Extracellular pH Changes and Accompanying Cation Shifts During Ouabain-Induced Spreading Depression

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Menna, G., C. K. Tong, and M. Chesler. Extracellular pH changes and accompanying cation shifts during ouabain-induced spreading depression. J. Neurophysiol. 83: 1338–1345, 2000. Interstitial ionic shifts that accompany ouabain-induced spreading depression (SD) were studied in rat hippocampal and cortical slices in the presence and absence of extracellular Ca\(^{2+}\). A double-barreled ion-selective microelectrode specific for H\(^+\), K\(^+\), Na\(^+\), or Ca\(^{2+}\) was placed in the CA1 stratum radiatum or midcortical layer. Superfusion of 10\(\mu\)M ouabain caused a rapid, negative, interstitial voltage shift (2–10 mV) after 3–5 min. The negativity was accompanied by a rapid alkaline transient followed by prolonged acidosis. In media containing 3 mM Ca\(^{2+}\), the alkalosis induced by ouabain averaged 0.07 ± 0.01 unit pH. In media with no added Ca\(^{2+}\) and 2 mM EGTA, the alkaline shift was not significantly different (0.09 ± 0.02 unit pH). The alkaline transient was unaffected by inhibiting Na\(^+\)-H\(^+\) exchange with ethylisopropylamiloride (EIPA) or by blocking endoplasmic reticulum Ca\(^{2+}\) uptake with thapsigargin or cyclopiazonic acid. Alkaline transients were also observed in Ca\(^{2+}\)-free media when SD was induced by microinjecting high K\(^+\). The late acidification accompanying ouabain-induced SD was significantly reduced in Ca\(^{2+}\)-free media and in solutions containing EIPA. The ouabain-induced SD was associated with a rapid but relatively modest increase in [K\(^+\)]\(_o\). In the presence of 3 mM external Ca\(^{2+}\), the mean peak elevation of [K\(^+\)]\(_o\) was 12 ± 0.62 mM. In Ca\(^{2+}\)-free media, the elevation of [K\(^+\)]\(_o\) had a more gradual onset and reached a significantly larger peak value, which averaged 22 ± 1.1 mM. The decrease in [Na\(^+\)]\(_o\) that accompanied ouabain-induced SD was somewhat greater. The [Na\(^+\)]\(_o\) decreased by averages of 40 ± 7 and 33 ± 3 mM in Ca\(^{2+}\) and Ca\(^{2+}\)-free media, respectively. In media containing 1.2 mM Ca\(^{2+}\), ouabain-induced SD was associated with a substantial decrease in [Ca\(^{2+}\)]\(_o\), that averaged 0.73 ± 0.07 mM. These data demonstrate that in comparison with conventional SD, ouabain-induced SD exhibits ion shifts that are qualitatively similar but quantitatively diminished. The presence of external Ca\(^{2+}\) can modulate the phenomenon but is irrelevant to the generation of the SD and its accompanying alkaline pH transient. Significance of these results is discussed in reference to the propagation of SD and the generation of interstitial pH changes.

INTRODUCTION

Extracellular ionic shifts associated with spreading depression (SD) have been described in a variety of preparations across several species (Kraig and Nicholson 1978; Kraig et al. 1983; Mutch and Hansen 1984; Nicholson et al. 1977; Somjen 1984; Vyskocil et al. 1972). Increasing significance has been attributed to ionic changes during SD in view of their putative role in ischemic brain injury (Balestrino et al. 1989; Iijima et al. 1992; Nedergaard and Astrup 1986). The hallmarks of these phenomena include a rapid, propagating increase in interstitial potassium concentration ([K\(^+\)]\(_o\), etc.) to ≈50 mM, associated with a negative shift in interstitial electric potential. Abrupt [Na\(^+\)]\(_o\) and [Cl\(^-\)]\(_o\) decreases of similar magnitude occur with the [K\(^+\)]\(_o\) change, along with a decrease in [Ca\(^{2+}\)]\(_o\) from 1 to 0.1 mM. The ionic displacements are typically accompanied by pH changes consisting of a rapid interstitial alkaline shift followed by prolonged acidosis (Kraig et al. 1983; Mutch and Hansen 1984; Nicholson et al. 1984; Somjen 1984). These endogenous pH\(_o\) shifts can significantly influence the initiation and propagation of SD (Tong and Chesler 1999).

The mechanisms that give rise to these pH\(_o\) shifts, and their relationship to the processes responsible for SD, are not understood. Related factors implicated in the generation of SD include the elevation of [K\(^+\)]\(_o\) and the release of glutamate (Grafstein 1956; Van Harreveld 1959). Evidence suggests that the accompanying influx of Ca\(^{2+}\) plays a significant role in SD (Jing et al. 1993). Elevation of internal Ca\(^{2+}\) may serve to activate vesicular release of glutamate, furthering the regenerative process responsible for SD propagation. In addition, it has been suggested that resulting waves of intracellular Ca\(^{2+}\) are linked to the mechanism of SD (Cornell-Bell et al. 1990; Kunkler and Kraig 1998; Nedergaard 1994).

The interstitial pH\(_o\) shifts tied to SD could also be related to the influx of Ca\(^{2+}\). Synchronous neural activity generates an alkaline pH\(_o\) transient with a marked dependence on external Ca\(^{2+}\) (Griffithchenko and Chesler 1996; Paalasmaa and Kaila 1996; Paalasmaa et al. 1994; Smith et al. 1994). It has been suggested that such alkalinizations arise from the Ca\(^{2+}\)-H\(^+\) exchange property of the plasmalemmal CaATPase (Schwiening et al. 1993). Thus it is compelling to speculate that the rapid alkalization at the onset of SD may also be triggered by the large initial influx of Ca\(^{2+}\) (Nicholson et al. 1977).

Although Ca\(^{2+}\) ions may play a significant role in SD, the fundamental properties of this phenomenon may not be reliant on the influx of Ca\(^{2+}\). In brain slices, SD has been reported in low Ca\(^{2+}\) solutions (Snow et al. 1983; Yaari et al. 1986). In addition, a form of SD can be triggered in the absence of external Ca\(^{2+}\) by the application of ouabain and, on occasion, by focal elevation of extracellular K\(^+\). This Ca\(^{2+}\)-independent event was recorded as an increase in light transmittance associated with a small negative shift of extracellular potential that propagated more slowly than conventional SD (Basarsky et al. 1998).

It is unknown whether the ionic shifts that accompany the ouabain-induced SD variant are qualitatively or quantitatively...
similar to those associated with conventional SD. This information would help place such events within the established context of SD and provide insights into the mechanisms of propagation. Moreover, investigating the interstitial pH changes accompanying this form of SD could directly address their relationship to Ca$^{2+}$ ions. We therefore studied the ionic changes that accompany ouabain-induced SD in the presence and absence of external Ca$^{2+}$. Some of the results appeared in an abstract (Menna et al. 1999).

**METHODS**

Slices of hippocampus and overlying cortical regions (300–400 µM thick) were prepared from anesthetized adult Sprague-Dawley rats (4–5 wk old). Cortical slices were used in only one set of experiments in which ethylisopropyl amiloride was used to block Na$^+$/H$^+$ exchange (see RESULTS). Slices were incubated for ≥90 min in artificial cerebral spinal fluid (ACSF) at room temperature and then transferred to a submersion-style slice chamber maintained at 32°C. The ACSF contained (in mM) NaCl 124, NaHCO$_3$ 26, KCl 3, CaCl$_2$ 3, MgCl$_2$ 1.5, Na$_2$HPO$_4$ 1, glucose 10, equilibrated with 95% O$_2$-5% CO$_2$ to achieve a nominal pH of 7.4. Because the voltage change on an ion-selective microelectrode depends on the logarithm of the ion concentration, the lower baseline [Ca$^{2+}$]o allows for an increased voltage response per absolute change in [Ca$^{2+}$]o. For the recordings of [Ca$^{2+}$]o, the concentration of CaCl$_2$ was therefore reduced to 1.2 mM. In HEPES-buffered ACSF, NaHCO$_3$ was omitted and 26 mM HEPES was titrated with NaOH to a pH of 7.4. NaCl was adjusted to maintain a constant sodium concentration. Cyclopiazonic acid and thapsigargin were obtained from Sigma and Alomone Laboratories, respectively. Benzolamide was a gift from Lederle Laboratories.

Solutions without added CaCl$_2$ contained 2 mM EGTA. NaHCO$_3$ was increased to 28 mM to compensate for acidity caused by EGTA. In three hippocampal slices, Ca$^{2+}$-selective microelectrodes were used to record the decrease in [Ca$^{2+}$]o during transition to Ca$^{2+}$-free ACSF with EGTA. The [Ca$^{2+}$]o decreased to ~1 µM within 5 min. In experiments in which SD was studied in the absence of Ca$^{2+}$, slices were superfused with 0 Ca$^{2+}$/EGTA solution for 15–20 min before the addition of ouabain.

Investigating the Ca$^{2+}$-dependence of SD-related alkaline shifts was a particular focus of the present study. A bicarbonate-dependent alkalization that is not dependent on Ca$^{2+}$ entry can be triggered by GABA$_A$ receptors (Chesler and Kaila 1992). Because the occurrence of such pH changes would have confounded the interpretation of results, 100 µM picrotoxin was added to the ACSF to block GABA$_A$ receptors. To ensure similar conditions during the [K$^+$]/[Na$^+$] and [Ca$^{2+}$]o studies, picrotoxin was added during these experiments as well. Studies of [K$^+$]/[Na$^+$] and pH in the absence of picrotoxin suggested that ionic changes during SD were similar whether or not this drug was present (see RESULTS).

Construction of double-barreled ion-sensitive microelectrodes (tip diameter 3–5 µm) and the method of recording and calibrating extracellular ion shifts have been described in Chesler and Chan (1988). Ion-sensitive barrels responsive to K$^+$, Na$^+$, Ca$^{2+}$, and pH contained Fluka ion-selective cocktails 60398, 71178, 21048, and 95291, respectively. The ion electrodes were calibrated with appropriate reference solutions spanning the ion concentration range encountered during the experiments. Because the Na$^+$-specific cocktail (Fluka 71178) does not have negligible sensitivity to K$^+$, the response was tested in calibration solutions containing 30 mM K$^+$, which was near the upper range of [K$^+$] encountered in these experiments. With this background [K$^+$], the Na$^+$-selective microelectrodes displayed a slope response of 50–54 mV per decade change in [Na$^+$] between 150 and 75 mM, which was the concentration range relevant for these experiments. Potentials on the reference barrels were subtracted continuously from the ion barrel potentials to yield the ion signal, which was monitored on a chart recorder.

Electrodes were lowered into the stratum radiatum of hippocampal slices or the midcortical layer of cortex slices to a tissue depth of ~150 µm. In most experiments, superfusion of 100 µM ouabain was used to induce SD. In some cases, slices were studied in an interface chamber and SD was initiated by a brief (20–100 ms) ejection of 1.2 M KCl into the CA1 stratum radiatum via a micropipette connected to a Picospritzer (General Valve Corp.). In Ca$^{2+}$-free media, however, SD could not be reliably induced by KCl injection, limiting the use of this protocol to just a few experiments. Measurements of potential changes were obtained directly from the chart record. Statistics were expressed as means ± SE. Comparisons were made with a two-tailed t-test. Values of n refer to the number of slices.

**RESULTS**

**SD induced by ouabain**

In hippocampal slices, superfusion of 100 µM ouabain induced SD after 3–5 min, as evidenced by a sudden negative DC shift between 2 and 10 mV (Basarsky et al. 1998). Among 84 slices from all experiments, the mean DC shift was −3.6 ± 0.2 mV. There was no significant difference in the amplitude of the DC shift in 3 mM Ca$^{2+}$ versus Ca$^{2+}$-free solution (see DISCUSSION).

**Shifts in extracellular pH during ouabain-induced SD**

In ACSF containing 3 mM Ca$^{2+}$, the negative DC shift was accompanied by rapid alkalization (Fig. 1A). The alkaline transient was followed by slow acidosis, as has been noted for SD in vivo (Kraig et al. 1983; Mutch and Hansen 1984; Somjen 1984). In media containing 100 µM picrotoxin, the initial alkalization averaged 0.07 ± 0.01 unit pH and was followed by a mean net acid shift of 0.21 ± 0.04 unit pH (n = 5). When picrotoxin was omitted, the alkaline and acid shifts were not significantly altered, averaging 0.08 ± 0.01 and 0.24 ± 0.03, respectively (n = 6).

In a number of studies, extracellular alkaline shifts evoked
by neural activity were inhibited after external Ca$^{2+}$ was removed (Grichtchenko and Chesler 1996; Paalasmaa and Kaila 1996; Paalasmaa et al. 1994; Smith et al. 1994). However, the alkalinization accompanying the ouabain-evoked SD was unaffected by withdrawal of Ca$^{2+}$ (Fig. 1B). In 0 Ca$^{2+}$ ACSF containing 2 mM EGTA, the mean alkalosis at the onset of SD was 0.09 ± 0.02 unit pH ($n = 6$), which was not significantly different from the alkalosis in media containing Ca$^{2+}$. The subsequent acidosis averaged 0.11 ± 0.02 unit pH, which was significantly diminished ($P < 0.05$) compared with the mean acid shift in the presence of Ca$^{2+}$.

To determine whether or not the Ca$^{2+}$-independent alkaline shift was a feature limited to SD induced by ouabain, we conducted experiments in an interface chamber, using microinjection of 1.2 M KCl to trigger SD. The resulting responses were recorded by a proximal double-barreled pH microelectrode ($pH_o$ and $V_1$) and a more distal single-barreled voltage electrode ($V_2$) as illustrated in Fig. 2A. In 0 Ca$^{2+}$ ACSF containing 2 mM EGTA, the mean alkalosis at the onset of SD was 0.09 ± 0.02 unit pH ($n = 6$), which was not significantly different from the alkalosis in media containing Ca$^{2+}$. The subsequent acidosis averaged 0.11 ± 0.02 unit pH, which was significantly diminished ($P < 0.05$) compared with the mean acid shift in the presence of Ca$^{2+}$.

Does bicarbonate efflux cause the alkalosis during ouabain-induced SD?

Efflux of bicarbonate ions across anion channels can cause a rapid extracellular alkaline shift (Kaila and Voipio 1987). The inclusion of picrotoxin in the ACSF precluded the generation of such responses via GABA$_A$ anion channels; however, alternate anion pathways could not be excluded. Benzolamide is a poorly permeant drug that blocks the dehydration of carbonic acid catalyzed by interstitial carbonic anhydrase, and thereby inhibits bicarbonate-mediated alkaline shifts. In contrast, external alkalosis that arises from a bicarbonate-independent net proton sink is amplified by this carbonic anhydrase inhibitor (Chesler and Kaila 1992). To distinguish these two forms of alkalosis, we observed the $pH_o$ shifts during ouabain-evoked SD in 0 Ca$^{2+}$ ACSF containing 10 μM benzolamide. Under these conditions, the SD was associated with an alkaline shift (Fig. 3A) of 0.19 ± 0.04 unit pH ($n = 6$) that was significantly greater than the mean alkalosis in the absence of benzolamide ($P < 0.05$). The slow acid shift was not significantly affected by benzolamide ($P = 0.6$), averaging 0.12 ± 0.02 unit pH.

FIG. 2. Recording of SD evoked by K$. A$: experimental arrangement. SD was elicited by ejection of 1.2 M KCl via a micropipette in the CA1 stratum radiatum. Recordings were made with a dual-barrel pH microelectrode ~500 μM distant and a single-barrel microelectrode located 500 μM closer to the CA3 region. B: ejection of KCl ($\downarrow$) elicited SD. The initial pH response was an acid shift that was interrupted by an alkaline transient (*), which resulted in a triphasic waveform. The onset at the second electrode ($V_2$) revealed a propagation velocity of ~1 mm/min. Solutions contained 100 μM picrotoxin.

FIG. 3. Ouabain-induced alkaline shift is not dependent on bicarbonate. A: in ACSF containing 10 μM benzolamide, a robust alkaline shift was noted that was significantly greater than control responses. The solution contained 100 μM picrotoxin. B: ouabain-evoked SD could be elicited in bicarbonate-free ACSF buffered with HEPES. In this solution, an early alkaline shift was still noted.
To more directly address whether or not bicarbonate was necessary for the alkaline shift, ouabain-induced SDs were studied in bicarbonate-free ACSF buffered with 26 mM HEPES. In this solution, an alkaline-acid sequence still occurred. The alkaline transient averaged 0.05 ± 0.01 (n = 4) and was followed by a mean acid shift of 0.04 ± 0.01 unit pH (Fig. 3B).

Pharmacological studies of pH shifts during ouabain-induced SD

In view of the Na⁺ influx accompanying SD, it is plausible that slowing or reversal of Na⁺-H⁺ exchange could underlie interstitial alkalosis. To test this hypothesis, ouabain-induced SD was studied in the presence and absence of the Na⁺-H⁺ exchange blocker ethylisopropylamiloride (EIPA), a derivative of amiloride. Because Na⁺-H⁺ exchange in hippocampal CA1 pyramidal neurons is insensitive to amiloride (Raley-Susman et al. 1991; Schwiening and Boron 1994), recordings were made in cortical slices. In cortical neurons the Na⁺-H⁺ exchanger is inhibited by amiloride (Ou-yang et al. 1993).

In control cortical slices, superfusion of ouabain caused a negative DC shift and typical alkaline-acid transients that averaged 0.08 ± 0.02 and 0.26 ± 0.05 unit pH, respectively (n = 4) (Fig. 4A). In the presence of 100 μM EIPA, superfusion of ouabain did not readily induce SD. In five slices in which SD was evoked, an alkaline-acid sequence occurred that averaged 0.08 ± 0.02 and 0.10 ± 0.04 unit pH, respectively (Fig. 4B). The mean acid shift in EIPA was smaller than the control value (P < 0.05); however, the alkalosis was not significantly different from the control.

The persistence of the alkaline shift in the nominal absence of extracellular Ca²⁺ indicates that influx of Ca²⁺ ions is not necessary for the generation of this pH change. However, this observation does not exclude the involvement of Ca²⁺ release from internal stores. To test whether or not Ca²⁺ liberated from endoplasmic reticulum (ER) played a role in generating the alkalosis, ouabain-induced SD was induced after slices were superfused for 20–30 min with 0 Ca²⁺ ACSF containing cyclopiazonic acid or thapsigargin, which are inhibitors of Ca²⁺ uptake into the endoplasmic reticulum. In ACSF containing 10 μM cyclopiazonic acid, the ouabain-induced SD in hippocampal slices was associated with an early alkaline shift of 0.07 ± 0.13 unit pH (n = 5), which was not significantly different from controls in the absence of the drug (P = 0.6). A similar response occurred in 5 μM thapsigargin, where the mean alkaline shift was 0.09 ± 0.01 unit pH (n = 4). Basarsky and colleagues (1998) reported no increase in [Ca²⁺]ᵢ in hippocampal slices during ouabain-induced SD in Ca²⁺-free me-
Shifts in $[K^+]_o$, $[Na^+]_o$, and $[Ca^{2+}]_o$ during ouabain-induced SD

In normal Ca$^{2+}$-containing ACSF, superfusion of ouabain had little or no effect on $[K^+]_o$ until the onset of a negative DC shift, whereupon $[K^+]_o$ underwent a sudden increase to a mean value of $12 \pm 0.62$ mM ($n = 6$) (Fig. 5A). In the absence of picrotoxin, the elevation of $[K^+]_o$ was similar, averaging $10.9 \pm 1.08$ mM ($n = 7$).

In 0 Ca$^{2+}$ ACSF, the elevation of $[K^+]_o$ began with a gradual increase as ouabain was washed into the slice (Fig. 5B). The negative DC shift was accompanied by a second, more rapid $[K^+]_o$ elevation to a peak value of $22 \pm 1.1$ mM ($n = 7$). The $[K^+]_o$ elevation in 0 Ca$^{2+}$ ACSF was significantly greater than the level attained in the presence of external Ca$^{2+}$ ($P < 0.001$), although both values were considerably smaller than the $[K^+]_o$ increase reported for conventional SD in hippocampal slices (Yaari et al. 1986).

Recordings of $[Na^+]_o$ also revealed a relatively small ionic shift (Fig. 6). In normal ACSF containing Ca$^{2+}$, the ouabain-induced SD caused a mean decrease in $[Na^+]_o$ of $40 \pm 7$ mM ($n = 6$). In 0 Ca$^{2+}$ ACSF, the change in $[Na^+]_o$ was not significantly different ($P = 0.5$), decreasing by $33 \pm 3$ mM ($n = 7$).

Recordings of interstitial Ca$^{2+}$ were obtained in ACSF containing 1.2 mM Ca$^{2+}$. A lower baseline [Ca$^{2+}$]$_i$ was used in these experiments to maximize the millivolt response per change in [Ca$^{2+}$]$_i$. In this solution, the ouabain-evoked SD was accompanied by a rapid decrease of [Ca$^{2+}$]$_i$ as shown in Fig. 7. The average decrease in [Ca$^{2+}$]$_i$ was $0.73 \pm 0.07$ mM to a mean value of $0.47 \pm 0.07$ mM.

**DISCUSSION**

The events contributing to conventional SD, although rare in normal tissue, are likely to play an important role in the way the brain responds to hypoxic and ischemic insults. Complete brain ischemia is shortly followed by an SD-like event (Hansen 1985), and focal ischemic lesions can be the source of multiple propagating SDs of long duration (Iijima et al. 1992; Nedergaard and Astrup 1986). In addition to the probable pathological importance of SD-like mechanisms, the study of such phenomena is useful for understanding brain ion homeostasis. The ionic shifts associated with SD-like events have a qualitative resemblance to those of normal activity but are far greater in magnitude and likely involve a commensurately greater role of channel- and transporter-mediated ion pathways. The SD variant elicited by superfusion of ouabain may be particularly useful in the dissection of such events because it can be reliably evoked in the absence of external Ca$^{2+}$ in a submersion-style brain slice chamber (Basarsky et al. 1998). By contrast, SD elicited by focal injection of K$^+$, although more faithful to established models of SD, could not be reliably elicited in Ca$^{2+}$-free media. Thus ouabain-induced SD was useful as a trigger of extracellular pH shifts, thereby providing a novel means by which the Ca$^{2+}$ dependence of these acid-base changes could be addressed. A second goal of this study was to measure the ionic shifts of ouabain-induced SD so that this event could be understood in relation to conventional SD.

In the adult brain, synchronous neuronal activity is associated with elevation of $[K^+]_o$ that does not breach a ceiling level of $\sim 12$ mM (Heinemann and Lux 1977). In contrast, SD has been characterized by an increase of $[K^+]_o$ that can exceed 50 mM (Kraig and Nicholson 1978; Nicholson et al. 1977; Vykocil et al. 1972). In the present study, SD initiated by ouabain resulted in a relatively small elevation of $[K^+]_o$. In media containing extracellular calcium, the mean elevation was only 12 mM and never exceeded 14 mM. This was surprising because inhibition of the Na$^+$-K$^+$ ATPase by ouabain might be expected to cause a higher elevation of $[K^+]_o$, as was noted by Kraig and coworkers (1983) in the cerebellar cortex. It is likely that the small elevation in K$^+$ was partly caused by egress of this ion from the tissue into the extracellular medium. However, there was a significantly greater increase of $[K^+]_o$ in the absence of extracellular Ca$^{2+}$, where the mean elevation reached 22 mM and the maximum was 28 mM. The tendency of extracellular Ca$^{2+}$ to limit the increase of $[K^+]_o$ was also evident during the introduction of ouabain. Before the onset of SD, $[K^+]_o$ gradually increased in the absence of Ca$^{2+}$ whereas in the presence of Ca$^{2+}$, the baseline $[K^+]_o$ remained stable until the occurrence of the negative voltage shift. It is unclear
why the baseline $[\text{K}^+]_o$ did not increase in media containing $\text{Ca}^{2+}$ as would be expected with inhibition of the $\text{Na}^+\text{-K}^+$ pump. A partial explanation may be the stabilizing effect of $\text{Ca}^{2+}$-dependent $\text{K}^+$ conductances, which would limit neuronal depolarization and cause a smaller efflux of $\text{K}^+$ ions. In this respect, effective depolarization brought about by loss of surface charge screening in $\text{Ca}^{2+}$-free media may also play a role.

Although the elevation of $[\text{K}^+]_o$ was greater in $\text{Ca}^{2+}$-free media, the presence or absence of $\text{Ca}^{2+}$ did not affect the mean extracellular DC shift. There was, however, considerable variability in the amplitude of the DC shift for similar elevations of $[\text{K}^+]_o$. These observations may stem from a variable site of initiation and a variable wave front of $[\text{K}^+]_o$ elevation when the SD-like event is initiated in different slice preparations. In addition, to the extent that standing extracellular currents contributed to the negative DC shifts, differences in interstitial volume or in the fluid level within the submersion chamber could have led to a variable shunting of such currents.

Ouabain-induced SD was associated with a shift in $[\text{Na}^+]_o$ of $\sim30$ mM. This observation distinguishes this SD from repetitive neuronal activity where the decrease in $[\text{Na}^+]_o$ is an order of magnitude smaller (Dietzel et al. 1982). However, in conventional SD, the decrease in $[\text{Na}^+]_o$ is far greater, typically exceeding 60 mM (Hansen and Zeuthen 1981; Kraig and Nicholson 1978). The relatively small decrease in $[\text{Na}^+]_o$ during ouabain-induced SD is consistent with the limited increase in $[\text{K}^+]_o$. Because net ionic shifts during SD will be determined by the sum of energetically uphill ion transport and downhill ionic fluxes, in the face of $\text{Na}^+\text{-K}^+$ pump blockade, the modest net ion shifts probably reflect a limited channel-mediated flux of $\text{Na}^+$ and $\text{K}^+$ compared with conventional SD. It is possible that ouabain caused a slow depolarization and early inactivation of voltage-gated channels, curtailing the movement of $\text{Na}^+$ and $\text{K}^+$.

Despite the modest size of the $[\text{K}^+]_o$ and $[\text{Na}^+]_o$ transients, ouabain-induced SD has been shown to propagate. In hippocampal slices, Basarsky and colleagues (1998) measured a conduction velocity of $\sim1$ mm per minute. This is considerably slower than other forms of SD that spread at greater velocities. In conventional SD, the decrease in $[\text{Na}^+]_o$ is far greater, typically exceeding 60 mM (Hansen and Zeuthen 1981; Kraig and Nicholson 1978). The relatively small decrease in $[\text{Na}^+]_o$ during ouabain-induced SD is consistent with the limited increase in $[\text{K}^+]_o$. Because net ionic shifts during SD will be determined by the sum of energetically uphill ion transport and downhill ionic fluxes, in the face of $\text{Na}^+\text{-K}^+$ pump blockade, the modest net ion shifts probably reflect a limited channel-mediated flux of $\text{Na}^+$ and $\text{K}^+$ compared with conventional SD. It is possible that ouabain caused a slow depolarization and early inactivation of voltage-gated channels, curtailing the movement of $\text{Na}^+$ and $\text{K}^+$.

The pH$_o$ transients that accompanied ouabain-induced SD were characterized by an early alkalosis followed by a prolonged acid shift. A similar pattern was noted whether or not $\text{Ca}^{2+}$ was present in the interstitial fluid. This alkaline-acid pattern has been reported for conventional SD in vivo (Kraig et al. 1983) and in vitro (Martins-Ferreira and Do Carmo 1987; Taira et al. 1992) and is also characteristic of synchronous neural activity in a variety of brain regions (Chesler 1990; Chesler and Kaila 1992).

The late acid shift is thought to arise from the generation of carbon dioxide and lactic acid because it can be blocked by metabolic inhibitors (Kraig et al. 1983) and it is accompanied by a large increase in interstitial carbon dioxide concentration (Taira et al. 1992). The appearance of late acidosis in the presence of ouabain indicates that an energetic load other than $\text{Na}^+\text{-K}^+$ ATPase was responsible for the generation of acid, as was noted by Kraig and colleagues (1983) in cerebellar cortex. Because acidosis was larger in the presence of external $\text{Ca}^{2+}$, a component of the acid response may be caused by mitochondrial $\text{Ca}^{2+}\text{-H}^+$ exchange (Meech and Thomas 1980). Suppression of late acidosis in the presence of EIPA suggests that $\text{Na}^+\text{-H}^+$ exchange played a role in bringing cellular acid into the interstitial compartment. In some preparations, stimulus-evoked acid shifts appeared to be similarly dependent on $\text{Na}^+\text{-H}^+$ exchange (Rose and Deitmer 1995; Walz 1989). However, in all of these instances, it remains uncertain whether or not the electrical responses that gave rise to the alkaline shifts were diminished by the $\text{Na}^+\text{-H}^+$ exchange inhibitor. In this context it is notable that in hippocampal slices, acid shifts associated with electrical stimulation (Voipio and Kaila 1993) and SD (Taira et al. 1992) could be explained solely by the accumulation of interstitial CO$_2$.

The onset of ouabain-induced SD was marked by a rapid interstitial alkaline shift. In ACSF containing bicarbonate, these experiments were performed in the presence of picrotoxin to eliminate possible contributions of GABAergic bicarbonate fluxes. It is plausible that GABAergic bicarbonate fluxes contribute to the pH$_o$ shifts during SD; however, this topic was not addressed.

The picrotoxin-resistant alkalosis that occurs during repetitive neural activity has several features in common with the SD alkaline transient. Both responses were unaffected by $\text{Na}^+\text{-H}^+$ exchange inhibitors (Chesler and Chan 1988), were increased by benzolamide, and persisted in HEPES-buffered media (Chesler and Kaila 1992). Unlike stimulus-evoked alkaline shifts, however, SD-associated alkalosis was unaffected by the removal of extracellular $\text{Ca}^{2+}$. It is possible that a $\text{Ca}^{2+}$-dependent component of the alkaline transient was obscured by masking effects of $\text{Ca}^{2+}$-dependent acidosis. Nonetheless, the persistence of a robust alkaline shift after removal of $\text{Ca}^{2+}$ stands in marked contrast to previous studies of stimulus-evoked alkalinations (Grichchenko and Chesler 1996; Paalasmaa and Kaila 1996; Paalasmaa et al. 1994; Smith et al. 1994). Moreover, the generation of a $\text{Ca}^{2+}$-independent alkaline shift was not a feature unique to the use of ouabain because a $\text{Ca}^{2+}$-independent alkaline transient also accompanied SD evoked by high $\text{K}^+$ (Fig. 2).

The alkaline transient was also unaffected by thapsigargin and cyclopiazonic acid, which would be expected to deplete $\text{Ca}^{2+}$ stores of the endoplasmic reticulum. These observations all suggest that the $\text{Ca}^{2+}\text{-H}^+$ exchange property of the plas-
malemmal Ca ATPase is not the basis for this pH change. An issue that arises is whether or not the same bicarbonate-independent mechanism accounts for the alkaline shifts during SD and during repetitive neuronal activity. The observed difference in Ca\textsuperscript{2+}-dependence is an argument for different mechanisms. Indeed, it was recently reported that a component of activity-dependent alkalosis can persist in the absence of external Ca\textsuperscript{2+} (Smith and Chesler 1999), which is consistent with more than one process. The relationship between the influx of Ca\textsuperscript{2+} and accompanying acid-base fluxes remains unclear.

In summary, these results demonstrate that ouabain-induced SD is associated with shifts in pH\textsubscript{o}, [K\textsuperscript{+}]o, [Na\textsuperscript{+}]o, and [Ca\textsuperscript{2+}]o that are qualitatively similar to those accompanying conventional SD but that display marked quantitative differences. The changes in [K\textsuperscript{+}]o and [Na\textsuperscript{+}]o are comparatively modest, which may be in part caused by exchange with the superfusion fluid. The decrease in [Ca\textsuperscript{2+}]o accompanying this form of SD can be substantial and may influence aspects of the SD but is not a necessary feature. The acid-base shifts show a mixed dependence on Ca\textsuperscript{2+}. The early alkaline transient, coinciding with the negative interstitial DC shift, appears to be independent of Ca\textsuperscript{2+} whereas the slow acidosis that follows is markedly diminished in the absence of Ca\textsuperscript{2+}. Whether or not these pH\textsubscript{o} shifts share the same mechanisms with the pH\textsubscript{0} changes accompanying normal synchronous neural activity remains to be determined.

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