kraig et al. 1983), cortex (Urbanics et al. 1978), and CA1 and CA3 regions of hippocampus (Jarolim et al. 1989; Walz 1989). The functional significance and mechanisms underlying these regional dependant pH shifts are not well understood.

There is evidence that neuronal excitability can be influenced by extracellular pH. The conductances of a variety of ion channels are altered by shifts in pH (Baukrowitz et al. 1999; Kiss and Korn 1999; Tombaugh and Somjen 1996), and these changes in conductance may modulate neuronal excitability. In addition, both GABA_A receptor current and N-methyl-D-aspartate (NMDA) receptor current are sensitive to extracellular pH. Extracellular alkalosis decreases the conductance through GABA_A receptor channels (Pasternack et al. 1992) and increases the NMDA receptor-mediated current (Tang et al. 1990; Traynelis and Cull-Candy 1990). Therefore activity-dependent extracellular pH shifts may be sufficient to affect neuronal excitability and the regional differences in activity-dependent pH changes may underlie regional differences in seizure susceptibility. In view of the pH sensitivity of neuronal excitability and regional diversity in the activity-dependent pH changes, we compared the extracellular pH response during seizure activity in the CA1 region and the dentate gyrus in vivo and in vitro.

METHODS

In vivo recording

Adult Sprague-Dawley rats (150–280 g, both sexes) were anesthetized with urethane (1.2–1.5 g/kg ip) and placed in a stereotaxic frame. The skull was exposed and burr holes drilled for electrode placement. A concentric bipolar electrode was placed in the CA3 region of the left hippocampus at an angle of 5° (AP, –3, ML, 3.5, depth 2.5–3.0 mm). Another stimulating electrode, fashioned from 0.01-in diam Teflon-coated stainless steel wire, was placed in the right angular bundle (AP, –8, ML, 4.4, depth 2.5–3.5 mm). A double-barrel hydrogen-sensitive microelectrode 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.

The hydrogen-sensitive microelectrodes were made according to established techniques (de Curtis et al. 1998). One barrel of the double-barreled electrode was silanized with 15% tri-N-butylchlorosilane (Alfrebro, Monroe, OH) in chloroform, and the tip was filled with the hydrogen ionophore II (Fluka Cocktail A). The electrode was then backfilled with a buffer solution [(in mM): 100 NaCl, 10 HEPES, and 10 NaOH, pH 7.5]. The reference barrel was filled with 2 M NaCl. The electrode was calibrated before each experiment in a series of standard solutions in artificial cerebrospinal fluid (ACSF; pH 6.0–8.0). The calibration solutions were similar to the ACSF, with...
NaHCO₃ substituted for the corresponding moles of NaCl. The pH-
sensitive microelectrodes had a response of 55–60 mV for a unit pH
change. The extracellular field activity and pH signal were amplified
and displayed on a chart recorder.

Administration of 20-Hz (300–600 μA, 0.3-ms biphasic pulses)
stimulus trains every 15 min to the CA3 region or the angular bundle
were used to initiate seizure activity in CA1 or the dentate gyrus,
respectively. At least five seizures were elicited in each recording
location, and the peak alkalinization and peak acidification were
measured for each stimulus train. These measurements were averaged
to produce a mean alkaline or acid peak change for that animal and
that stimulus/recording pair. Chemical convulsants, bicuculline and
kainic acid, were used to initiate spontaneous epileptiform activity.
Kainic acid (Sigma, St. Louis, MO) was dissolved in normal saline
(0.9%) at 6 mg/ml and administrated intraperitoneally at a single dose
of 12 mg/kg. (+)Bicuculline (Sigma) was dissolved in 1 N HCl at 5
g/ml and was diluted to 0.5 mg/ml with normal saline just prior to
injection. Bicuculline was administrated intravenously at a dose of
0.3–0.5 mg/kg, and the animal was monitored for 10–20 min. Re-
peated doses (up to 5) of bicuculline were administered to each animal
at 1-h intervals. Each animal received either bicuculline or kainic acid,
not both. For each animal receiving a chemical convulsant, recordings
were obtained from both CA1 and the dentate gyrus.

In vitro recording

Hippocampal slices were prepared by conventional methods from
Sprague-Dawley rats (100–200 g, both sexes). After anesthetizing the
rats (ketamine 25 mg/kg, xylazine 5 mg/kg, acepromazine 0.8 mg/kg
ip), the brains were removed. Transverse slices (400–500 μm)
through the hippocampus were cut with a Vibratome (Technical
Products International). Slices were placed in an interface-type cham-
er and continuously perfused with ACSF at 32°C under a stream of
humidified 95% O₂-5% CO₂. Composition of the ACSF was (in mM)
127 NaCl, 2 KCl, 1.5 MgCl, 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 h before electrophys-
iological recording was begun. A hydrogen-sensitive microelectrode
was placed in the cell body layer of CA1 or the dentate gyrus. A
bipolar tungsten stimulating electrode was positioned in the Schaffer
collaterals to stimulate CA1 or the perforant path to stimulate the
dentate gyrus. Stimulus trains (600–800 μA, 0.3-ms biphasic pulses
at 15 Hz) were used to initiate synchronized neuronal activity in both
CA1 and the dentate gyrus.

Nonsynaptic epileptiform activity was induced in the hippocampus
by changing to ACSF containing 0-added calcium and high potas-
sium. The potassium was raised to 5 mM to induce epileptiform
activity in the CA1 region and was raised to 8 mM to induce epilep-
tiform activity in the dentate gyrus. In both regions, this nonsynaptic
epileptiform activity can take more than 1 h to appear, but once it
appears, the interval between field bursts and the burst duration
remains stable for many hours (Bikson et al. 1999; Pan and Stringer
1996).

RESULTS

pH changes in CA1 and dentate gyrus in vivo during
synchronized neuronal activity

In urethan-anesthetized rats, 20-Hz stimulation of the CA3
region or the angular bundle caused reproducible changes in
the extracellular field potential and extracellular pH in CA1
and dentate gyrus (Fig. 1, n = 12 animals). In the CA1
pyramidal cell layer, stimulation was associated with a biphasic
pH response. At the start of the stimulus train, the pH of the
extracellular space became alkaline (0.05–0.1 pH units). This
was followed by an acidification (0.1–0.25 pH units). Above
and below the pyramidal cell layer, the pH shifts were similar
in shape to those recorded in the cell body layer. The magnitude
of the pH changes was maximal in the pyramidal cell layer
and proximal dendrites and then gradually declined as the
recording electrode was moved into stratum oriens or distal s.
radiatum and s. lacunosum-moleculare. Throughout the CA1
region, the acidification peaked after termination of the stim-
ulus train and after termination of the neuronal activity (Fig. 1
and see Fig. 2 A). In the dentate gyrus, stimulus trains to the
angular bundle induced only acidification (Fig. 1, n = 12
animals) in the granule cell layer. The magnitude of the pH
shift ranged from 0.2 to 0.3 pH units and was fairly constant
from s. moleculare through the hilus. In contrast to CA1, in
the dentate gyrus, the acidification peaked with the termination
of the neuronal discharge (Fig. 2 A).

To rule out the possibility that the difference in pH changes
between CA1 and the dentate gyrus depended on the electrical
stimulation, the pH changes during spontaneous seizures

FIG. 1. Laminar profile of extracellular pH shifts in the hippocampus in
vivo. Stimulus trains were administered to the contralateral CA3 while
advancing a dual barrel (pH-sensitive and DC potential) electrode through the
hippocampus. Shown are the extracellular pH changes at different laminae of
hippocampus, indicated to the left. The vertical dashed lines mark the onset and
termination of the 20-Hz, 30-s stimulus trains. Horizontal dashed lines mark
the extracellular pH baseline. Alkalinization is a downward change of the pH
tracing, while acidification is an upward change.
evoked by chemical convulsants were recorded. The pH responses during spontaneous seizure activity induced by bicuculline (0.3 mg/kg, \(n = 6\) animals) or kainic acid (12 mg/kg, \(n = 3\) animals) were similar to those evoked by stimulus trains. In CA1, the pH responses showed a biphasic pattern. At the onset of synchronized neuronal activity, marked by the negative shift of the extracellular DC potential, there was an initial alkalinization similar to that recorded during stimulus trains. This alkalinization was followed by an acidification. The amplitudes of the acidification and alkalinization were more variable than those seen during stimulation. In the dentate gyrus, only acidification was recorded (Fig. 2, B and C). The acidification was only observed when there was synchronous discharge of the granule cells associated with a negative shift of the DC potential.

**pH responses in vitro**

To further compare the pH responses in CA1 and the dentate gyrus, we measured the extracellular pH changes in slices (Fig. 3). The pH of the extracellular space in the slices was more acidic than the perfusing solution (7.15–7.18 in the center of the slice compared with 7.35–7.40 in the perfusing solution) and there was a pH gradient across the thickness of the slice. This gradient was first reported in 1987 (Schiff and Somjen 1987) and is now thought to be due to a gradient in pCO\(_2\) (Chesler et al. 1994; Voipio and Kaila 1993). All the recordings for the present study were obtained at the center of the slice (a depth of \(\sim 150\) \(\mu\)m). Stimulation of Schaffer collateral fibers (15 Hz, 30 s) evoked biphasic pH shifts in the CA1 region similar to those recorded in vivo during stimulation \((n = 6\) slices). Stimulation of synaptic inputs evoked only acid shifts in the dentate gyrus \((n = 6\) slices). In both CA1 and the dentate gyrus, the extracellular space continued to acidify after the termination of the stimulus trains. But in the dentate gyrus this poststimulus acidification was smaller than in CA1. The similarity of the in vitro pH changes to those observed in vivo suggests that differences in blood flow between CA1 and the dentate gyrus are not an important contributor to the different activity-dependent pH patterns measured in vivo (Chesler and Kaila 1992). However, the rate of recovery of the extracellular pH in vitro after the stimulus trains was significantly slower in both CA1 and the dentate gyrus. For both CA1 and the dentate gyrus, the mean half-time of recovery in vitro was 31.4 ± 1.7 (SE) s \((n = 6\) for both CA1 and dentate gyrus), while in vivo the mean half-time of recovery was 9.7 ± 0.6 s \((n = 6\) for both CA1 and dentate gyrus).

To test whether activity-dependent depolarization of glial cells may contribute to the extracellular pH changes in the hippocampus, extracellular pH was measured during stimulus trains in the presence of 1 mM barium (Fig. 3, \(n = 6\) slices, no
more than 1 slice from one animal). This concentration of barium has been shown to block glial depolarization (Chesler and Kraig 1989). In the presence of barium, the extracellular alkaline shifts were increased in CA1 from 0.04 to 0.11 pH units before to 0.11 to 0.02 pH units after perfusion with barium. In the dentate gyrus, barium caused the appearance of an initial alkalinization (up to 0.09 pH units, range between 0.06 and 0.13 pH units), so that the pH changes in the dentate gyrus now resembled the pattern of changes normally recorded in CA1. The effects of barium were reversible.

To determine whether the extracellular pH changes during synchronized neuronal activity are due to synaptic transmission, we recorded the pH shifts during seizure activity induced in nonsynaptic conditions (0-added calcium). Field bursts in CA1, induced by perfusion with 0-added Ca$^{2+}$ and 5 mM K$^+$ ACSF, were associated with acidification (Fig. 4A, n = 6 slices). At the onset of the extracellular DC shift, indicating the onset of the synchronized neuronal burst, there was no alkalinization. After termination of the field burst, the extracellular pH began to recover toward baseline, but the onset of the next field burst caused acidification again. Field bursts in the dentate gyrus, induced by perfusion with 0-added Ca$^{2+}$ and 8 mM K$^+$ ACSF, were associated only with acidification of the extracellular space (Fig. 4B, n = 10 slices). As in CA1, the onset of the extracellular DC shift was associated with acidification. During the field burst, the extracellular pH appears to reach a plateau value and then it recovers after termination of the field burst.

In some slices, spreading depression appeared spontaneously during perfusion with the 0-added calcium solutions (Fig. 5). In both CA1 and the dentate gyrus, the pH increased at the onset of the spreading depression. This initial alkalinization was followed by a more sustained acidification that recovered with the extracellular DC potential. To rule out the possibility that residual calcium in the bath or in the extracellular space within the slice underlies the initial alkalinization, EGTA (1 mM, n = 3 slices) or the calcium channel blocker cadmium chloride (0.2 mM, n = 3) was added to the 0-calcium ACSF. Neither EGTA nor cadmium chloride blocked the alkalinization during spreading depression (data not shown).

**DISCUSSION**

This study demonstrated that activity-dependent extracellular pH shifts differ between CA1 and the dentate gyrus. In the CA1 region, synchronized neuronal activity, induced by stimulus trains or chemical convulsants, was associated with bi-phasisic pH shifts consisting of an initial alkalinization followed by a slower acidification. In the dentate gyrus, synchronized neuronal activity was associated only with acidifi-
Depression episode in the dentate gyrus perfused with 0 Ca²⁺ was present, even in the ab-

Alkalinization of the extracellular space can occur in the dentate gyrus in vivo, at least during spreading depression through another mechanism. Activity-dependent extracellular alkaline shifts are present in both CA1 and the dentate gyrus. The later acid shift might decrease the neuronal excitability and neuronal activity may have important physiological significance. It has been shown that extracellular pH affects synaptic transmission and neuronal excitability. Since extracellular alkalosis decreases the conductance of GABA_A receptor channels (Pasternack et al. 1992) and increases the NMDA receptor current (Tang et al. 1990; Traynelis and Cull-Candy 1990), the early extracellular alkaline shift might increase (or sustain) excitability in the CA1 region prolonging the neuronal activity. The later acid shift might decrease the neuronal excitability and may be involved in seizure termination in both CA1 and the dentate gyrus (Xiong et al. 2000). Therefore the different extracellular pH patterns in CA1 and dentate gyrus may contribute to the difference in their propensity for epileptic activity.

Regional differences in glial function may explain the regional difference in pH regulation during neuronal activity. During neuronal activity, potassium released from active neurons will depolarize glial cells, resulting in the net secretion of acid from the glia (Chesler and Kaila 1992). In the dentate gyrus, this acid released from the glia may neutralize the initial extracellular alkaline shift. Blockade of the glial depolarization by barium (Ballanyi et al. 1987) would thus block the resulting secretion of acid (Chesler and Kraig 1989; Grichtchenko and Chesler 1994). This may “unmask” an alkalinization in the dentate gyrus, as has been shown in the dorsal horn of the spinal cord (Sykova et al. 1992). For this hypothesis to be true, one must propose regional differences in glial function between CA1 and the dentate gyrus. The density of the inward rectifying potassium channels, which account for the activity-dependent glial depolarization, has been shown to be lower in CA1 than in CA3 (D’Ambrosio et al. 1998; McKhann et al. 1997), but glia in the dentate gyrus have not been studied. Alternatively, recent work has suggested that the effect of barium may not be entirely due to the blockade of glial acid secretion. Barium may substitute for calcium and move through calcium channels and in this way also contribute to the alkaline shifts (Smith and Chesler 1999).

The regional difference in the activity-dependent pH patterns between CA1 and the dentate gyrus during synchronized neuronal activity may have important physiological significance. It has been shown that extracellular pH affects synaptic transmission and neuronal excitability. Since extracellular alkalosis decreases the conductance of GABA_A receptor channels (Pasternack et al. 1992) and increases the NMDA receptor current (Tang et al. 1990; Traynelis and Cull-Candy 1990), the early extracellular alkaline shift might increase (or sustain) excitability in the CA1 region prolonging the neuronal activity. The later acid shift might decrease the neuronal excitability and may be involved in seizure termination in both CA1 and the dentate gyrus (Xiong et al. 2000). Therefore the different extracellular pH patterns in CA1 and dentate gyrus may contribute to the difference in their propensity for epileptic activity.

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