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

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Smith, S. E. and M. Chesler. Effect of divalent cations on AMPA-evoked extracellular alkaline shifts in rat hippocampal slices. J. Neurophysiol. 82: 1902–1908, 1999. The generation of activity-evoked extracellular alkaline shifts has been linked to the presence of external Ca\(^{2+}\) or Ba\(^{2+}\). We further investigated this dependence using pH- and Ca\(^{2+}\)-selective microelectrodes in the CA1 area of juvenile, rat hippocampal slices. In HEPES-buffered media, alkaline transients evoked by pressure ejection of RS-HCl or another saline, containing EGTA. In HEPES media, addition of 300–500 μM Ba\(^{2+}\) caused the reappearance of an alkaline response. In approximately one-fourth of slices, a persistent alkaline shift, became indiscernible at a [Ca\(^{2+}\)]\(_{o}\) of 117 ± 29 μM. Addition of as little as 30–50 μM Ba\(^{2+}\) caused an unexpected enhancement of the alkaline shift. In this context, the effect of divalent Ca\(^{2+}\) blockers on alkaline shifts and the dependence of this pH response on the external concentration of Ca\(^{2+}\) and Ba\(^{2+}\), has not been investigated. In this study, we used pH- and Ca\(^{2+}\)-selective microelectrodes, and discrete concentrations of external Ba\(^{2+}\). To more fully examine these relationships, employing focal injections of AMPA to elicit extracellular alkaline shifts. In this protocol, the effect of divalent Ca\(^{2+}\) channel blockers on the alkaline shift also was studied. Ion substitution and divalent blocker experiments confirmed a large dependence on Ca\(^{2+}\) or Ba\(^{2+}\) ions. However, our data indicate that significant alkaline shifts can be elicited reliably in the presence of micromolar external Ba\(^{2+}\) and often can be evoked in the absence of either Ba\(^{2+}\) or Ca\(^{2+}\). Some of these results have appeared in an abstract (Smith et al. 1996).

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.

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Normal Ringer solution contained (in mM) 124 NaCl, 26 NaHCO₃,
3 KCl, 3 CaCl₂, 1.5 MgCl₂, 1 NaH₂PO₄, and 10 glucose, gassed with
95% O₂-5% CO₂ (pH 7.4). Ringer solution buffered with 26 mM
N-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES) had
NaHCO₃ omitted and was titrated to pH 7.4 with NaOH. In some
experiments, the Ca²⁺ was reduced to 1 mM to allow for greater
resolution of Ca²⁺ shifts detected with ion-selective microelectrodes.

Zero-Ca²⁺ Ringer had CaCl₂ omitted and contained 1 or 5 mM
ethylene glycol bis (beta-aminoethyl ether)-N,N,N',N'-tetraacetic
acid (EGTA). Solutions containing 5 mM EGTA were prepared with
35 mM NaHCO₃ to titrate the acidity of the EGTA and NaCl
reduced accordingly. All experiments were conducted in saline
containing 100 µM picrotoxin (PITX; Sigma Chemical). Solutions
containing CdCl₂ or NiCl₂ had NaH₂PO₄ 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) dis-
solved in 150 mM NaCl. In a small number of experiments, the
ejection pipette was filled with 10 mM sodium glutamate, and the
solution was titrated at pH 7.4 to neutralize the solution before
injection. In some experiments, the pressure ejection pipette was
filled with 10 mM sodium glutamate, and the solution was titrated at
pH 7.4 to neutralize the solution before injection.

Double-barreled pH electrodes were fabricated as previously de-
scribed (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 microelec-
trodes, calibrated in standard phosphate buffers, exhibited a 55- to
60-mV response per unit pH. Ion and reference signals were recorded
via high-impedance (>10¹³ 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
pyramidal or s. radiatum. The choice of region was governed only by
the ability of the tissue to generate sizable, repeatable alkaline re-
sponses. As no consistent differences were noted between the behav-
or of pHᵩ responses in s. pyramidale versus s. radiatum, the results
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 pHᵩ 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 pHᵩ 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
alcaline shift >0.05 unit pH was obtained in normal Ringer. In a few
experiments, pHᵩ 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 assump-
tions about the CO₂ 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

$$\beta = \frac{2.3[H^+][A_m][K_a]}{[\text{SID}]}$$

where Aₘ is the total concentration of HEPES and Kₐ 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

$$\Delta \text{SID} = \beta \times \Delta \text{pH}$$

where ΔpH is the mean amplitude of the alkalinization (Chesler
1990).

R E S U L T S

Baseline pHᵩ was similar in 26 mM bicarbonate or 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 pHᵩ was 7.15 ± 0.019 (range
7.08–7.23). In another 10 slices studied in 26 mM HEPES
Ringer, the baseline pHᵩ was 7.13 ± 0.015 (range 7.06–7.20).

Simultaneous measurement of pHᵩ and [Ca²⁺]ᵩ

In seven slices, we studied evoked pH shifts in 26 mM
HEPES-buffered Ringer. A pH- and a Ca²⁺-selective micro-
electrode 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 Ca²⁺ was reduced from 3 to 1 mM in

FIG. 1. Calcium dependence of the AMPA-evoked alkaline
shift. A: simultaneous recording of extracellular Ca²⁺ and pH
transients evoked by pressure ejection of 100 µM AMPA. B:
presure ejection of 10 mM glutamate elicited similar ion trans-
sients in media containing 50 mM DL-2-amino-5-phosphonoval-
eric acid (APV). C: alkaline response evoked by pressure ejec-
tion of AMPA was inhibited reversibly in 0-Ca²⁺ media
containing 1 mM EGTA. A rapid acid transient was unmasked
in the 0-Ca²⁺ media. All solutions were buffered with 26 mM
HEPES.
Alkaline shifts during washout of extracellular Ca$^{2+}$

For all seven slices, the peak alkaline pH shifts averaged 0.072 ± 0.015 unit pH and were associated with a peak fall in [Ca$^{2+}$]$_o$ of 295 ± 47 μM. Assuming an extracellular pH buffering capacity of 14.7 mM (see METHODS), the alkaline shifts were associated with a change in the extracellular strong ion difference (∆SID) equivalent to the addition of 1.06 ± 0.22 mM strong base. Over the seven slices, the ratio of the ∆SID:∆[Ca$^{2+}$]$_o$ ranged from 2.1–5.5 and averaged 3.65 ± 0.44.

Evoked alkaline shifts in zero-Ca$^{2+}$ media

In solutions containing EGTA and no added Ca$^{2+}$, 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$^{2+}$/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$_o$ changes and [Ca$^{2+}$]$_o$, adjacent recordings of the ion transients were made in slices during the washout and return of extracellular Ca$^{2+}$. Superfusion of zero-Ca$^{2+}$/HEPES-buffered Ringer (with 1 mM EGTA) caused a fall in extracellular [Ca$^{2+}$]$_o$ to micromolar levels within a few minutes (Fig. 2). During the Ca$^{2+}$ 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$^{2+}$]$_o$ caused a decrease in the amplitude of the alkaline shifts, accompanied by the appearance of an initial acid-going transient that eventually superseded the alkalization. The acid transient remained during much of the return of extracellular Ca$^{2+}$. 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$^{2+}$]$_o$ of 117 ± 29 μM, over a [Ca$^{2+}$]$_o$ range from 1 to 281 μM.

Evoked alkaline shifts in zero-Ca$^{2+}$ 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$^{2+}$-independent alkalinizations occurred in both bicarbonate (Fig. 3A) and HEPES-buffered Ringer (Fig. 3B) in which the measured [Ca$^{2+}$]$_o$ 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$^{2+}$]$_o$. In six slices studied in HEPES Ringer, alkaline shifts noted in the absence of external Ca$^{2+}$ 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$^{2+}$ channel blockers in bicarbonate-free Ringer

In HEPES-buffered Ringer, addition of 300 μM Cd$^{2+}$ 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$^{2+}$ alone reduced the alkaline shift by 36 ± 4% ($n = 7$, $P < 0.001$; Fig. 4B). When 300 μM Cd$^{2+}$ and 100 μM Ni$^{2+}$ were applied together, the AMPA-evoked alkalization was blocked completely in four slices (Fig. 4C).

To better define the Ca$^{2+}$ 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$^{2+}$ was added to the nimodipine-B.
Effect of Cd\(^{2+}\) was amplified three- to sevenfold (Fig. 5B) in addition to the Cd\(^{2+}\) alone (\(n = 5\)). The evoked alkaline shift was reduced 65\% (\(P < 0.001\); 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 \(\mu\)M CNQX, 50 \(\mu\)M APV, and 100 \(\mu\)M PiTX) by repetitive (50 Hz) antidromic activation of the CA1 pyramidal neurons. After addition of 100 \(\mu\)M Ni\(^{2+}\), the antidromic alkaline shifts were increased by 171\% (\(n = 4\), \(P < 0.05\); 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 \(\mu\)M). With the extracellular carbonic anhydrase inhibited, a comparatively small AMPA ejection pulse was used to elicit an average alkaline shift of 0.10 \(\pm\) 0.02 unit pH (\(n = 7\)). Under these conditions, Ni\(^{2+}\) inhibited the alkaline responses. After addition of 50–100 \(\mu\)M Ni\(^{2+}\) to the benzolamide-containing saline, the AMPA-evoked alkaline shifts were reduced to 73\% of controls, \((P < 0.05)\) averaging 0.078 \(\pm\) 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, Ba\(^{2+}\) 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 \(\mu\)M Ba\(^{2+}\) was, respectively, 40\% \(\pm\) 3 and 51\% \(\pm\) 5\% of the responses obtained in 3 mM Ca\(^{2+}\).

Like the control responses in normal Ca\(^{2+}\), the alkalizations elicited in 500 \(\mu\)M Ba\(^{2+}\) were amplified by benzolamide.

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

In bicarbonate-buffered Ringer, addition of 300 \(\mu\)M Cd\(^{2+}\) alone (Fig. 5A) reduced the AMPA-evoked alkaline shift by 67\% \(\pm\) 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 \(\mu\)M Cd\(^{2+}\) plus 100 \(\mu\)M Ni\(^{2+}\), the AMPA-evoked alkaline shift was reduced 65\% \(\pm\) 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 \(\mu\)M 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 \(\mu\)M Ni\(^{2+}\) increased the AMPA-evoked alkaline shift by 154\% \(\pm\) 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 \(\mu\)M CNQX, 50 \(\mu\)M APV, and 100 \(\mu\)M PiTX) by repetitive (50 Hz) antidromic activation of the CA1 pyramidal neurons. After addition of 100 \(\mu\)M Ni\(^{2+}\), the antidromic alkaline shifts were increased by 171\% (\(n = 4\), \(P < 0.05\); Fig. 6B).
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 en-
tirety 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, electrically evoked 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 concen-
trations 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

![FIG. 6. Paradoxical effect of Ni$^{2+}$ in bicarbonate Ringer. A: addition of 100 μM Ni$^{2+}$ consistently increased the AMPA-evoked alkaline shift. B: alkaline shifts evoked by repetitive antidromic stimulation were enhanced similarly after addition of Ni$^{2+}$. C: in the presence of 10 μM benzolamide, Ni$^{2+}$ reduced the alkaline shift.](http://jn.physiology.org/)

![FIG. 7. AMPA-evoked alkaline shift is supported by Ba$^{2+}$. A: barium-dependent alkaline shift in HEPES Ringer. After block of the response in 0-Ca$^{2+}$/EGTA, the EGTA was washed out. Alkaline shift remained inhibited in the 0-Ca$^{2+}$/HEPES Ringer. After addition of 500 μM Ba$^{2+}$, a smaller AMPA-evoked alkaline shift could be elicited. B: barium-dependent alkaline shift in bicarbonate Ringer. After superfusion of 0-Ca$^{2+}$/EGTA Ringer, followed by 0-Ca$^{2+}$ Ringer, the alkaline shift was abolished. Response returned after addition of 500 μM Ba$^{2+}$. Barium-dependent alkaline shift was amplified by addition of 10 μM benzolamide.](http://jn.physiology.org/)
protons (in a ratio of 2 \( \text{H}^+ : 1 \text{Ba}^{2+} \)), then the expected extracellular \( \Delta S_{\text{SID}} \) would have been 100 \( \mu \text{M} \). With a mean measured alkaline shift of \( \sim 0.03 \) unit pH and a calculated buffering capacity of 15 m\( \text{M} \), the experimental \( \Delta S_{\text{SID}} \) would be \( \sim 450 \) \( \mu \text{M} \). 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 \( \text{Ba}^{2+} \) ions by this transporter and cycling of \( \text{Ba}^{2+} \), 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 \( \sim 0.03 \) unit pH, corresponding to a \( \Delta S_{\text{SID}} \) of 450 \( \mu \text{M} \). Although these alkalizations 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 can 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 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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