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Lowering of extracellular pH suppresses low-Mg^{2+}-induces seizures in combined entorhinal cortex-hippocampal slices

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Abstract Lowering $[Mg^{2+}]_o$ induces epileptiform bursting in hippocampus and entorhinal cortex (EC), presumably by activation of *N*-methyl-D-aspartate (NMDA) receptors. Since increasing $[H^+]_o$ has been shown to reduce NMDA receptor activation, we hypothesized that this could contribute to anticonvulsant actions of acidic pH. To test this, we studied the effects of raising extracellular PCO_2 (20.6%, pH = 6.7) or lowering extracellular pH (6.7 or 6.2) on low-Mg^{2+}-induced epileptiform discharges. Lowering the pH to 6.7 by either means increased the interval between seizure-like events (SLEs), decreased the maximal amplitude of SLEs, and, if the site of seizure generation was at a distance from the recording site, acidification slowed the rate of seizure propagation. In contrast, the duration of SLEs was unaffected by acidic pH or high PCO_2. Raising PCO_2 or lowering pH to 6.7 also blocked early (8–10 min) but not late (> 20 min) phases of status-like discharges. All effects of the extracellular pH changes were fully reversible. Further lowering of extracellular pH to 6.2 completely and reversibly blocked both SLEs and status-like discharges. Our data show that the effects of high PCO_2 and low pH on seizures in the EC in vitro may be dose-dependent and consistent with induction by proton blockade of NMDA receptors. Thus, blockade of NMDA currents by protons may be an important component of the anticonvulsant action of extracellular acidosis. The results also suggest that acidosis may be a desirable property for new antiepileptic treatments.

Key words Acidosis · Seizures · Brain slices · Rat

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Introduction

Lowering of extracellular magnesium concentration ($[Mg^{2+}]_o$) induces epileptiform activity in a number of cortical structures (Anderson et al. 1986; Mody et al. 1987; Stanton et al. 1987; Thomson et al. 1985; Walther et al. 1986; Wong and Prince 1990), including the hippocampus and entorhinal cortex (EC; Dreier and Heinemann 1991; Jones 1989; Jones and Heinemann 1988; Stanton et al. 1987; Walther et al. 1986). In the hippocampus, short recurrent discharges (40–150 ms) dominate the low-$[Mg^{2+}]_o$-induced epileptiform activity (Herron et al. 1985; Köhr and Heinemann 1987; Mody et al. 1987; Stanton et al. 1987). However in the EC, low $[Mg^{2+}]$ induces two types of epileptiform activity with characteristics similar to those of seizures observed in intact animals. The *early* prolonged seizure-like events (SLEs; Fig. 1A) are characterized by up to 100-s long, sustained, negative d.c. potential shifts, with superimposed tonic and clonic-like discharges. Previous studies have shown that the SLEs either start in the subiculum, the EC, or the neighboring temporal cortex (Dreier and Heinemann 1991). From each of these sites, other regions are recruited with some delay. With prolonged exposure to low $[Mg^{2+}]_o$ (typically 60–90 min after the onset of early SLEs), the epileptiform activity changes from cyclical long-duration negativities to continuous, recurrent *late* discharges (status-like events; Fig. 1C; Dreier and Heinemann 1991; Walther et al. 1986; Wong and Prince 1990). These discharges persist as long as the $[Mg^{2+}]_o$ remains low.

While the early SLEs can be blocked with clinically used anticonvulsants such as valproic acid, phenytoin, carbamazepine, and phenobarbital, status-like activity is relatively resistant to these drugs (Dreier and Heinemann 1990; Heinemann et al. 1991). It is well established that all these types of low-Mg^{2+} epileptiform activity involve a major contribution of *N*-methyl-D-aspartate (NMDA) receptor activation, since elevating $[Mg^{2+}]_o$, or applying NMDA antagonists such as 2-amino-5-phosphonopentanoic acid (AP5), ketamine, or dextrophan, blocks these discharges (Dreier and Heinemann 1990; Herron et al. 1985; Köhr and Heinemann 1987; Stanton et al. 1987). However, it is not known what factors render the late status-like events more resistant to drugs.

Changes in extracellular PCO_2 and pH can be associated with, and modify, neuronal activity in vivo (Balestrino and Somjen 1988; Withrow 1972; Woodbury and Karler 1960) and in vitro (Balestrino and Somjen 1988; Caspers and Speckmann 1972; Chen and Chesler 1992a, b, c; Somjen 1984). Extracellular acidification suppresses low-Mg^{2+}-induced seizures in the cingulate cortex (Aram and Lodge 1987). This effect may be due to blockade of the NMDA subtype of glutamate receptors (Grantyn and Lux 1988; Tang et al. 1990; Traynelis and Cull-Candy 1990).

This study determined the effects of two levels of acidosis in the amplitude, duration and rate of spread of early SLEs and late status-like events induced by reducing $[Mg^{2+}]_o$ in combined EC-hippocampal slices.

Materials and methods

Experiments were performed on 30 slices prepared from rat brain by standard methods (Stanton et al. 1987) using a vibratome (Camden Instruments). Male Sprague-Dawley rats (Taconic Farms; 55–65 days old) were decapitated under deep ether anesthesia. The brain was removed and washed in cold artificial cerebrospinal fluid (ACSF). Slices were cut in an approximately horizontal plane from the temporal lobe to include the hippocampal formation, the parahippocampal region, and EC (Dreier and Heinemann 1991). Slices were transferred to an interface-type chamber and continuously perfused with prewarmed (34–35° C), oxygenated (95% O_2/5% CO_2) ACSF containing (in mM): NaCl 126; KCl 5; NaH_2PO_4 1.25; $MgCl_2$ 2 or 0; $CaCl_2$ 2; $NaHCO_3$ 26; and glucose 10. The pH of the ACSF, corrected for temperature, was 7.3 ± 0.06 [mean $\pm$ SEM, $[H^+]_o$ approximately 50 nM (Church and McLennan 1989)]. We used two methods to change the pH of the ACSF. In the first method, a pH of 6.7 ± 0.06 was produced by bubbling ACSF with a gas mixture of 79.4% O_2/20.6% CO_2 ($PCO_2 = 156$ Torr). In the second method, this same pH was achieved by adding HCl (10–12 mM) dropwise to ACSF bubbled with 95% O_2/5% CO_2. Both the reservoir of ACSF and the chamber bath were bubbled with the gas mixtures, allowing for almost instantaneous changes in the composition of the chamber gas. The pH was continuously monitored with a pH meter (Beckmann pH model 34), until the desired pH was reached and maintained. A pH of 6.2 ± 0.06 was reached by titration with larger quantities of HCl (18–20 mM), during continuous gassing with 95% O_2/5% CO_2. All pH adjustments were done at the experimental temperature of 34° C. The osmolarity of all solutions ranged between 300 and 310 mosmd.

To ensure the viability of the slices, Schaffer collateral-evoked potentials were examined in area CA1 of the hippocampus prior to starting an experiment. These potentials were evoked with a teflon-insulated bipolar platinum stimulating electrode positioned into the Schaffer collateral/commissural axons of stratum radiatum in field CA1. Synaptically evoked field potentials and epileptic activity were recorded with glass micropipettes filled with 2M NaCl (resistance 1–5 MΩ). Only slices with a single population spike that was greater than 2 mV were included in the study. After verifying that the slices were healthy, we positioned the recording electrode into the deep layers of the medial EC and perfused with Mg^{2+}-free ACSF to induce spontaneous epileptiform activity.

We evaluated the effects of increased PCO_2 (or decreased pH) on the duration and peak amplitude (Fig. 1A) of slow, negative d.c. potentials associated with SLEs as well as the intervals between SLEs. Despite the use of only one electrode in the EC, we were able to obtain an assessment of the time required for seizure spread from a remote generator site to the EC, assuming the site of initiation was at a distance from the recording electrode and did not change during the experiment. To achieve this, we used the observation that in the EC, a small, early negative potential shift occurs synchronously with the onset of seizure activity at the remote generator. This small, negative d.c. shift precedes by several seconds the actual spread of the SLE to the EC, characterized by a large deflection in the d.c. potential and large extracellular ionic shifts (Dreier and Heinemann 1991). Therefore, a consistent change in this delay (prepotential duration) was presumed to be an effect on seizure propagation time. The effect of pH on status epilepticus-like activity was tested in its early phase (8–10 min after the spontaneous onset of status epilepticus-like activity; Fig. 1B) and in its late phase (≥ 20 min after onset; Fig. 1C).

Statistical evaluations were performed using an analysis of variance for repeated measures (baseline, treatment, and wash), with post-hoc Fisher protected least significant differences (PLSD) test. The level of significance was preset to 5% ($P < 0.05$).

Results

Effect of lowering extracellular pH to 6.7 on early paroxysmal SLEs in Mg^{2+}-free medium

The recording of typical *early* SLE and late status-like events in Fig. 1 was obtained from layer V of the medial EC, 43 min after the onset of perfusion with Mg^{2+}-free ACSF at normal pH (7.3; $[H^+]_o$ 50 nM). The observed discharge patterns are similar to previous observations of low-$[Mg^{2+}]_o$-induced epileptiform discharges (Stanton et al. 1987). The parameters we measured were peak amplitude, duration, and interval between SLEs (as indicated in Fig. 1A for a typical early SLE). In Fig. 1B, a transitional state is shown where the discharges during SLEs resemble clonic afterdischarges. Figure 1C is from the same slice 84 min after the onset of Mg^{2+} washout and is typical of late recurrent status epilepticus-like discharges.

Figure 2 illustrates a time delay between SLEs at two recording sites in the EC and subiculum. In this particular example, the event began in the subiculum, and only after a delay of approximately 7 s was the EC secondarily recruited. However, a small negative shift of the d.c. field potential (associated with a small rise in $[K^+]_o$, not shown) can already be noted in the EC during the time when the seizure event is restricted to the subiculum (Fig. 2, arrow). Thus, in slices where the generator was remote to the EC recording site, the time delay between the onset of the fast prepotential and the actual seizure

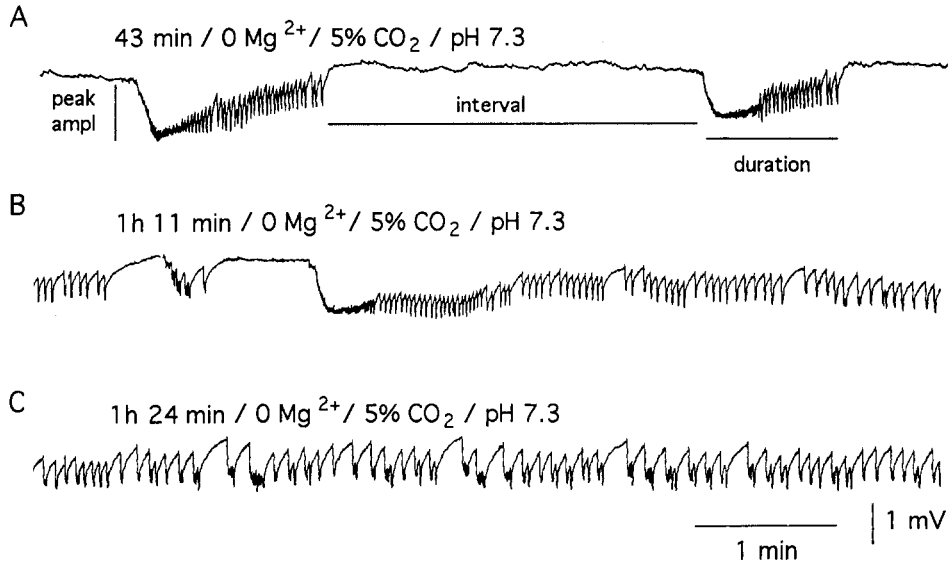

Fig. 1A-C Time course of the spontaneous development of epileptiform activity induced by Mg^{2+}-free artificial cerebrospinal fluid (ACSF) in rat entorhinal cortex slice. **A** Individual tonic-clonic seizure-like events (SLEs) and parameters measured: peak amplitude of SLE, interval between SLEs, and duration of SLE. **B** The transition phase between SLEs and status epilepticus-like activity arising from the period of clonic afterdischarges of a SLE. **C** Status-like activity. Time after onset of the last change in the low-magnesium perfusion, the percentage of CO_2 in the bubbling gasses (balance of oxygen), and actual pH of ACSF perfusate is shown above each trace

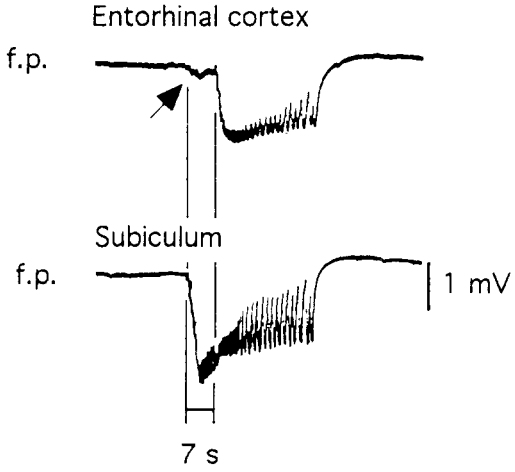

Fig. 2 Simultaneous recording of a low-Mg^{2+}-induced seizure-like event (SLE) in the subiculum and enta-hinal cortex (EC). It has been previously shown that in the combined slices the seizure spread is irregular. The epileptiform activity (SLE) was first recorded in the subiculum; after 7 s the SLE was recorded also in the EC. Simultaneously with recording of the seizure onset in the subiculum, a small, negative d.c. shift was observed in the EC (*arrow*). The change in the duration (defined as a delay from the onset of the small, negative d.c. shift to the onset of SLE) of the small d.c. shift induced by our treatments was used to measure the change in the rate of the spread of seizure activity, assuming that the site of the seizure origin did not change throughout the experiment. (*f. p.* fast prepotential)

onset (prepotential duration) can be used to assess the relative effect of $[H^+]_o$ on seizure spread.

The effect of lowering extracellular pH ($pH_o = 6.7$) by increasing extracellular PCO_2 on low-Mg^{2+} SLEs is illustrated in Fig. 3A. In three out of nine slices, SLEs in the EC were completely blocked by pH_o of 6.7. In the remaining six slices, $pH_o = 6.7$ caused a reversible and statistically significant increase in the duration of the interval between SLEs (Fig. 3A, Table 1). Similarly, elevating PCO_2 also significantly decreased the peak amplitude of SLEs (Table 1). In contrast, the duration of SLEs was not significantly altered by high extracellular PCO_2. As with H^+ application, all effects of high CO_2 were fully reversible after a change of extracellular PCO_2 back to 5%.

In six out of nine slices treated with Mg^{2+}-free ACSF at normal pH, small, negative d.c. prepotentials were observed in the EC preceding the onset of the SLE, indicating a powerful excitatory drive originating at a site remote to the recording electrode (Fig. 2). In slices where lowering pH_o to 6.7 did not completely block SLEs, we compared time delays between the small, negative d.c. shift and the SLE in normal $pH_o = 6.7$. Acidified pH caused a significant increase in the duration of these prepotentials (defined as a delay between the onset of the prepotential and the onset of SLE). Assuming that the site of seizure initiation did not change, this suggests that acidification slowed the rate of seizure spread (Table 1; prepotential duration, $PCO_2 = 20.6\%$).

To control for the possible actions of CO_2 not attributable to extracellular acidification per se, we also examined the effects of lowering pH_o by adding HCl directly. The effect of lowering pH of the ACSF directly (pH 6.7; $[H^+]_o$ 200 nM) on early SLEs is illustrated in Fig. 4A. In one out of six slices, acidic pH completely blocked SLEs, while, in the remaining five slices, the interval between SLEs was significantly increased and peak amplitudes were significantly decreased (Table 1, Fig. 4A; pH 6.7, PCO_2 5%). In five of six slices, there were no small negative prepotentials observed, suggesting that in these experiments the site of seizure initiation was relatively close to the recording site. In the one case where a prepotential was observed, lowering the pH increased its duration. As in the experiments with elevated

Table 1 Effects of high PCO_2 and low pH on seizure-like events (*SLE*) induced in Mg^{2+}-free medium (Mean ±SEM)

SLE	$PCO_2 = 20.6\%$, pH = 6.7					$PCO_2 = 5\%$, pH = 6.7				
	Baseline	CO_2 20.6%	Wash	*F* value	*P* value	Baseline	pH 6.7	Wash	*F* value	*P* value
Interval (min)	2.59 ±0.9	8.36 ±2.35*,***	1.85 ±0.62	$F_{(2,10)} = 7.81$	$P < 0.01$	5.2 ±1.48	8.58 ±1.28*,***	3.6 ±1.07	$F_{(2,8)} = 17.57$	$P < 0.01$
Amplitude peak (mV)	1.92 ±0.29	1.32 ±0.19*,***	1.68 ±0.24	$F_{(2,8)} = 11.59$	$P < 0.01$	1.4 ±0.07	0.9 ±0.04*,***	1.38 ±0.14	$F_{(2,8)} = 25.57$	$P < 0.001$
Prepotential duration (s)	3.6 ±1.2	16.2 ±4.2*,***	5.4 ±2.4	$F_{(2,10)} = 7.41$	$P < 0.05$	n.a.	n.a.	n.a.	n.a.	n.a.
Duration of SLEs (min)	1.43 ±0.35	1.55 ±0.29	1.83 ±0.31	$F_{(2,10)} = 2.14$	$P = 0.168$	n.s. 1.06 ±0.08	1.5 ±0.04*	1.64 ±0.14*	$F_{(2,8)} = 9.7$	$P < 0.01$

* Significantly different ($P < 0.05$) from baseline value; ** significantly different ($P < 0.05$) from wash value; *n.s.* nonsignificant; *n.a.* not applicable; ANOVA for repeated measures with post-hoc Fisher PLSD test

Fig. 3A–C The effects of raising extracellular PCO_2 to 20.6% (pH 6.7) on epileptiform activity induced by Mg^{2+}-free ACSF in the entorhinal cortex of combined slices. **A** High PCO_2 increased the interval between SLEs, slowed the spread of seizure activity as measured by duration of the prepotential, and decreased the peak amplitude of SLEs. *First trace*, baseline SLEs in Mg^{2+}-free medium; *second trace*, effect of increased PCO_2 (pH = 6.7) – there is an increase in the interictal interval duration; *third trace*, complete reemerging of SLEs when PCO_2 was returned to baseline value (5%). The *arrows* indicates the onset of prepotentials used to assess the rate of seizure spread. **B** High PCO_2 reverses the transient phase of clonic-like discharges into discrete SLEs. *First trace*, baseline SLEs in Mg^{2+}-free medium; *second trace*, development of transition phase; *third trace*, increased PCO_2 converts the transition phase back to discrete SLEs; *fourth trace*, complete reemergence of status-like activity when PCO_2 was returned to the baseline value (5%). **C** High PCO_2 does not significantly affect late recurrent discharges. *First trace*, late recurrent discharges in Mg^{2+}-free medium; *second trace*, the acidification by high PCO_2 caused only a slight decrease in amplitude and increase in frequency of the individual discharges constituing late status-like activity, however, there was no reversal back to individual SLEs (pH = 6.7); *third trace*, late recurrent discharges after PCO_2 was changed back to baseline

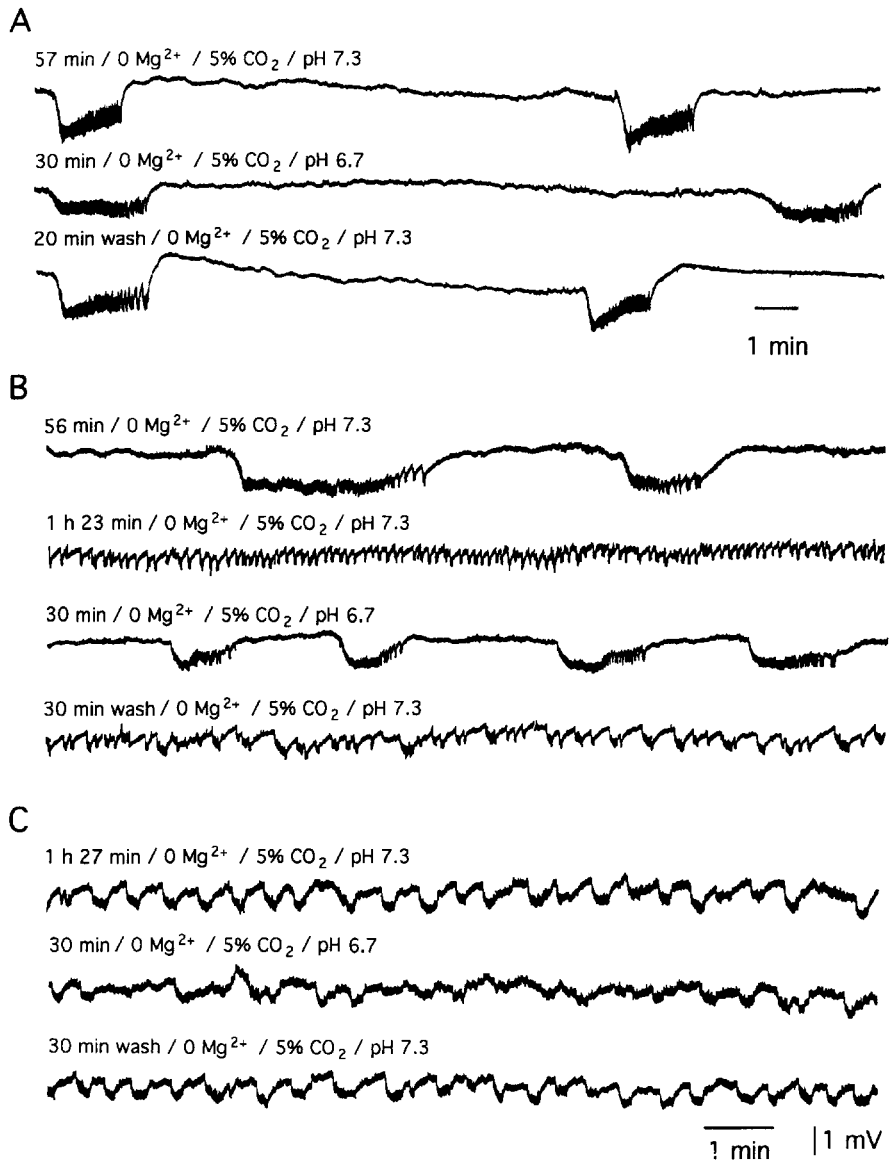

Fig. 4A–C The effects of lowering extracellular pH by adding HCl (pH 6.7) on epileptiform activity induced by Mg^{2+}-free ACSF. **A** Acidic extracellular pH increases the interval between SLEs and decreases the peak amplitude of SLEs. *First trace*, baseline SLEs in Mg^{2+}-free medium; *second trace*, lowering pH 6.7 by acidification (at constant PCO_2) prolonged the interval between SLEs and reduced SLE amplitude; *third trace*, reemergence of seizure discharges when pH was returned to 7.3. **B** Low pH converts the discharges during the transition from SLEs to status-like activity back into discrete SLEs. *First trace*, baseline SLEs in Mg^{2+}-free medium; *second trace*, transition phase; *third trace*, acidified pH (6.7) reversed the discharges of the transition phase back to discrete SLEs; *fourth trace*, progression to recurrent status epilepticus-like activity when pH was changed back to 7.3. **C** Acidic extracellular pH (6.7) does not suppress late recurrent status-like discharges *First trace*, late recurrent status-like discharges; *second trace*, acidification of ACSF to pH = 6.7 changed the pattern of later recurrent status-like discharges; however, neither blockade nor reversal to individual SLEs was observed. *Third trace*, late recurrent discharges when extracellular pH was returned back to 7.3

PCO_2, there was no significant effect on the duration of SLEs.

When pH_o was further lowered to 6.2 ($[H^+]_o$ 630 nM), a complete block of the SLEs was observed in all six slices within 30 min (Fig. 5A). Upon reperfusion with pH 7.3 ($[H^+]_o$ 50 nM) low-Mg^{2+} ACSF, SLEs regularly recurred within 20 min.

Effect of lowering extracellular pH on late status epilepticus-like stages of low-Mg^{2+}-induced epileptiform activity

After prolonged (> 1 h) perfusion in Mg^{2+}-free medium, epileptiform activity changed in character from discrete SLEs to status epilepticus-like discharges (Fig. 1C). Between the discrete SLEs and status discharges, a transition period was observed, during which rapidly recurring epileptiform discharges similar to clonic afterdischarges of a seizure-like event were seen interspersed with variable silent periods (Fig. 1B). Under control conditions, once established, the late recurrent discharges never spontaneously changed back to the pattern of discrete SLEs. This is consistent with previous findings (Dreier and Heinemann 1991; Walther et al. 1986).

The effect of high PCO_2 (pH 6.7) on epileptiform discharges 8–10 min into the transition period is illustrated in Fig. 3B. In one out of eight slices, lowering pH to 6.7 blocked all epileptiform activity. In the remaining slices ($n = 7$), elevating ACSF PCO_2 reverted the transitional discharge patterns back to discrete SLEs. Compared with initial SLEs, the amplitudes of SLEs that reemerged in high PCO_2 were significantly smaller. As long as CO_2 was kept at 20.6% (for up to 1 h), no status epilepticus-like activity recurred. However, when the PCO_2 was returned to baseline (5%), transitional, and eventually status-like discharges rapidly reappeared.

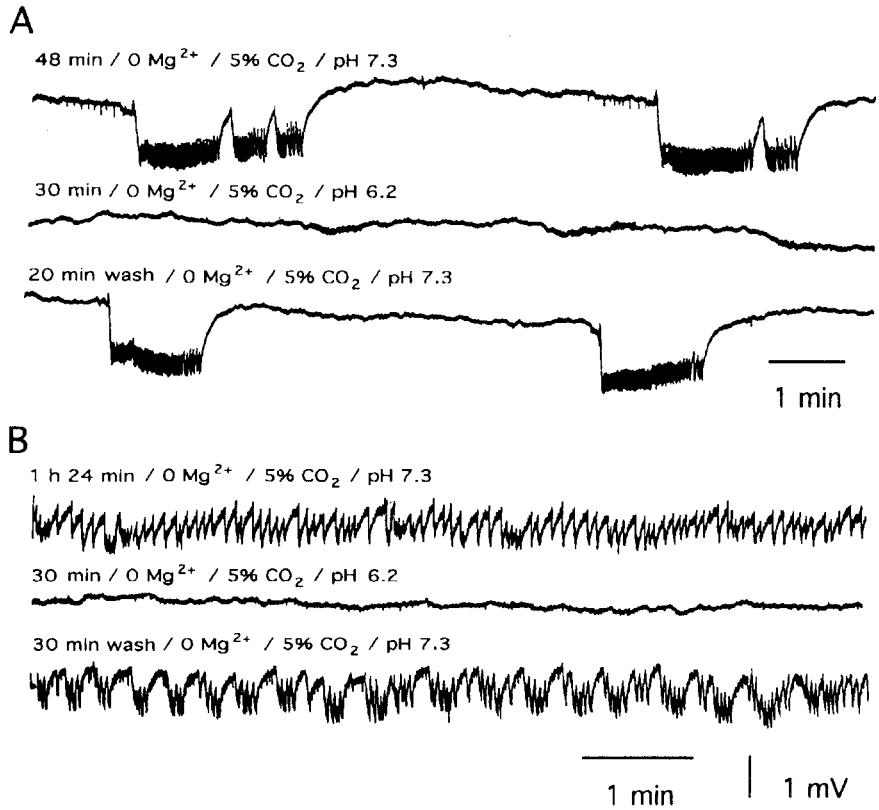

Fig. 5A, B Acidification of the extracellular pH to 6.2 by adding HCl reversibly completely blocks both SLEs and late recurrent discharges. **A** Lowering pH of the ACSF to 6.2 completely suppresses low-magnesium-induced SLEs. *First trace*, baseline SLEs in Mg^{2+}-free medium; *second trace*, SLEs were completely blocked by pH 6.2; *third trace* complete reemergence of SLEs when pH was returned to 7.3. **B** Lowering pH to 6.2 also completely blocked low-Mg^{2+}-induced late recurrent status-like discharges. *First trace*, late recurrent discharges in Mg^{2+}-free medium, *second trace*, blockade by pH = 6.2; *third trace*, complete reemergence of status-like activity when extracellular pH was returned to 7.3

It was relatively easy to test the effect of high PCO_2 on early status epilepticus-like activity, since pH_o was lowered almost instantaneously upon switching gases, due to a rapid penetration of CO_2 (bubbled through both the ACSF reservoir and the chamber) through the tissue. However, the wash-in phase of directly acidified ACSF took longer to equilibrate. However, in three experiments, status epilepticus-like discharges appeared just before low-pH ACSF perfusion began. In these slices, status-like activity was reversed to discrete SLEs by $pH_o = 6.7$ (Fig. 4B).

In contrast, later than 20 min after the onset of spontaneous status epilepticus-like activity, changing extracellular pH to 6.7 either with high PCO_2 ($n = 6$) or $[H^+]$ ($n = 5$) neither blocked status-like discharges nor converted them to discrete SLEs. In fact, the acidification with high PCO_2 caused a slight decrease in amplitude and increase in frequency of the individual status discharges. Acidification with HCl moderately decreased both amplitude and frequency of discharges (Figs. 3, 4C). However, late status-like activity was relatively resistant to alteration by acidic pH_o.

While established status activity was relatively resistant to blockade by $pH_o = 6.7$ ($[H^+]_o = 200$ nM), further lowering of pH_o to 6.2 ($[H^+]_o = 630$ nM) by adding larger quantities of HCl to the ACSF completely blocked even late status epilepticus-like activity ($n = 9$; Fig. 5B). As with other effects of acidic pH_o, this block was reversible, with recurrent status discharges reappearing rapidly upon return to normal pH (7.3; $[H^+]_o = 50$ nM).

Discussion

As previously reported (Dreier and Heinemann 1990; Walther et al. 1986), lowering of $[Mg^{2+}]_o$ induces a series of seizure-like events in the EC which progressively change in character from discrete seizure-like events, through a transition phase, to continuous status epilepticus-like activity. In comparison with a previous study (Dreier and Heinemann 1990), we more often observed a transient state of activity similar to clonic afterdischarges of SLEs at the transition from early SLEs to late recurrent status-like discharges. Furthermore, the presence of interictal discharges prior to development of SLEs was less prevalent than in slices cut by tissue chopper from Wistar rats (Dreier and Heinemann 1991). This could be due either to differences in the preparation or in the strain of rats used (Sprague-Dawley versus Wistar). Late status discharges of longer duration were usually seen 15–20 min after this transient period. These time-dependent changes in low-Mg^{2+}-induced epileptiform activity are quite similar to the course of intractable status epilepticus in vivo both in humans (Treiman et al. 1990) and in animals (Lothman et al. 1989; Louvel and Heinemann 1983; Walton and Treiman 1988a, b).

The present findings show that lowering extracellular pH suppresses in vitro seizures induced by Mg^{2+}-free medium, either when initiated in the EC or elsewhere within the slice. This action seems to be dose-dependent, i.e., pH_o 6.2 had a stronger anticonvulsant action than pH_o 6.7. In addition, the effects of increased PCO_2 ap-

pear to be virtually the same as the action of ACSF solution acidified by HCl, suggesting that a significant portion of the anticonvulsant action of high PCO_2 may be through reducing extracellular pH.

It has been shown previously that removing Mg^{2+} from the extracellular space causes epileptiform activity (Anderson et al. 1986; Stanton et al. 1987; Walther et al. 1986), which is believed to result, in large part, from relieving the voltage-dependent Mg^{2+} block of NMDA receptors (Mayer et al. 1984). This low-$[Mg^{2+}]_o$ epileptiform activity is blocked by low concentrations of the specific NMDA receptor antagonist AP5 (Stanton et al. 1987). Recent studies have found that increased $[H^+]_o$ potently blocks NMDA-gated currents (Tang et al. 1990; Traynelis and Cull-Candy 1990) with a 50% inhibitory concentration (IC_{50}) at about pH 7.3. There is also a study showing that the manipulation of extracellular pH can influence the NMDA component of synaptically evoked EPSPs (Taira et al. 1993). Therefore, it is likely that the anticonvulsant effects of acidified extracellular medium also involve an inhibition of NMDA receptor transmission. Similarly, it has been reported that acidotic extracellular pH suppresses, and alkalotic pH enhances, responses of neurons to microiontophoretically administered kainate, quisqualate, and NMDA (Church and McLennan 1989). However, a recent study of H^+ actions on kainic acid (KA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) whole cell currents showed that the IC_{50} for these actions were pH_o 5.7 and 6.3, respectively. Thus NMDA is the only excitatory amino acid receptor modulated by $[H^+]$ within the physiologic range of pH (Traynelis and Cull-Candy 1990, 1991). These data are consistent with the hypothesis that the mechanism of anticonvulsant action of low pH/high PCO_2 in our studies and others (Aram and Lodge 1987), probably involves an important contribution from NMDA receptor blockade.

Since the block of NMDA currents by H^+ is approximately half-maximal at normal physiologic pH (Traynelis and Cull-Candy 1990), a proconvulsant effect of high (alkaline) pH could involve an unblocking of NMDA receptors (Aram and Lodge 1987; Traynelis and Cull-Candy 1990). Alkalinization of both normal medium and low-$[Mg^{2+}]_o$ (0.2–0.5 mM) medium has been shown to provoke spontaneous epileptiform discharges similar to those elicited by Mg^{2+}-free medium (Aram and Lodge 1987). Indeed, such an action may contribute to the convulsant effect of hyperventilation in some epileptic patients (Kaplan and Lesser 1993). This is an interesting alternative to the hypothesis that hyperventilation causes seizure activity by lowering of brain interstitial $[Ca^{2+}]_o$. In fact, studies on hyperventilation-induced variations in $[Ca^{2+}]_o$ suggest that this effect is probably too small to account for the proconvulsant effects of hyperventilation (Balestrino and Somjen 1988; Balestrino et al. 1986).

NMDA receptors play an important role not only in acute seizures but also in progressive epileptogenesis (Miller et al. 1986; Velíšek and Mareš 1992). Blocking NMDA receptor activation with either competitive or noncompetitive antagonists markedly slows the rate of development of kindled seizures (Morrell 1991). By regulating postsynaptic influx of Ca^{2+} into neurons (MacDermott et al. 1986), NMDA receptors induce long-term potentiation (LTP) of synaptic strength (Collingridge et al. 1983) and Ca^{2+}-mediated delayed neuronal death (Choi 1988). By reducing NMDA receptor activation, protons may be a physiologic mechanism which regulates LTP, suppresses epileptogenesis, and limits excitotoxic neuronal death.

There are several other mechanisms that may contribute to the anticonvulsant effects of extracellular acidosis. The inhibitory action of high concentrations of protons on AMPA and kainate currents may explain the decrease in the amplitude of SLEs and individual late status discharges (Church and McLennan 1989). A similar acidosis-induced decrease in the amplitude of electrically induced field potentials was observed in the CA3 region of the hippocampus (Jarolimek et al. 1989; Somjen 1984). In our studies, an additional factor in the actions of directly acidified ACSF may be the slight increase in $[Cl^-]_o$. This could enhance influx of Cl^- intracellularly through $GABA_A$ channels, thus inducing membrane hyperpolarization, which may prevent epileptiform bursting. However, computing a reversal potential for Cl^- revealed a 3.73-mV change after adding 20 mM HCl. This is less than 4% change, which is unlikely to have a significant influence on bursting.

There are also many nonspecific effects of increased extracellular proton concentrations that may contribute to its anticonvulsant actions. Acidosis increases tonic block of sodium channels by protons (Hille 1968). It also neutralizes negatively charged groups on the outer membrane surface to promote membrane hyperpolarization (Hille 1968). Moreover, extracellular acidosis increases the levels of free, unbound $[Ca^{2+}]_o$ potentially stabilizing the membrane (Somjen et al. 1987). In our experiments, acidification through increased PCO_2, may include a contribution from the depression of membrane excitability due to the local anesthetic effect of CO_2 (Esplin et al. 1972). In addition, the anticonvulsant effects of CO_2 may involve a disruption of amino acid and carbohydrate metabolism (Woodbury and Karler 1960).

Although a possibility, it seems unlikely that decreasing $[O_2]_o$ could be a significant contribution to the effects we observed. In our experiments, the maximal reduction of PCO_2 was from a normal value of 95% to 79.4%. For those partial pressures of O_2, the theoretical solubility in the ACSF (at 35° C) is 1.06 mM and 0.88 mM, respectively. This is still well above the normal physiologic concentration of oxygen transported to tissues in the body ($\approx$0.1 mM O_2) and the saturation of O_2 regulation of mitochondrial respiration ($\approx$0.32 mM O_2). Therefore, this mild hypoxia seems unlikely to produce any significant changes in slice energetics.

Our findings, coupled with previous studies (Aram

and Lodge 1987; Tang et al. 1990; Traynelis and Cull-Candy 1990), allow for several speculations. First, hyperventilation-provoked seizures could well be due to extracellular alkalosis. Second, the anticonvulsant actions of acetazolamide (Woodbury and Karler 1960; a carbonic anhydrase inhibitor) may also be mediated by acidification. By inhibiting carbonic anhydrase, acetazolamide should cause accumulation of protons in the extracellular space (Heuser et al. 1975). Third, in designing antiepileptic regimens it may be more advisable to use drug forms that acidify, although it is unclear how much this can influence extracellular brain pH. Fourth, the anticonvulsant actions of valproic acid and ketogenic diet may be mediated, in part, through extracellular brain acidosis (Dean 1993; Uthman and Wilder 1993). Fifth, the extracellular acidification induced by epileptic seizure may be important in the self-termination of seizures (Caspers and Speckmann 1972; Somjen 1984). Lastly, the acidification of brain extracellular space may also play a significant role in attenuating the consequences of neuronal hypoxic/ischemic injuries (Kaku et al. 1993).

In conclusion, our data support the hypothesis that extracellular acid-base balance and CO_2 levels in the brain may play an important role in controlling seizure susceptibility by dynamically modulating NMDA receptor activity.

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