Effect of Divalent Cations on AMPA-Evoked Extracellular Alkaline Shifts in Rat Hippocampal Slices

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INTRODUCTION

Neuronal activity is accompanied by rapid extracellular alkalization that can arise from at least two sources (Chesler and Kaila 1992). Activation of GABA-A receptors leads to the efflux of bicarbonate across the anion channel and a consequent rise in extracellular pH (pHᵢ) (Chen and Chesler 1990, 1992a; Kaila and Voipio 1987; Kaila et al. 1990). In addition, there is a bicarbonate-independent alkaline shift that can be elicited by activation of ionotropic glutamate receptors (Chen and Chesler 1992a; Chesler and Chan 1988) or by direct electrical stimulation of a neuronal population (Chen and Chesler 1992b; Grichtchenko and Chesler 1996; Paalasmaa and Kaila 1996; Tong and Chesler 1999). Speculation on the mechanism of the bicarbonate-independent alkaline transient has centered on the role of calcium ions.

In giant nerve cells of the snail, alkaline shifts in surface pH have been linked to Ca²⁺ entry and its subsequent extrusion by a plasmalemmal Ca²⁺-ATPase (Schwiening et al. 1993). This transporter imports two protons per extruded Ca²⁺ (Niggl et al. 1982) and therefore may account for the bicarbonate-independent alkaline shift in vertebrate preparations. Observations consistent with this hypothesis have been noted in mammalian brain slices. Extracellular alkaline shifts evoked by glutamate agonists or by direct electrical stimulation were abolished in the absence of external Ca²⁺ (Grichtchenko and Chesler 1996; Paalasmaa and Kaila 1996; Paalasmaa et al. 1994; Smith et al. 1994). In addition, cytoplasmic acid shifts sensitive to CaAT-Pase inhibitors have been linked to neuronal Ca²⁺ entry (Trapp et al. 1996). On the other hand, Ba²⁺, which is thought to be poorly extruded by this transporter (Kwan et al. 1990; Zhao and Dhalla 1988), also was able to support these alkaline shifts (Grichtchenko and Chesler 1996; Tong and Chesler 1999).

The relationship between extracellular Ca²⁺ shifts and the alkaline transient, and the dependence of this pH response on the external concentration of Ca²⁺ and Ba²⁺, has not been investigated. In this study, we used pH- and Ca²⁺-selective microelectrodes, and discrete concentrations of external Ba²⁺, to more fully examine these relationships, employing focal injections of AMPA to elicit extracellular alkaline shifts. In this context, the effect of divalent Ca²⁺ channel blockers on the alkaline shift also was studied. Ion substitution and divalent blocker experiments confirmed a large dependence on Ca²⁺ entry and its subsequent extrusion by the plasmalemmal Ca²⁺-ATPase (Schwiening et al. 1993). This transporter imports two protons per extruded Ca²⁺. These data indicate that an activity-dependent alkaline shift is largely dependent on the entry of Ca²⁺ or Ba²⁺ via voltage-gated calcium channels. However, sizable alkaline transients still can be generated with little or no external presence of these ions. Implications for the mechanism of the activity-dependent alkaline shift are discussed.

METHODS

Procedures were carried out with approval of the NYU Medical Center Institutional Animal Care and Use Committee. All efforts were made to minimize animal suffering and reduce the number of animals used to the minimum required for our study. Long Evans rat pups, bred in-house (P12–18 of either sex), were anesthetized and rapidly decapitated. Hippocampal slices (300–400 μm) were prepared on a Vibratome in ice-cold Ringer, then incubated at room temperature until use. Experiments were conducted at 28–32°C in a submersion-style chamber with constant superfusion of the Ringer solution at 3–5 ml per min.
Normal Ringer solution contained (in mM) 124 NaCl, 26 NaHCO$_3$, 3 KCl, 3 CaCl$_2$, 1.5 MgCl$_2$, 1 NaH$_2$PO$_4$, and 10 glucose, gassed with 95% O$_2$–5% CO$_2$ (pH 7.4). Ringer solution buffered with 26 mM N-hydroxymethylpiperazine-N’-2-ethanesulfonic acid (HEPES) had NaHCO$_3$ omitted and was titrated to pH 7.4 with NaOH. In some experiments, the Ca$^{2+}$ was reduced to 1 mM to allow for greater resolution of Ca$^{2+}$ shifts detected with ion-selective microelectrodes. Zero-Ca$^{2+}$ Ringer had CaCl$_2$ omitted and contained 1 or 5 mM ethylene glycol bis (beta-aminoethyl) ether)-N, N’, N’-tetraacetic acid (EGTA). Solutions containing 5 mM EGTA were prepared with 35 mM NaHCO$_3$ to titrate the acidity of the EGTA and had NaCl reduced accordingly. All experiments were conducted in saline containing 100 μM picrotoxin (PTX; Sigma Chemical). Solutions containing CdCl$_2$ or NiCl$_2$ had NaH$_2$PO$_4$ omitted. The pressure ejection pipettes had tip diameters of ~5 μm and contained 100 μM RS-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) dissolved in 150 mM NaCl. In a small number of experiments, the ejection pipette was filled with 10 mM sodium glutamate, and the Ringer contained 50 μM D-2-amino-5-phosphonovaleric acid (APV) to block activation of N-methyl-D-aspartate receptors. APV and 6-cyano-7-nitroquinoline-2,3-dione (CNQX) were purchased from Tocris Cookson. Benzolamide was a gift of Dr. T. H. Maren and Lederle Laboratories, Pearl River, NY.

Double-barreled pH electrodes were fabricated as previously described (Chesler and Chan 1988). Reference barrels were back-filled with 1 M NaCl. The silanized pH-sensitive barrels were back-filled with 150 mM NaCl plus phosphate buffer (pH 6.87) and front-filled with a pH-sensitive liquid cocktail (Fluka 95291). The pH microelectrodes, calibrated in standard phosphate buffers, exhibited a 55- to 60-mV response per unit pH. Ion and reference signals were recorded via high-impedance (>$10^{13}$ Ω) operational amplifiers wired for unity gain. Extracellular DC potential was monitored and subtracted from the signal on the ion-sensitive barrel.

The pH electrode and the pressure ejection pipette were mounted on a Narashige dual micromanipulator with their tips ~50 μm apart. The array was lowered to a tissue depth of ~150 μm in the CA1 stratum pyramidale or s. radiatum. The choice of region was governed only by the ability of the tissue to generate sizable, repeatable alkaline responses. As no consistent differences were noted between the behavior of pH$_o$ responses in s. pyramidale versus s. radiatum, the results from these regions were pooled. To avoid movement of the electrode tips relative to one another, the array was not moved after being positioned in the tissue. Therefore baseline pH$_o$ was checked only at the start and at the end of experiments when the array was moved between tissue and bath. The pH of the extracellular space was more acid than the bath, typically ranging between 7.0 and 7.20.

To elicit pH$_o$ shifts, a Picospritzer (General Valve) was used to deliver pressure pulses (40 psi) of AMPA at regular intervals (1–5 min.). The duration of the agonist pulse was adjusted until a consistent alkaline shift >0.05 unit pH was obtained in normal Ringer. In a few experiments, pH$_o$ shifts were evoked by antidromic stimulation (50 Hz, 5 s) of the CA1 pyramidal neurons using a bipolar stimulating electrode placed in the alveus. The unfiltered signals were measured as a change in voltage on a strip chart recorder and archived to video tape. Means were presented with standard errors. Values of $n$ referred to the number of slices. Amplitudes of the alkaline shifts were compared using a paired $t$-test.

In bicarbonate Ringer, the interstitial buffering of alkaline shifts depends on the extracellular carbonic anhydrase activity and assumptions about the CO$_2$ partial pressure (Chesler 1990). Because these factors are uncertain, experiments requiring estimates of interstitial buffering capacity were conducted in HEPES Ringer. The buffering capacity (β) of the extracellular fluid was estimated as:

$$\beta = \frac{2.3[H^+][A_{tot}][K_b]}{([H^+] + K_b)^2} \tag{1}$$

where $A_{tot}$ is the total concentration of HEPES and $K_b$ is the dissociation constant (Roos and Boron 1981). The equivalent amount of strong base added to the extracellular fluid during an alkaline shift was referred to as the change in strong ion difference (ΔSID) and was estimated as:

$$\Delta SID = \beta \times \Delta pH \tag{2}$$

where ΔPpH is the mean amplitude of the alkalization (Chesler 1990).

**RESULTS**

Baseline pH$_o$ was similar in 26 mM bicarbonate versus 26 mM HEPES-buffered media as has been previously reported (Gottfried and Chesler 1994). In a sample of 10 slices in bicarbonate saline, the baseline pH$_o$ was 7.15 ± 0.019 (range 7.08–7.23). In another 10 slices studied in 26 mM HEPES Ringer, the baseline pH$_o$ was 7.13 ± 0.015 (range 7.06–7.20).

**Simultaneous measurement of pH$_o$ and [Ca$^{2+}$]$_o$**

In seven slices, we studied evoked pH shifts in 26 mM HEPES-buffered Ringer. A pH- and a Ca$^{2+}$-selective microelectrode were used to record simultaneous ion shifts (Fig. 1, A and B) evoked by AMPA ($n = 5$) or glutamate ($n = 2$, in 50 μM APV). The bath Ca$^{2+}$ was reduced from 3 to 1 mM in

![Fig. 1](http://jn.physiology.org/DownloadedFrom) Calcium dependence of the AMPA-evoked alkaline shift. **A**: simultaneous recording of extracellular Ca$^{2+}$ and pH transients evoked by pressure ejection of 100 μM AMPA. **B**: pressure ejection of 10 mM glutamate elicited similar ion transients in media containing 50 μM D-2-amino-5-phosphonovaleric acid (APV). **C**: alkaline response evoked by pressure ejection of AMPA was inhibited reversibly in 0-Ca$^{2+}$ media containing 1 mM EGTA. A rapid acid transient was unmasked in the 0-Ca$^{2+}$ media. All solutions were buffered with 26 mM HEPES.
Evoked alkaline shifts in zero-Ca\textsuperscript{2+} with a peak fall in [Ca\textsuperscript{2+}]\textsubscript{o} shifts averaged 0.072 ± 0.027 pH unit. For all seven slices, the peak alkaline shift persisted in the nominal absence of extracellular calcium. These extracellular Ca\textsuperscript{2+}-independent alkaline shifts became indiscernible at an average [Ca\textsuperscript{2+}]\textsubscript{o} range from 1 to 281 μM.

Alkaline shifts during washout of extracellular Ca\textsuperscript{2+}

In solutions containing EGTA and no added Ca\textsuperscript{2+}, the agonist-evoked alkaline shift was abolished reversibly in 41 slices (34 slices bathed in bicarbonate saline and 7 slices bathed in HEPES saline). In many cases, transition to the zero-Ca\textsuperscript{2+}/EGTA Ringer caused the alkaline shift to be superseded by a rapid acid shift (Fig. 1C) as noted previously (Smith et al. 1994). This acid transient could be abolished by CNQX (not shown), indicating that it was triggered by the activation of AMPA receptors and was not a mechanical or electrical artifact. The mechanism of this acid transient was not explored.

To better resolve the relationship between the pH\textsubscript{o} changes and [Ca\textsuperscript{2+}]\textsubscript{o}, adjacent recordings of the ion transients were made in slices during the washout and return of extracellular Ca\textsuperscript{2+}. Superfusion of zero-Ca\textsuperscript{2+} with 1 mM EGTA-buffered Ringer caused a fall in extracellular [Ca\textsuperscript{2+}]\textsubscript{o} to micromolar levels within a few minutes (Fig. 2). During the Ca\textsuperscript{2+} washout, AMPA was intermittently pressure-ejected from a nearby micropipette to follow the effect on the evoked alkaline shift. As shown in Fig. 2, the reduction of [Ca\textsuperscript{2+}]\textsubscript{o} caused a decrease in the amplitude of the alkaline shifts, accompanied by the appearance of an initial acid-going transient that eventually superseded the alkalinization. The acid transient remained during much of the return of extracellular Ca\textsuperscript{2+}. These observations suggested that a persistent component of the alkaline shift could have been obscured by the early acid transient (see following text). In eight slices, the alkaline shifts became indiscernible at an average [Ca\textsuperscript{2+}]\textsubscript{o} of 117 ± 29 μM, over a [Ca\textsuperscript{2+}]\textsubscript{o} range from 1 to 281 μM.

Evoked alkaline shifts in zero-Ca\textsuperscript{2+} media

In a subset of experiments (15 of 59 slices), a component of the alkaline shift persisted in the nominal absence of extracellular calcium. These extracellular Ca\textsuperscript{2+}-independent alkalinizations occurred in both bicarbonate (Fig. 3A) and HEPES-buffered Ringer (Fig. 3B) in which the measured [Ca\textsuperscript{2+}]\textsubscript{o} was <1 μM. In bicarbonate buffer, these alkaline shifts averaged 0.032 ± 0.004 unit pH (n = 9) and were 42 ± 8% of the paired control responses in normal [Ca\textsuperscript{2+}]\textsubscript{o}. In six slices studied in HEPES Ringer, alkaline shifts noted in the absence of external Ca\textsuperscript{2+} averaged 0.027 ± 0.006 unit pH and were 28 ± 3% of the paired controls. These persistent responses were abolished by CNQX (not shown), indicating that they were not electrical or mechanical artifacts.

Effect of Ca\textsuperscript{2+} channel blockers in bicarbonate-free Ringer

In HEPES-buffered Ringer, addition of 300 μM Ca\textsuperscript{2+} was able to reversibly reduce the AMPA-evoked alkaline shift. The mean decrease of the response was 55 ± 2% (n = 5, P < 0.001; Fig. 4A). Addition of 100 μM Ni\textsuperscript{2+} alone reduced the alkaline shift by 36 ± 4% (n = 7, P < 0.001; Fig. 4B). When 300 μM Ca\textsuperscript{2+} and 100 μM Ni\textsuperscript{2+} were applied together, the AMPA-evoked alkalinization was blocked completely in four slices (Fig. 4C).

To better define the Ca\textsuperscript{2+} entry pathway associated with activation of the alkaline shift, we tested the effect of nimodipine, an L-type calcium channel antagonist. In seven slices, addition of 10 μM nimodipine to HEPES-buffered Ringer inhibited the AMPA-evoked alkaline shift by 30 ± 7% (P < 0.05). When 100 μM Ni\textsuperscript{2+} was added to the nimodipine-

![Image](http://jn.physiology.org/DownloadedOn/2017-10-20T13:30:57Z)

FIG. 2. Simultaneous measurement of [Ca\textsuperscript{2+}]\textsubscript{o} and pH\textsubscript{o} transients during washout and return of bath calcium. Calcium-free solution contained 5 mM EGTA. Solution buffered with 26 mM HEPES.

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FIG. 3. Persistence of the AMPA-evoked alkaline shift in the absence of extracellular Ca\textsuperscript{2+}. A: alkaline response evoked in 0-Ca Ringer (1 mM EGTA) buffered with 26 mM bicarbonate. B: alkaline response evoked in 0-Ca Ringer (1 mM EGTA) buffered with 26 mM HEPES.
containing Ringer, the alkaline shifts were further reduced to 51 ± 3% of control (n = 5, P < 0.001; not shown).

**Effect of Cd\(^{2+}\) and Ni\(^{2+}\) in bicarbonate Ringer**

In bicarbonate-buffered Ringer, addition of 300 μM Cd\(^{2+}\) alone (Fig. 5A) reduced the AMPA-evoked alkaline shift by 67 ± 4% (n = 4, P < 0.05). Whereas addition of Cd\(^{2+}\) plus Ni\(^{2+}\) abolished the response in HEPES Ringer, Ni\(^{2+}\) produced no additional block in bicarbonate Ringer (Fig. 5A). In the presence of 300 μM Cd\(^{2+}\) plus 100 μM Ni\(^{2+}\), the AMPA-evoked alkaline shift was reduced 65 ± 5% (n = 4, P < 0.02), which was not significantly different from the effect of Cd\(^{2+}\) alone (n = 4, P = 0.7). When 10 μM benzolamide was added in addition to the Cd\(^{2+}\) and Ni\(^{2+}\), the remaining alkaline shift was amplified three- to sevenfold (Fig. 5B). The residual response therefore originated from a proton sink, typical of alkaline shifts activated by ionotropic glutamate receptors (Chen and Chesler 1992c).

Further dissection of these effects revealed a paradoxical action of Ni\(^{2+}\) when applied alone. In 11 slices, addition of 50–100 μM Ni\(^{2+}\) increased the AMPA-evoked alkaline shift by 154 ± 9% (P < 0.001; Fig. 6A). The enhancement by Ni\(^{2+}\) was not specific to the response evoked by AMPA receptors. In four slices, alkaline transients were evoked in the absence of synaptic transmission (in 20 μM CNQX, 50 μM APV, and 100 μM PiTX) by repetitive (50 Hz) antidromic activation of the CA1 pyramidal neurons. After addition of 100 μM Ni\(^{2+}\), the antidiromic alkaline shifts were increased by 171 ± 12% (n = 4, P < 0.05; Fig. 6B).

This enhancement of the alkaline shift would be expected if Ni\(^{2+}\) had an inhibitory effect on the interstitial carbonic anhydrase. To test this hypothesis, we prevented the possibility of such inhibition by bathing slices in benzolamide (10–20 μM). With the extracellular carbonic anhydrase inhibited, a comparatively small AMPA ejection pulse was used to elicit an average alkaline shift of 0.10 ± 0.02 unit pH (n = 7). Under these conditions, Ni\(^{2+}\) inhibited the alkaline responses. After addition of 50–100 μM Ni\(^{2+}\) to the benzolamide-containing saline, the AMPA-evoked alkaline shifts were reduced to 73 ± 11% of controls, (P < 0.05) averaging 0.078 ± 0.02 unit pH (Fig. 6C).

**Replacement of Ca\(^{2+}\) with Ba\(^{2+}\)**

Although the AMPA-evoked alkaline shift was blocked in media containing 1–5 mM EGTA and no added Ca\(^{2+}\), a component of the response could be restored by adding Ba\(^{2+}\). In these experiments, Ca\(^{2+}\) first was removed from the interstitial space by superfusion with Ringer containing 1 mM EGTA and no added Ca\(^{2+}\). Before addition of Ba\(^{2+}\), the EGTA was washed out of the slice. Periodic ejection of AMPA confirmed that the alkaline shift remained blocked. With subsequent addition of Ba\(^{2+}\), a significant component of the alkalization returned. In HEPES (n = 12; Fig. 7A) and bicarbonate media (n = 13; Fig. 7B), the alkaline shift elicited by 500 μM Ba\(^{2+}\) was, respectively, 40 ± 3 and 51 ± 5% of the responses obtained in 3 mM Ca\(^{2+}\).

Like the control responses in normal Ca\(^{2+}\), the alkalinations elicited in 500 μM Ba\(^{2+}\) were amplified by benzolamide.
that these responses were unrelated to extracellular Ca$^{2+}$, we used a Ca$^{2+}$-free bicarbonate Ringer that contained 2 mM Ba$^{2+}$ plus 5 mM EGTA to provide a calculated free-Ba$^{2+}$ concentration of 37 μM. In five slices bathed in this solution, the AMPA-evoked alkaline shift was 0.025 ± 0.01 unit pH and averaged 41 ± 10% of the control responses in 3 mM Ca$^{2+}$ (Fig. 9B).

**DISCUSSION**

**Relationship between divalent cation shifts and the alkaline transient**

Simultaneous recording of the alkaline shift and the [Ca$^{2+}$]$_{o}$ transient allowed comparisons of the net ionic changes after AMPA ejection. If the measured fall in [Ca$^{2+}$]$_{o}$ represented a cellular Ca$^{2+}$ load, which subsequently was extruded in entirety by a plasmalemmal CaATPase, a ΔSID:Δ[Ca$^{2+}$]$_{o}$ ratio of 2:1 would be anticipated (Niggli et al. 1982). Using a HEPES buffering power of 15 mM, the mean AMPA-evoked alkaline shift (0.072 unit pH) corresponded to a change in the strong ion difference (ΔSID) of ~1 mM (equivalent to the addition of this concentration of strong base to the extracellular fluid). Because the corresponding measured fall in [Ca$^{2+}$]$_{o}$ averaged ~300 μM, the ΔSID:Δ[Ca$^{2+}$]$_{o}$ ratio would range between 3 and 4. However, the limited response times of the pH and Ca$^{2+}$ electrodes and the diffusion time between pressure ejection and ion electrodes sites would have distorted these measurements. It is therefore likely that both the ΔSID and the cellular Ca$^{2+}$ load were greater. Thus, order of magnitude comparisons of Ca$^{2+}$ and pH responses are probably the best that can be expected using these techniques. However, it should be noted that that evoked intracellular Ca$^{2+}$ load was likely handled by mechanisms in addition to the CaATPase, such as Na$^{+}$-Ca$^{2+}$ exchange (Blaustein 1988), uptake into endoplasmic reticulum (Markram et al. 1995), or sequestration by mitochondria (Wang and Thayer 1996). To the extent that a plasmalemmal CaATPase did not fully handle the intracellular Ca$^{2+}$ load, the calculated ratios would have been greater.

In a previous study, Ba$^{2+}$-dependent alkaline shifts could be supported by Ba$^{2+}$ but required millimolar concentrations of this ion (Grichtchenko and Chesler 1996). Using AMPA microejection to evoke the pH response, micromolar concentrations of Ba$^{2+}$ were sufficient. The ability of 50 μM Ba$^{2+}$ to support the alkaline shift can be considered in the context of H$^{+}$ antiport. If the entire extracellular concentration of 50 μM Ba$^{2+}$ had entered cells and was extruded in exchange for
protons (in a ratio of 2 H\(^+\):1Ba\(^{2+}\)), then the expected extracellular \(\Delta S_{\text{SID}}\) would have been 100 \(\mu\)M. With a mean measured alkaline shift of \(~0.03\text{ unit pH}\) and a calculated buffering capacity of 15 mM, the experimental \(\Delta S_{\text{SID}}\) would be \(~450\text{ \(\mu\)M}\). In view of the limited response time of the pH electrodes, the actual \(\Delta S_{\text{SID}}\) is likely to have been larger. To generate the observed alkaline shifts using the plasmalemmal CaATPase therefore would have required efficient extrusion of Ba\(^{2+}\) ions by this transporter and cycling of Ba\(^{2+}\), whereby ions entered cells and were extruded multiple times in exchange for protons.

**Alkaline shifts in the absence of extracellular Ca\(^{2+}\)**

In one-fourth of the slices, alkaline shifts were elicited in the nominal absence of external Ca\(^{2+}\) or Ba\(^{2+}\). These shifts averaged \(~0.03\text{ unit pH}\), corresponding to a \(\Delta S_{\text{SID}}\) of 450 \(\mu\)M. Although these alkalinations were not noted in the majority of slices, the appearance of an acid transient at low [Ca\(^{2+}\)]\(_o\) may have obscured such changes, as suggested by Fig. 2. For the plasmalemmal CaATPase hypothesis to be considered in this context, the extruded Ca\(^{2+}\) would have had to originate from internal stores. This possibility has not been excluded by the present experiments.

**Effect of Ca\(^{2+}\) channel blockers on the alkaline shift**

In HEPES-buffered solution, simultaneous addition of Cd\(^{2+}\) and Ni\(^{2+}\) completely blocked the AMPA-evoked alkaline shift, consistent with a requirement for Ca\(^{2+}\) entry via voltage-gated channels. In isolation, these blockers may roughly distinguish between low- and high-voltage-activated Ca\(^{2+}\) channels (Bean 1989; Fox et al. 1987). The block of roughly one-third of the alkaline shift by Ni\(^{2+}\) was similar to the effect of this cation on spike-induced Ca\(^{2+}\) entry in postnatal CA1 pyramidal cells (Christie et al. 1995) and indicates that both low- and high-voltage-activated Ca\(^{2+}\) channels trigger the pH response. Nimodipine was about half as effective as Cd\(^{2+}\) alone, suggesting that L-type Ca\(^{2+}\) channels were responsible for triggering approximately one-fourth of the alkaline shift. Nimodipine blocked a similar fraction of somatic Ca\(^{2+}\) entry in postnatal CA1 pyramidal neurons (Christie et al. 1995). Neuronal entry of Ca\(^{2+}\) via AMPA receptors is likely to have played a minor role because the AMPA receptor subtype with significant Ca\(^{2+}\) permeability is confined to the less prominent GABAergic interneurons (Geiger et al. 1995; Racca et al. 1996).

In bicarbonate Ringer, Cd\(^{2+}\) and Ni\(^{2+}\) did not fully abolish the alkaline shift. Partial binding of these cations by bicarbonate ions might have contributed to the incomplete inhibition. In addition, because Ni\(^{2+}\) alone had a paradoxical enhancing effect, the combination of Ni\(^{2+}\) and Cd\(^{2+}\) would be less effective than anticipated. The Ni\(^{2+}\)-mediated enhancement of the alkaline shift was dependent on bicarbonate and was occluded by benzolamide. These observations are consistent with a partial reduction of extracellular carbonic anhydrase activity mediated by Ni\(^{2+}\). Inhibition of this enzyme by a variety of divalent cations has been reported (Maren 1967; Puscas et al. 1989). These effects of Ni\(^{2+}\) on activity-dependent pH\(_o\) shifts may warrant consideration in other studies where this divalent ion is used in brain slices.

In summary, the present data indicate that the influx of Ca\(^{2+}\) or Ba\(^{2+}\) across voltage-gated Ca\(^{2+}\) channels is an important component in the steps that lead to extracellular alkalization after activation of AMPA receptors. The results are in agreement with other studies of activity-dependent alkaline shifts and can be interpreted to support the involvement of a plasmalemmal CaATPase. However, alkaline responses could be obtained at extremely low concentrations of external Ba\(^{2+}\) and in many instances occurred in the absence of extracellular Ca\(^{2+}\). These results indicate that the entry of Ca\(^{2+}\) and its subsequent extrusion by the plasmalemmal CaATPase cannot explain all of the properties of activity-dependent alkaline shifts. Involvement of internal Ca\(^{2+}\) stores or alternative pathways for transmembrane H\(^+\) movement are possibilities that have not been excluded.

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