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Mechanisms of H^+ and Na^+ Changes Induced by Glutamate, Kainate, and D-Aspartate in Rat Hippocampal Astrocytes

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The excitatory transmitter glutamate (Glu), and its analogs kainate (KA), and D-aspartate (D-Asp) produce significant pH changes in glial cells. Transmitter-induced pH changes in glial cells, generating changes in extracellular pH, may represent a special form of neuronal–glial interaction. We investigated the mechanisms underlying these changes in intracellular H^+ concentration ($[H^+]_i$) in cultured rat hippocampal astrocytes and studied their correlation with increases in intracellular Na^+ concentration ($[Na^+]_i$), using fluorescence ratio imaging with 2′,7′-bis(carboxyethyl)-5,6-carboxyfluorescein (BCECF) or sodium-binding benzofuran isophthalate (SBFI). Glu, KA, or D-Asp evoked increases in $[Na^+]_i$; Glu or D-Asp produced parallel acidifications. KA, in contrast, evoked biphasic changes in $[H^+]_i$, alkaline followed by acid shifts, which were unaltered after Ca^{2+} removal and persisted in 0 Cl^--saline, but were greatly reduced in CO_2/HCO_3^--free or Na^+-free saline, or during 4,4′-diisothiocyanato-stilbene-2,2′-disulphonic acid (DIDS) application. The non-NMDA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) blocked KA-evoked changes in $[H^+]_i$ and $[Na^+]_i$, indicating that they were receptor–ionophore mediated. In contrast, CNQX increased the $[H^+]_i$ change and decreased the $[Na^+]_i$ change induced by Glu. D-Asp, which is transported but does not act at Glu receptors, induced $[H^+]_i$ and $[Na^+]_i$ changes that were virtually unaltered by CNQX. Our study indicates that $[Na^+]_i$ increases are not primarily responsible for Glu- or KA-induced acidifications in astrocytes. Instead, intracellular acidifications evoked by Glu or D-Asp are mainly caused by transmembrane movement of acid equivalents associated with Glu/Asp-uptake into astrocytes. KA-evoked biphasic $[H^+]_i$ changes, in contrast, are probably attributable to transmembrane ion movements mediated by inward, followed by outward, electrogenic Na^+/HCO_3^- cotransport, reflecting KA-induced biphasic membrane potential changes.

Key words: glial cell; glutamate; kainate; aspartate; intracellular sodium; intracellular pH; ion regulation; glutamate transport; ionotropic glutamate receptor; Na^+/HCO_3^- cotransport

A well-known action of neurotransmitters in the brain is alteration of intra- and extracellular Na^+, K^+, Ca^{2+}, and/or Cl^- concentrations by activation of specific receptors or uptake mechanisms. In addition, many transmitters, like the excitatory amino acid L-glutamate (Glu) and its analog D-aspartate (D-Asp) induce an acidification in both neurons and glial cells (Bouvier et al., 1992; Deitmer and Munsch, 1992; Brookes and Turner, 1993; Deitmer and Schneider, 1996). Changes in glial cell pH can influence extracellular pH and, in so doing, potentially modulate the function of adjacent neurons (Ransom, 1992; Kaila et al., 1993; Taira et al., 1993, Gottfried and Chesler, 1994; Rose and Deitmer, 1994; Newman, 1996). It is, therefore, of broader neurobiological interest to elucidate the mechanisms that cause neurotransmitter-induced changes in glial pH.

The Glu- or D-Asp-induced intraglial acidification was suggested to be caused by a Glu transporter that exchanges intracellular K^+ and OH^- with extracellular Na^+ and Glu or D-Asp (Fig. 1*A*) (Bouvier et al., 1992). Surprisingly, kainate (KA), a Glu agonist, which is not a substrate for transport-mediated Glu uptake (Kimelberg et al., 1989), also caused an acidification in leech glial cells and cerebral astrocytes (Deitmer and Munsch, 1992; Brune and Deitmer, 1995).

Several mechanisms could account for the KA-induced acidification of glial cells. KA is a non-NMDA Glu-receptor agonist that binds to ionotropic Glu receptors and opens cation channels, generating Na^+ influx, and sometimes Ca^{2+} influx, and K^+ efflux (Fig. 1*A*) (von Blankenfeld and Kettenmann, 1991). The KA-induced acidification might be caused by influx of H^+ down its electrochemical gradient through these cation channels (Chen and Chesler, 1992; Deitmer and Munsch, 1992). Another hypothesis is that an increase in intracellular Ca^{2+} (Muller et al., 1992; Jabs et al., 1994; Munsch et al., 1994) activates a plasma membrane Ca^{2+}/H^+-ATPase, extruding Ca^{2+} in exchange for extracellular H^+ (Fig. 1*B*) (Schwiening et al., 1993; Paalasmaa et al., 1994).

Alternatively, the KA-induced acidification of glial cells could involve inhibition of cellular acid extrusion after an increase in intracellular Na^+ ($[Na^+]_i$). This hypothesis is attractive, because glial cells maintain a relatively alkaline intracellular pH primarily by Na^+-dependent transporters including Na^+/H^+ exchange, Na^+/HCO_3^- cotransport, and Na^+-dependent Cl^-/HCO_3^- exchange (Fig. 1*C*) (Chesler, 1990; Deitmer and Rose, 1996). A reduction in the inwardly directed Na^+ gradient after increases in $[Na^+]_i$ could, therefore, lead to decreased acid extrusion and intracellular acidification. To test this hypothesis, we studied the correlation between changes in intracellular H^+ concentration ($[H^+]_i$) and $[Na^+]_i$ induced by Glu, KA, or D-Asp in cultured rat hippocampal astrocytes using fluorescence ratio imaging with

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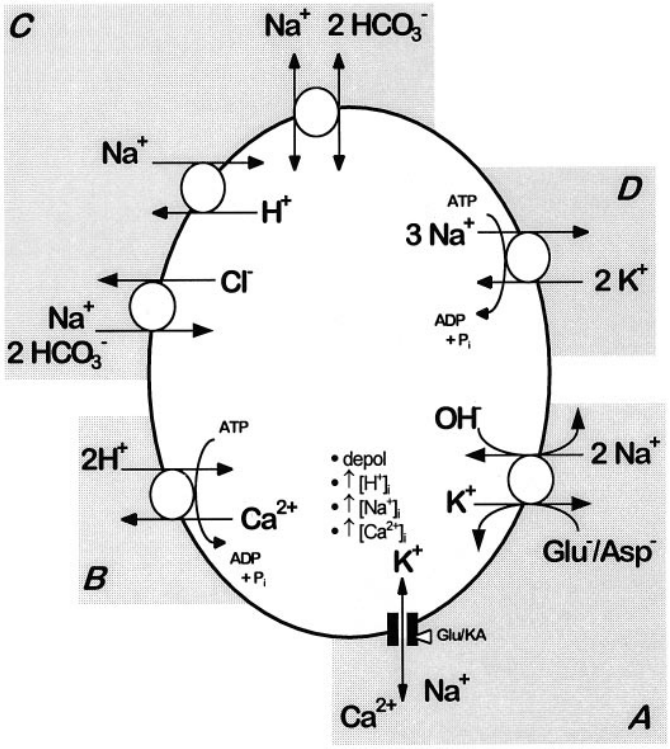

Figure 1. Model showing possible interrelationships between Glu-induced ion fluxes and $[H^+]_i$ regulation in hippocampal astrocytes. *Glu* and *D-Asp* are substrates for the electrogenic Glu transporter (*A*, *hatched area*), which exchanges extracellular Glu^- or D-Asp^- and 2 Na^+ for intracellular K^+ and OH^-. This causes cellular depolarization and acidification. Activation of ionotropic non-NMDA receptors by Glu or KA (indicated by *arrowhead*) depolarizes the cells by influx of Na^+, and sometimes Ca^{2+}, and efflux of K^+ through cation channels (*A*). Various secondary changes in $[H^+]_i$ could be caused by these actions of Glu. Additional intracellular acidification could originate secondary to increase in intracellular Ca^{2+} activating a Ca^{2+}/H^+ pump in the plasma membrane (*B*), and/or because of the increase in $[Na^+]_i$ attenuating cellular acid secretion by Na^+/H^+ exchange and Na^+-dependent Cl^-/HCO_3^- exchange (*C*). Cellular depolarization could cause intracellular alkalinization by stimulating inwardly directed electrogenic Na^+/HCO_3^- cotransport (*C*); a late hyperpolarization follows a period of elevated $[Na^+]_i$ attributable to Na^+, K^+-ATPase activity (*D*), and this could activate outwardly directed Na^+/HCO_3^- cotransport resulting in intracellular acidification.

BCECF-AM and SBFI-AM. We found that $[Na^+]_i$ increases are not primarily responsible for Glu- or KA-induced acidifications in hippocampal astrocytes. Our study confirms that the acidification evoked by Glu or D-Asp was most likely attributable to transport-mediated uptake and strongly suggests that KA-evoked $[H^+]_i$ changes were attributable to altered activity of Na^+/HCO_3^- co-transport in response to membrane potential changes.

MATERIALS AND METHODS

Cell cultures. Cell cultures of astrocytes were prepared as described previously (Sontheimer et al., 1991). To summarize, newborn Sprague–Dawley rats were narcotized with CO_2 and decapitated. Hippocampi were removed and exposed to 20 U/ml Papain (Worthington, Freehold, NJ) for 30 min. After trituration, cells were plated onto poly-D-lysine-coated (Sigma, St. Louis, MO) coverslips at a density of 80/mm^2 and grown in an incubator at 37°C and 5% CO_2/95% O_2. Cells were fed every 4 d with DMEM/F12 (Dulbecco's modified Eagle's medium) + 10% bovine serum (JRA Scientific, Lexington, KS). These cultures contained >95% astrocytes, as judged by immunostaining for glial fibrillary acidic protein (GFAP). The cells grew to confluence in about 10 d, and cells from 10–17 d *in vitro* were used for experiments.

For mixed neuron–glia cultures, rat hippocampi [embryonic day (E) 19] were removed and dissociated by a 10 min enzyme exposure to 10 U/ml Papain and trituration. The dissociated cells were plated onto poly-L-ornithine/laminin-coated (Sigma) coverslips in DMEM/F12 medium + 10% bovine serum. After 24 hr in culture, the cells were transferred into Neurobasal medium with B27 (50:1) (Life Technologies, Grand Island, NY), half of which was subsequently exchanged every 5–7 d. At day 8 in culture, the mitotic inhibitor cytosine-b-d-arabinofuranoside (ARA-C) (Sigma) was added to a final concentration of 5 μM to inhibit glial cell division. Measurements were made using cells between 14 and 21 d in culture. Neurons were identified by their morphological appearance in the light microscope and studies were limited to large pyramidal cells with at least three processes (length $\geq$ 4 times the cell diameter). Our experience is that these cells are always GFAP-negative and have typical neuronal electrical characteristics when impaled, including spontaneous action and synaptic potentials (our unpublished observations).

Solutions. The standard saline contained (in mM): 115.75 NaCl, 3 KCl, 2 $MgSO_4$, 2 $CaCl_2$, 1.25 NaH_2PO_4, 23 $NaHCO_3$, and 10 glucose, and was continuously bubbled with 5% CO_2/95% O_2 resulting in a pH of 7.38. The CO_2/HCO_3^--free saline was titrated to a pH of 7.4 with NaOH or HCl and contained the same amount of KCl, $MgSO_4$, $CaCl_2$, NaH_2PO_4, and glucose, but 126.25 mM NaCl and 25 mM HEPES.

The fluorescent dyes for measurement of intracellular H^+ (acetoxymethylester of 2′,7′-bis(carboxyethyl)-5,6-carboxyfluorescein (BCECF-AM) and Na^+ (acetoxymethylester of sodium-binding benzofuran isophthalate (SBFI-AM) were obtained from Teflabs (Austin, TX). The non-NMDA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) was obtained from Research Biochemical Incorporated (Natick, MA). All other drugs and chemicals were obtained from Sigma. Except for CNQX and gramicidin, which were first dissolved in dimethyl sulfoxide (DMSO), and monensin and nigericin, which were dissolved in ethanol, the drugs were added directly to the saline before use.

The solutions for intracellular calibration of the BCECF ratio signal contained (in mM): 30 Na^+, 120 K^+, 30 Cl^-, 120 gluconic acid, and 1 $MgSO_4$, and were adjusted to different pH with either 10 mM piperazine-N,N′-bis[2-ethane-sulfonic acid] (PIPES), for pH $\leq$ 6.9) or HEPES (pH $\geq$ 7.2). Nigericin (10 μM) and gramicidin (3 μM) were added for equilibration of extra- and intracellular pH. Solutions for calibration of SBFI's sensitivity to $[Na^+]_i$ contained (in mM): 150 (K^+ + Na^+), 30 Cl^-, 120 gluconic acid, 1 $MgSO_4$, 10 HEPES, adjusted to pH 7.2 with KOH. Gramicidin (3 μM), monensin (10 μM), and ouabain (1 mM), were used to equilibrate extra- and intracellular Na^+ (Rose and Ransom, 1996).

Dye loading. Cells were loaded with the AM-ester of BCECF (20 μM) (15 min) or SBFI (10 μM) (90 min) in CO_2/HCO_3^--free saline at room temperature (20–22°C). Pluronic acid (0.1%) was added to improve dye uptake. Experiments were performed in a closed chamber, which was perfused with CO_2/HCO_3^--buffered solution, warmed to 37°C, at a standard flow rate of 4 ml/min. Because of a dead space between the switching site and the chamber, solution exchange at standard flow rate required 20–22 sec from the time of switching. To compensate for this fact, solution changes are indicated 20 sec later in the figures than performed in the original experiment.

Measurement and calibration of intracellular pH and [Na^+]. The experiments were performed with a Nikon-Diaphot-TMD inverted epifluorescence microscope equipped with a 40× epifluorescence oil objective. Cells were excited every 5 sec with dual digikrom 120 monochromators (CVI Laser Corporation, Albuquerque, NM) at 440 and 490 nm (for pH measurement) or 345 and 385 nm (for Na^+ measurement); five video frames were averaged for each excitation period. Background fluorescence was minimal (<1%), therefore, no background subtraction was performed. Emission fluorescence was collected above 510 nm by a GenIISys image intensifier system connected to a video camera (MTI CCD72, Dage-MTI, Michigan City, IN). Data were quantified by an image acquisition program from Georgia Instruments (Roswell, GA) and analyzed by a personal computer. During data analysis, two temporally adjacent data points were averaged to improve signal-to-noise ratio.

To determine absolute intracelluar pH (pH_i) and intracellular H^+ concentration ($[H^+]_i$) (see below), respectively, for each individual cell, we used a one-point calibration technique as described by Boyarski et al. (1988). Cells were perfused with a calibration solution containing nigericin and gramicidin (see above) titrated to pH 7.2 at the end of each experiment. Data were normalized for the 440/490 nM ratio of this calibration solution and fitted to a variant of a pH titration curve, determined for 75 cells on six different coverslips with solutions titrated to pH 6.3, 6.6, 6.9, 7.2, 7.5, and 7.8. Every three to four experiments, we

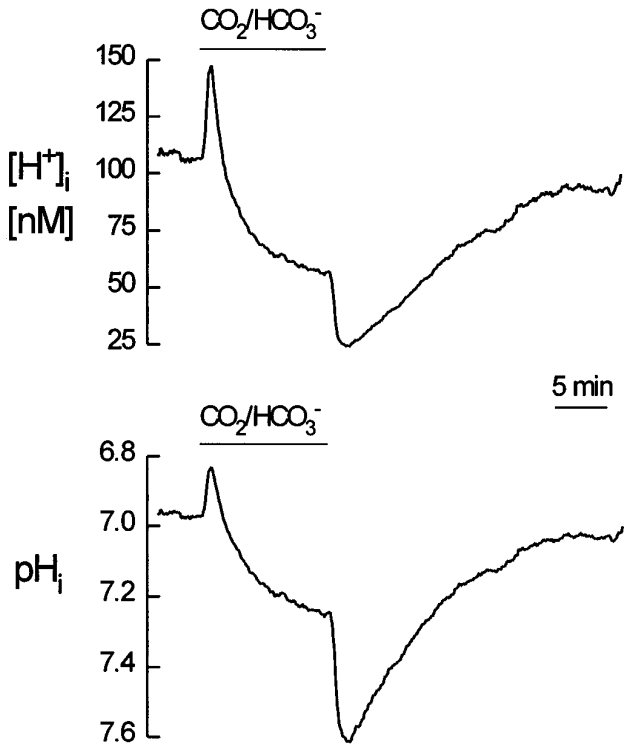

Figure 2. Relationship between pH_i and intracellular H^+ concentration. The distortion caused by presenting changes in intracellular H^+ concentration ($[H^+]_i$) as changes in pH_i is illustrated here. A hippocampal astrocyte was switched from a HEPES-buffered, CO_2/HCO_3^--free saline to a CO_2/HCO_3^--buffered saline and back. The changes in $[H^+]_i$ are shown along with the changes in pH_i. In the pH_i tracing, the absolute magnitude of the alkaline shift appears larger than the acid shift, whereas the $[H^+]_i$ trace reveals just the opposite. To avoid these distortions, all subsequent data are presented as $[H^+]_i$.

checked for the accuracy of this calibration procedure by performing a two-point calibration with solutions titrated to pH 7.2 and 7.4.

As described in detail by Rose and Ransom (1996), the 345/385 nm ratio of intracellular SBFI changes monotonically in hippocampal astrocytes with changes in $[Na^+]_i$ between 0 and 50 mM. For intracellular calibration of $[Na^+]_i$, cells were therefore perfused with two or three calibration solutions (containing gramicidin, monensin, and ouabain) (see above) with different Na^+ concentrations (0, 30, and 50 mM Na^+); this was done at the end of each experiment.

Each experiment with astrocyte cultures allowed analysis of 3–11 individual cells, and was repeated on at least four different coverslips, unless stated otherwise. Data on hippocampal neurons were obtained from four single cells on four different coverslips. Data are presented as means ± SD and were statistically analyzed by paired or unpaired Student's *t* test where appropriate (significance level, $p \leq 0.001$, unless stated otherwise).

Intracellular H^+ concentration more clearly reflects experimental changes than intracellular pH. pH is a logarithmic scale, introduced to conveniently handle differences in acidity over several orders of magnitude occurring in chemical systems. When referring to the cytosol of living cells, however, the use of a pH scale seems neither necessary nor reasonable, because cellular acidity usually is restricted to values between pH 7.7 and 6.0, reflecting H^+ concentrations from ~20–1000 nM. Apart from this, the pH scale has the serious disadvantage that similar pH changes at different baseline pH values might incorrectly suggest similar changes in acidity to the observer.

Figure 2 illustrates this problem graphically by showing the linearized changes in intracellular H^+ ($[H^+]_i$) in a hippocampal astrocyte on switching from a HEPES-buffered to a CO_2/HCO_3^--buffered saline and back (*upper trace*), compared with intracellular pH changes for the same recording (*lower trace*). Whereas the lower trace visually suggests that the initial acidification caused by addition of CO_2 is much smaller than the alkalinization on its removal, the upper trace shows that the opposite is true for this cell; the final decrease in $[H^+]_i$ concentration is actually smaller than the initial increase in $[H^+]$. We believe therefore that it is more sensible to illustrate cellular acidity using a linear scale and have, for reasons of simplicity, expressed acidity in terms of $[H^+]$. (In actuality, $[H^+]$ does not represent single H^+ ions, which are practically nonexistent in cells, but rather the concentration of H_3O^+ molecules.)

RESULTS

Glu can activate several mechanisms in cultured astrocytes (compare Fig. 1), such as (1) ionotropic non-NMDA receptors (also engaged by the nondesensitizing Glu agonist KA (von Blankenfeld and Kettenmann, 1991), (2) metabotropic Glu receptors (e.g., trans-(±)-1-amino-(1S,3R)-cyclopentadicarboxylic acid (*t*-ACPD) are selective agonists for this site) (Schoepp and Conn, 1993), and (3) electrogenic Na^+-coupled uptake of Glu (Bouvier et al., 1992). Glu receptors of the NMDA type are not commonly expressed in astrocytes, but have been reported (Porter and McCarthy, 1994; Steinhauser et al., 1994).

These different mechanisms can be stimulated selectively by Glu analogs. KA activates the non-NMDA receptors but is not transported (Kimelberg et al., 1989). D-Asp is a substrate for the Glu transporter, but is only a very poor substrate for the ionotropic receptor (Erecinska and Silver, 1990). To analyze the mechanisms of Glu-induced changes in astrocytic $[H^+]_i$, we used D-Asp in addition to Glu and KA, to better distinguish between receptor- versus uptake-mediated effects. We did not study the actions of *t*-ACPD, because it changes intracellular Ca^{2+}, but not $[H^+]_i$, in astrocytes (Brune and Deitmer, 1995). To allow uniform comparison between the actions of different agonists, all substances were applied for 1 min at a concentration of 1 mM by bath perfusion, unless otherwise stated.

$[H^+]_i$ changes induced by Glu, KA, and D-Asp

Baseline intracellular H^+ concentration ($[H^+]_i$) in hippocampal astrocytes was 54.6 nM in standard CO_2/HCO_3^--buffered saline (Table 1) corresponding to a pH_i of 7.26.

Bath application of 1 mM Glu for 1 min elicited $[H^+]_i$ changes in every cell investigated ($n = 94$). The majority of cells (80%) responded to Glu with a pure acidification that started either directly at the onset of the Glu perfusion or up to 30 sec later. The acid shift developed with a maximal slope of 4.1 nM/min and reached its peak of 15.6 nM ~5–10 min after the Glu was removed from the bath (Table 1, Fig. 3*A*). In 20% of the cells, this acidification was preceded by a small alkaline shift (≤ 4 nM) (Fig. 3*B*).

KA application changed $[H^+]_i$ in 82% of the cells. The KA-induced $[H^+]_i$ changes were biphasic; a decrease in $[H^+]_i$ by 9.0 nM was followed by an increase of 10.3 nM (Table 1). The alkaline shift reached its peak ~40–70 sec after the onset of the KA-perfusion, whereas the acid shift developed slowly and peaked after 5–10 min (Fig. 3). D-Asp application elicited a slow increase in $[H^+]_i$ in all astrocytes investigated. The D-Asp-induced increase in $[H^+]_i$ averaged 10.4 nM and had a similar time course and maximal slope (5.3 nM/min) as the Glu-induced acid shift (Table 1, Fig. 3*A*).

The Glu-induced membrane depolarization of cultured astrocytes decreased with repetitive applications (Kettenmann and Schachner, 1985). Similar decreases in evoked changes in $[H^+]_i$ were observed in the present study. Amplitudes and slopes of the evoked transients during second and third applications (intervals between pulses, 15–20 min) were reduced in comparison to the first pulse ($p \leq 0.025$) (data not shown). The amplitudes of the second and third Glu-evoked changes, compared with the first, were 87 ± 34% (mean ± SD) and 83 ± 44%, respectively ($n =$

Table 1. $[H^+]_i$ and $[Na^+]_i$ transients induced by Glu, KA, or D-Asp under different conditions

		CO_2/HCO_3^-	CNQX (%)	HEPES
Baseline	$[H^+]_i$ (nM)	54.6 ± 14.9 (289)	57.3 ± 12.8 (29)	**81.7 ± 27.5 (179)
	$[Na^+]_i$ (mM)	15.0 ± 4.9 (223)	15.7 ± 5.7 (61)	**11.9 ± 5.7 (83)
Glu	Δ $[H^+]_i$	15.6 ± 4.2 (75)	**138 ± 25 (29)	**43.7 ± 25 (104)
	Δ $[Na^+]_i$	13.2 ± 4.5 (60)	**52 ± 13 (29)	*12.0 ± 3.7 (32)
KA	Δ $[H^+]_i$	−9.0/10.3 ± 4.8/6.4 (179)	**0 (39)	**4.9 ± 4.5 (194)
	Δ $[Na^+]_i$	23.4 ± 6.1 (82)	**0 (50)	*21.6 ± 6.2 (82)
D-Asp	Δ $[H^+]_i$	10.4 ± 2.9 (26)	100 ± 23 (26)	
	Δ $[Na^+]_i$	6.1 ± 1.0 (64)	*89 ± 15 (52)	

Baseline values of intracellular H^+ and Na^+ concentrations ($[H^+]_i$, in nM; and $[Na^+]_i$, in mM) in standard CO_2/HCO_3^--buffered saline, in CO_2/HCO_3-buffered saline containing 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (25 μM), and in HEPES-buffered, CO_2/HCO_3^--free saline. The second, third, and fourth rows list changes in $[H^+]_i$ and $[Na^+]_i$ induced by Glu, KA, or D-Asp (each applied at 1 mM for 1 min). Note that KA produces a biphasic shift (alkaline–acid), and values for both peaks are shown. Changes in CNQX-containing saline are expressed in % as compared with standard CO_2/HCO_3^--buffered saline. Shown are means ± SD and number of cells. Negative numbers represent decreases in $[H^+]_i$. Asterisks indicate significant differences as compared with control values in CO_2/HCO_3^--buffered saline; $^{**}p \leq 0.001$; $^{*}p \leq 0.025$.

44). Likewise, KA-induced $[H^+]_i$ changes also decremented with repetition; the second and third responses were 84 ± 10% and 82 ± 12% of the original response ($n = 47$). $[H^+]_i$ changes elicited by D-Asp, however, showed virtually no attenuation with repetitive application (97 ± 27% and 95 ± 29%, respectively; $n = 29$). To facilitate comparison of evoked changes in $[H^+]_i$ before and after various manipulations, we corrected the second and third responses for these expected changes (i.e., in data expressed as percentage change).

CO_2/HCO_3^- dependence of Glu- and Ka-induced $[H^+]_i$ changes

Regulation of baseline $[H^+]_i$ in glial cells is dependent on the presence of CO_2/HCO_3^-, partly because of its role as a buffer system and partly because of HCO_3^--carrying transporters (see Fig. 1) (Boyarski et al., 1993; Deitmer and Rose, 1996). To investigate the influence of CO_2/HCO_3^- on the Glu- and KA-induced $[H^+]_i$ changes, we performed experiments in HEPES-buffered, CO_2/HCO_3^--free saline. As expected (Pappas and Ransom, 1993), baseline $[H^+]_i$ increased significantly to 81.7 nM in this saline (Table 1), corresponding to a pH_i of 7.09.

In CO_2/HCO_3^--free saline the amplitude of the Glu-induced acid shift increased about threefold to 43.7 nM (Table 1), and its maximal slope increased to 45.0 nM/min (Fig. 3*B*). These effects can most likely be attributed to the two- to threefold reduction in intracellular buffering capacity in the absence of CO_2/HCO_3^- (Thomas, 1976). Cells that showed an alkaline response in $CO_2/$

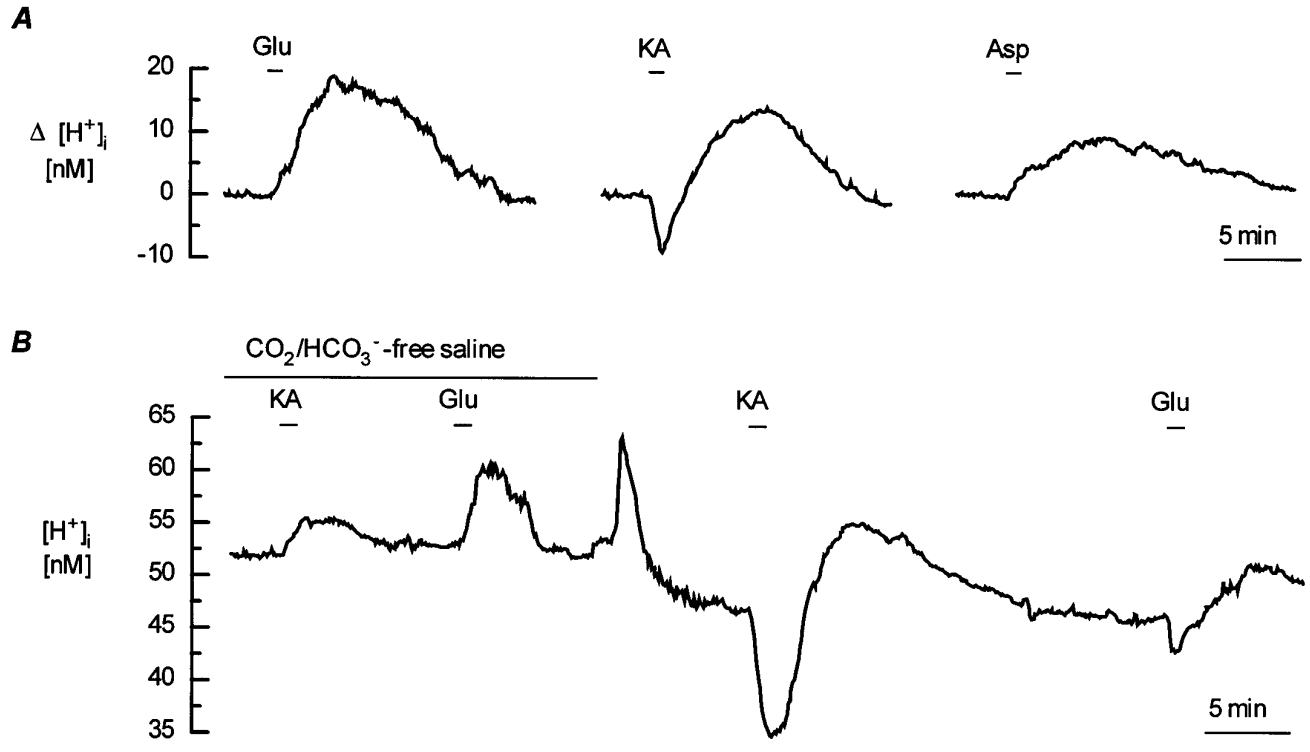

Figure 3. $[H^+]_i$ changes evoked by Glu, KA, or D-Asp in CO_2/HCO_3^--containing or CO_2/HCO_3^--free solution. *A* illustrates the typical changes in intracellular H^+ concentration ($\Delta[H^+]_i$) caused by bath application of Glu, KA, or D-Asp in standard, CO_2/HCO_3^--buffered saline. Substances were applied for 1 min (indicated by *bars*) at a concentration of 1 mM. These recordings were from three different cells. To facilitate comparison, baseline $[H^+]_i$ was set to 0 in all cells. *B*, Record showing changes $[H^+]_i$ caused by 1 min bath application (indicated by *bars*) of KA or Glu (1 mM) in HEPES-buffered, CO_2/HCO_3^--free saline, and CO_2/HCO_3^--buffered saline.

HCO_3^-, did not show this alkaline shift in CO_2/HCO_3^--free saline ($n = 13$) (Fig. 3*B*).

In contrast to their uniform reaction in standard, CO_2/HCO_3^--buffered saline, the astrocytes' response to KA was more variable in CO_2/HCO_3^--free saline. Seventy-eight percent of cells responded to KA in the absence of CO_2/HCO_3^-. Of the responders, most (67%) acidified at KA application (Fig. 3*B*), whereas 33% showed a very small alkaline shift (<2 nM) (data not shown). The average KA-induced increase in $[H^+]_i$ was 4.9 nM (Table 1). Surprisingly, some cells ($n = 19$) that did not react to KA in HEPES-buffered saline responded with robust biphasic $[H^+]_i$ changes in standard, CO_2/HCO_3^--buffered saline. These results strongly suggested that the KA-induced alkaline-acid shifts in standard saline were attributable to HCO_3^- movement across the plasma membrane. Otherwise the $[H^+]_i$ changes would have increased in CO_2/HCO_3^--free saline because of the amplifying effect of reduction in intracellular buffering power (see above) (Thomas, 1976).

Origin of KA-induced $[H^+]_i$ changes in CO_2-buffered saline

The amplitude of the biphasic alkaline-acid transient elicited by KA application in CO_2/HCO_3^--buffered saline increased with increasing KA concentrations from 0.1 to 2.0 mM ($n = 49$; application for 2 min) (Fig. 4). The KA-induced $[H^+]_i$ shifts were unaltered during Ca^{2+} removal (Table 2, Fig. 5*A*). Replacement of extracellular Cl^- by gluconic acid led to a significant decrease in baseline $[H^+]_i$ (Table 2, Fig. 5*B*). This effect was probably attributable to removal of acidifying Cl^-/HCO_3^- exchange in these astrocytes, as reported earlier (Mellergard et al., 1993; Shrode and Putnam, 1994). Removal of Cl^- also could alkalinize astrocytes by depolarizing them and activating inward Na^+/HCO_3^- cotransport (Pappas and Ransom, 1994), but astrocyte membrane potential appears to be insensitive to the transmembrane gradient of Cl^- (Ransom and Goldring, 1973). In the absence of Cl^-, the KA-induced alkalinization was reversibly reduced to 52% ($p \leq 0.0001$) of the control response, and the KA-induced acidification increased to 129% ($p \leq 0.01$) (Table 2, Fig. 5*B*).

Replacement of extracellular Na^+ by *N*-methyl-D-glucamine (NMDG), (115.75 mM) and choline (23 mM) strongly acidified the cells (Table 2, Fig. 6*A*). The KA-induced alkaline transient was abolished in some cells and, on average, was reversibly diminished to 18% of the control value. The acid transient was abolished completely (Fig. 6*A*). Similar effects were observed after application of the anion transport blocker (4,4′-diisothiocyanatostilbene-2,2′-disulphonic acid (DIDS) (0.5 mM). After an initial alkaline transient, baseline $[H^+]_i$ increased strongly (Table 2, Fig. 6*B*). The KA-evoked alkaline shift was reduced to 25% of the control value, and the acid shift was abolished (Fig. 6*B*). The effects of DIDS, as expected (Brune et al., 1994; O'Connor et al., 1994), were not fully reversible.

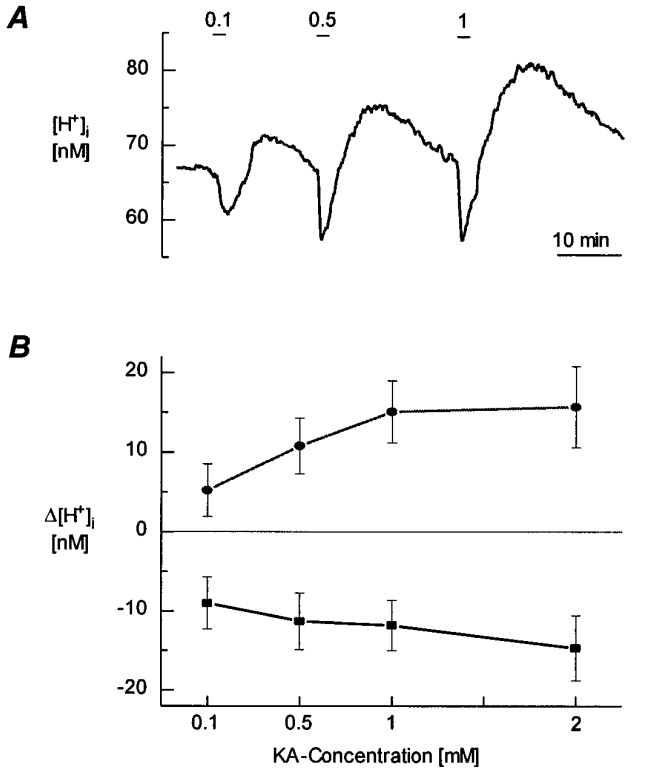

Figure 4. Concentration dependence of kainate-induced $[H^+]_i$ changes. *A*, The biphasic KA-induced responses in $[H^+]_i$ increased with KA concentration. All applications were for 2 min periods; KA was applied at 0.1, 0.5, and 1 mM (indicated by *bars*). *B*, Graphic summary of the amplitudes of biphasic alkaline-acid transients elicited by 2 min bath applications of KA at 0.1, 0.5, 1, and 2 mM in CO_2/HCO_3^--buffered saline. Shown are the mean values of 49 cells; bars indicate SD.

In contrast to hippocampal astrocytes, we observed only a rapid acidification, but no alkaline shift, in cultured hippocampal neurons after KA application. This was true in both CO_2/HCO_3^--buffered (Fig. 7) and CO_2/HCO_3^--free saline (data not shown). In CO_2/HCO_3^--buffered saline, KA application rapidly increased neuronal $[H^+]_i$ by 22.8 ± 10.3 nM from a baseline of 75.4 ± 18.6 nM ($n = 4$).

Taken together, these results suggested that the glial-specific, biphasic $[H^+]_i$ changes after application of KA were attributable to a CO_2/HCO_3^-- and Na^+-dependent transport mechanism sensitive to DIDS that can operate (although with reduced amplitude) in the absence of external Cl^-.

Table 2. Kainate-induced $[H^+]_i$ changes under different conditions

	Control	0 Ca^{2+}	0 Cl^-	0 Na^+	DIDS
	$n = 289$	$n = 41$	$n = 31$	$n = 44$	$n = 42$
$[H^+]_i$ (nM)	54.6 ± 14.9	50.5 ± 5.7	**30.3 ± 5.2	**171.1 ± 56.6	**108.5 ± 20.3
Alkaline (%)	100	96 ± 34	**52 ± 31	**18 ± 29	**25 ± 29
Acid (%)	100	102 ± 27	*129 ± 51	**0	**0

Baseline values of $[H^+]_i$ (in nM) and $[H^+]_i$ changes (shown as % of control) in CO_2/HCO_3^--buffered saline (control), after removal of extracellular Ca^{2+} (0 Ca^{2+}), Cl^- (0 Cl^-), or Na^+ (0 Na^+), or after addition of the anion transport blocker 4,4′-diisothiocyanato-stilbene-2,2′-disulphonic acid (DIDS) (0.5 mM) are shown. Percentage values in the second and third rows indicate relative amplitudes of kainate-induced alkaline and acid shifts in comparison to the control. Shown are n = number of cells, and means ± SD. Asterisks indicate significant differences as compared with control values in CO_2/HCO_3^--buffered saline; **$p \leq 0.001$; *$p \leq 0.01$.

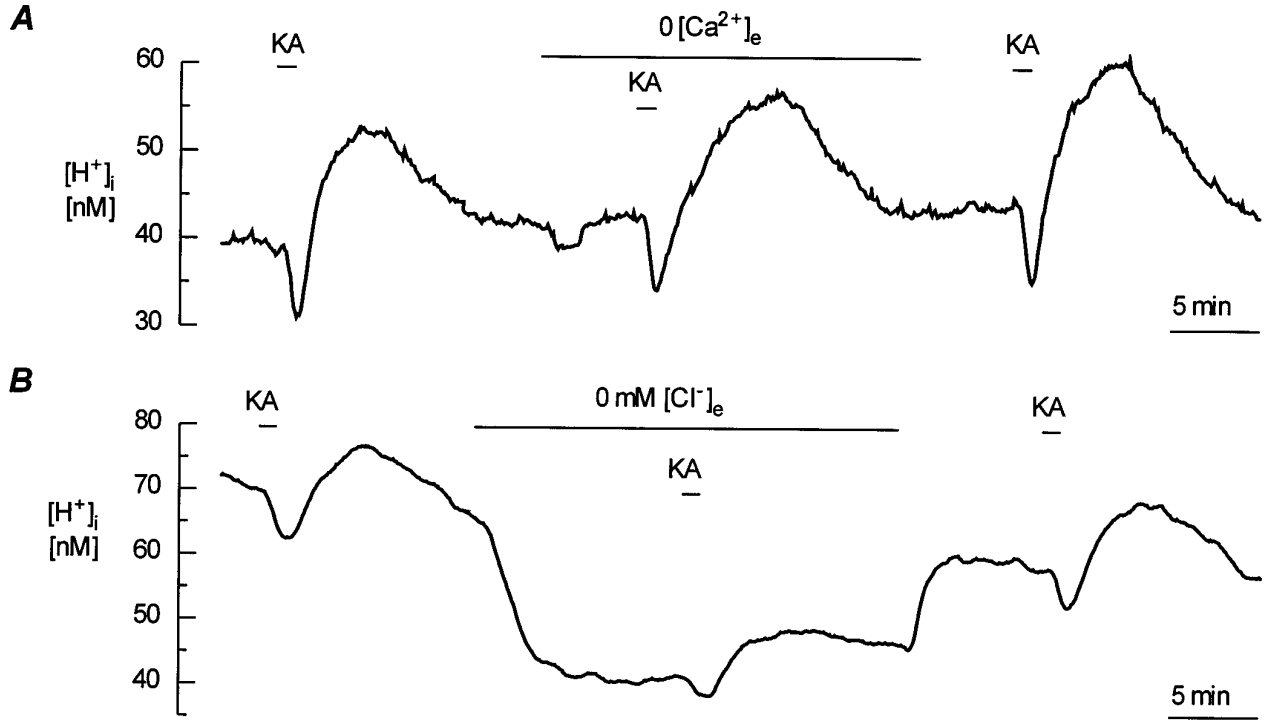

Figure 5. Ca^{2+}- and Cl^--dependence of kainate-induced $[H^+]_i$ changes. *A*, Alkaline-acid transients elicited by 1 mM KA applications for 1 min (indicated by *bars*) in standard CO_2/HCO_3^--buffered saline (2 mM Ca^{2+}) and after removal of extracellular Ca^{2+} (0 $[Ca^{2+}]_e$, solution contained 0.5 mM EGTA). The KA response was unchanged in the absence of $[Ca^{2+}]_e$. *B*, Alkaline-acid transients elicited by 1 mM KA application for 1 min (indicated by *bars*) in standard CO_2/HCO_3^--buffered saline (containing 122.75 mM Cl^-) and after replacement of extracellular Cl^- by gluconic acid (0 $[Cl^-]_e$). Removing Cl^- caused an alkaline shift probably because of reverse Cl^-/HCO_3^- exchange. Note that the KA response was preserved qualitatively in the absence of $[Cl^-]_e$.

$[Na^+]_i$ changes induced by Glu, KA, and D-Asp

In agreement with an earlier study (Rose and Ransom, 1996), baseline $[Na^+]_i$ in hippocampal astrocytes was about 15 mM in standard saline (Table 1). Application of either Glu, KA, or D-Asp rapidly increased $[Na^+]_i$ within 30–60 sec, followed by a recovery to baseline within 10 min (shown for Glu in Figs. 8, 10; KA in Figs. 8, 9; D-Asp in Fig. 11). This was true for every cell investigated, although the absolute amplitudes of the $[Na^+]_i$ signals differed significantly between the three agonists. The average $[Na^+]_i$ increase was highest with KA (23.4 mM), followed by Glu (13.2 mM), and D-Asp (6.1 mM) (Table 1). The maximal slopes of $[Na^+]_i$ increase (in mM/min) induced by these three agonists were 22.1 ± 2.4 (n = 43), 12.8 ± 9.0 (n = 30), and 9.3 ± 5.6 (n = 26), respectively. During repetitive applications, amplitude and slopes of the $[Na^+]_i$ transients decreased as described for the $[H^+]_i$ changes (data not shown) (see above).

In CO_2/HCO_3^--free saline, baseline $[Na^+]_i$ decreased significantly to 11.9 mM (Table 1), indicating a contribution of inwardly directed Na^+/HCO_3^- cotransport and/or Na^+-dependent Cl^-/HCO_3^- exchanger to baseline $[Na^+]_i$ in these cells under standard conditions (Rose and Ransom, 1996). Both Glu- and KA-induced $[Na^+]_i$ increases were slightly reduced in comparison to CO_2/HCO_3^--buffered saline ($p \leq 0.025$) (Table 1).

Comparison of $[H_+]_i$ and $[Na^{+]}i$ changes

A direct comparison of the Glu- and KA-induced $[Na^+]_i$ and $[H^+]_i$ transients is shown in Figure 8. In CO_2/HCO_3^--buffered saline, Glu-induced acidifications started up to 30 sec later than the increases in $[Na^+]_i$. $[H^+]_i$ continued to rise for minutes after $[Na^+]_i$ was already decreasing and only started to recover when $[Na^+]_i$ had nearly reached baseline again (Fig. 8*A*, *solid traces*). The same differences also were true for D-Asp-induced $[Na^+]_i$ and $[H^+]_i$ changes (see Fig. 11).

In CO_2/HCO_3^--free saline, in contrast, the time course of the Glu-induced $[H^+]_i$ increase more closely matched that of the $[Na^+]_i$ transient (Fig. 8*A*, *dashed traces*). Both $[H^+]_i$ and $[Na^+]_i$ increases started at about the same time, and the acidification reached its peak earlier than in CO_2/HCO_3^--buffered saline. The $[H^+]_i$ recovery to baseline, however, was still slightly delayed compared with the $[Na^+]_i$ recovery (Fig. 8*A*, *dashed traces*).

The time course and occurrence of KA-induced $[Na^+]_i$ and $[H^+]_i$ transients differed greatly. In CO_2/HCO_3^--buffered saline, the KA-evoked $[Na^+]_i$ increase was paralleled by an alkalinization. The subsequent acidification started about when $[Na^+]_i$ reached its highest amplitude and persisted even when $[Na^+]_i$ had reached baseline again (Fig. 8*B*, *solid traces*). In contrast to this, KA-induced acidifications were strongly reduced or even absent in CO_2/HCO_3^--free saline, whereas the $[Na^+]_i$ increases only decreased slightly (Fig. 8*B*, *dashed traces*). This comparison demonstrates that large increases in $[Na^+]_i$ are not necessarily associated with an acidification of hippocampal astrocytes in CO_2/HCO_3^--free saline.

Effect of CNQX on $[H^+]_i$ and $[Na^+]_i$ changes induced by Glu, KA, and D-Asp

To investigate receptor-mediated effects of Glu and its agonists, we applied CNQX (25 μM), which selectively blocks activation of ionotropic non-NMDA receptors. CNQX perfusion did not alter baseline $[H^+]_i$ or $[Na^+]_i$ in astrocytes (Table 1), and all of its effects were reversible.

CNQX completely blocked $[H^+]_i$ and $[Na^+]_i$ transients evoked by KA in both CO_2/HCO_3^--free (data not shown) and $CO_2/$

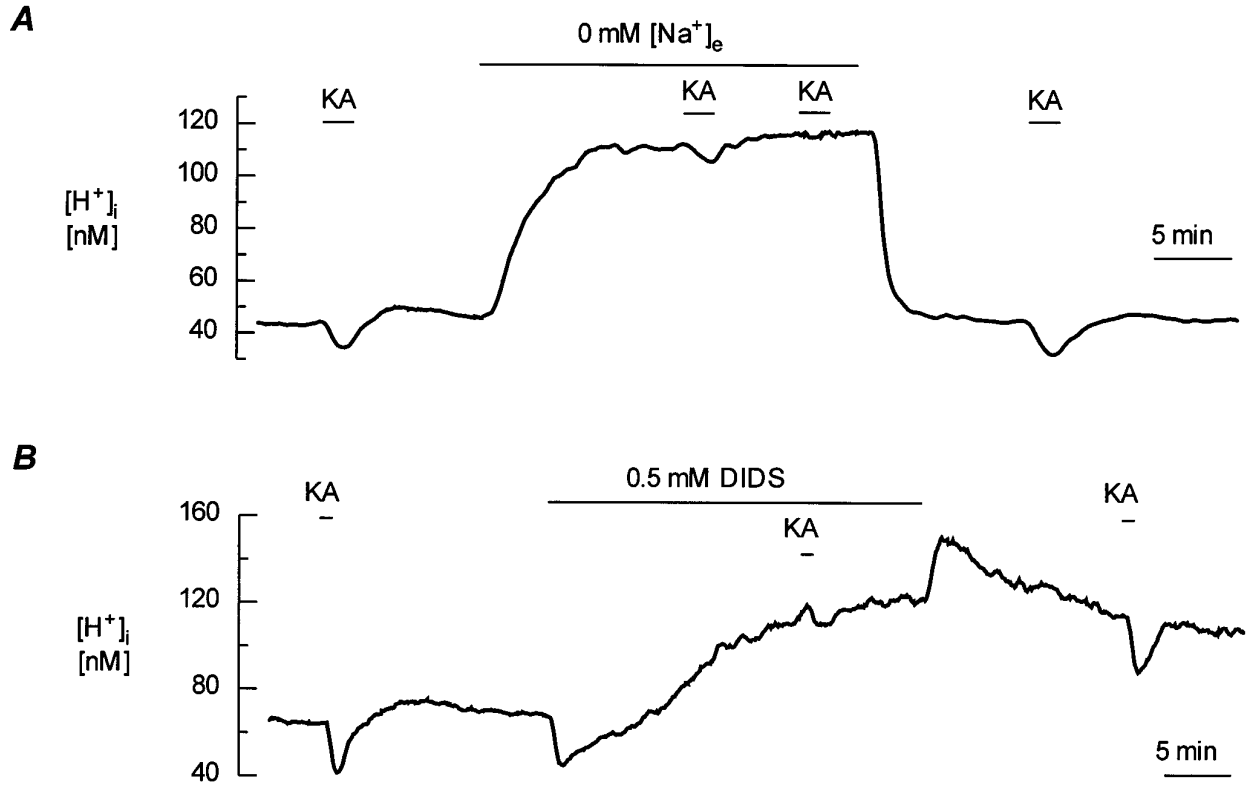

Figure 6. Na^+ dependence and influence of DIDS on kainate-induced $[H^+]_i$ changes. *A*, Alkaline-acid transients elicited by 1 mM KA application for 2 min (indicated by *bars*) in standard CO_2/HCO_3^--buffered saline and after replacement of extracellular Na^+ by NMDG and choline (0 $[Na^+]_e$). Removal of Na^+ caused a marked acid shift, because it blocks or reverses acid-exporting mechanisms. The KA-induced changes in $[H^+]_i$ were reversibly blocked in the absence of Na^+. *B,* Alkaline-acid transients elicited by 1 mM KA application for 1 min (indicated by *bars*) are shown in standard CO_2/HCO_3^--buffered saline and during application of the anion transport blocker DIDS (0.5 mM). DIDS caused a partially reversible acid shift, because it blocks acid-exporting mechanisms. The KA response was largely blocked by DIDS.

HCO_3^--buffered saline (Table 1, Fig. 9), confirming that these effects of KA were solely attributable to receptor activation.

In contrast to this, CNQX significantly *increased* Glu-induced $[H^+]_i$ changes to 138%, and *decreased* $[Na^+]_i$ changes to 52% of control values (Table 1, Fig. 10). These results allow several straightforward and important conclusions concerning the mechanisms of the Glu-induced $[H^+]_i$ and $[Na^+]_i$ changes. They show that about half of the Glu-induced $[Na^+]_i$ increase was attributable to receptor activation, and, therefore, most likely caused by Na^+ influx through the receptor–ionophore complex. Furthermore, they indicate that the observed acidification was not only independent of receptor activation, but was in fact probably dampened by it. In addition, this experiment demonstrated that the intraglial acidification on Glu application was not a direct effect of the increase in $[Na^+]_i$, confirming the impression that $[Na^+]_i$ increases are not of necessity followed by increases in $[H^+]_i$ in hippocampal astrocytes (see above).

The D-Asp-induced acidification was virtually unaltered by CNQX (100%) (Table 1), whereas the $[Na^+]_i$ transient was slightly reduced to 89% ($p \leq 0.025$) (Table 1, Fig. 11), consistent with the expectation that D-Asp is a very poor substrate for the ionotropic receptor, but is readily transported into astrocytes.

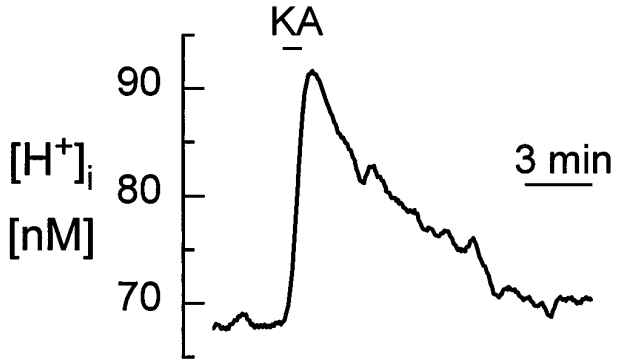

Figure 7. Neuronal $[H^+]_i$ changes induced by KA. KA application (1 mM for 1 min) (indicated by *bar*) rapidly increased $[H^+]_i$ in a cultured hippocampal neuron in CO_2/HCO_3^--buffered saline. Unlike the situation in astrocytes, KA never evoked alkaline shifts in neurons.

DISCUSSION

Origin of Glu- and D-Asp-evoked acidifications

In agreement with studies on cerebral astrocytes (Brookes and Turner, 1993; Brune and Deitmer, 1995), L-Glu or D-Asp acidified hippocampal astrocytes. Our results indicate that these acidifications are caused primarily by a transporter that exchanges Glu and Na^+ for intracellular OH^- and K^+, as demonstrated for retinal glial cells (Bouvier et al., 1992) (see Fig. 1*A*). Glu-induced intracellular acidification is not a phenomenon restricted to cell cultures; Glu uptake via this transporter also produced an intracellular acidification in rat hippocampal slices (Amato et al., 1994).

Our conclusion is supported by several lines of evidence. In CO_2/HCO_3^--free saline, Glu-induced $[H^+]_i$ increases were closely paralleled by $[Na^+]_i$ increases. The CNQX-resistant, D-Asp-induced $[H^+]_i$ increases implied that these acidifications were not

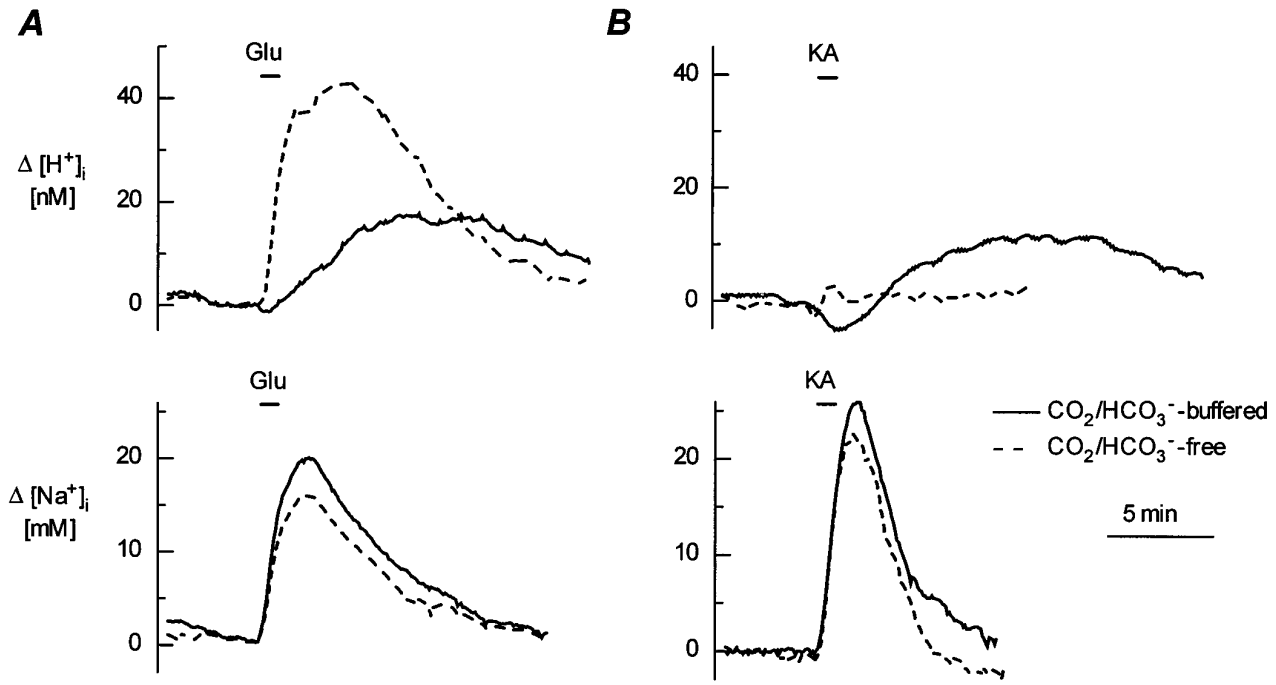

Figure 8. Comparison between glutamate- and kainate-induced $[Na^+]_i$ and $[H^+]_i$ transients. *A*, Recordings showing changes in intracellular Na^+ and H^+ concentrations ($\Delta[Na^+]_i$, $\Delta[H^+]_i$) induced by application of Glu (1 mM for 1 min) (indicated by *bars*) in CO_2/HCO_3^--buffered saline (*solid lines*) and CO_2/HCO_3^--free saline (*dashed lines*). Although Glu-induced $[H^+]_i$ changes were greatly altered when switching between CO_2/HCO_3^--free and CO_2/HCO_3^--containing solution, very small alterations were seen in the induced changes in $[Na^+]_i$. *B*, Recordings showing KA-induced $[Na^+]_i$ and $[H^+]_i$ changes. KA (1 mM) was applied for 1 min as indicated by the *bars*. Again, the significant changes in KA-induced $[H^+]_i$ transients caused by switching from CO_2/HCO_3^--containing solution to CO_2/HCO_3^--free solution were not associated with significant changes in the $[Na^+]_i$ transients. *A*, *B*, Na^+ and H^+ measurements were obtained from different cells.

attributable to non-NMDA receptor activation with H^+ influx through receptor-coupled ion channels. Likewise Glu-induced acidifications were resistant to CNQX (they actually increased), demonstrating that they too were not caused by ionotropic non-NMDA receptor activation. Glu transport is voltage dependent (Brew and Attwell, 1987), and if CNQX partially blocked the Glu-induced depolarization (Bowman and Kimelberg, 1984; Kettenmann and Schachner, 1985), Glu uptake and acidification would be increased. Reduction of Glu-induced depolarization also might have reduced a depolarization-dependent alkalinization process, thereby magnifying the acidification (see below).

The stoichiometry for coupled transport of Glu^-/D-Asp^-, K^+,

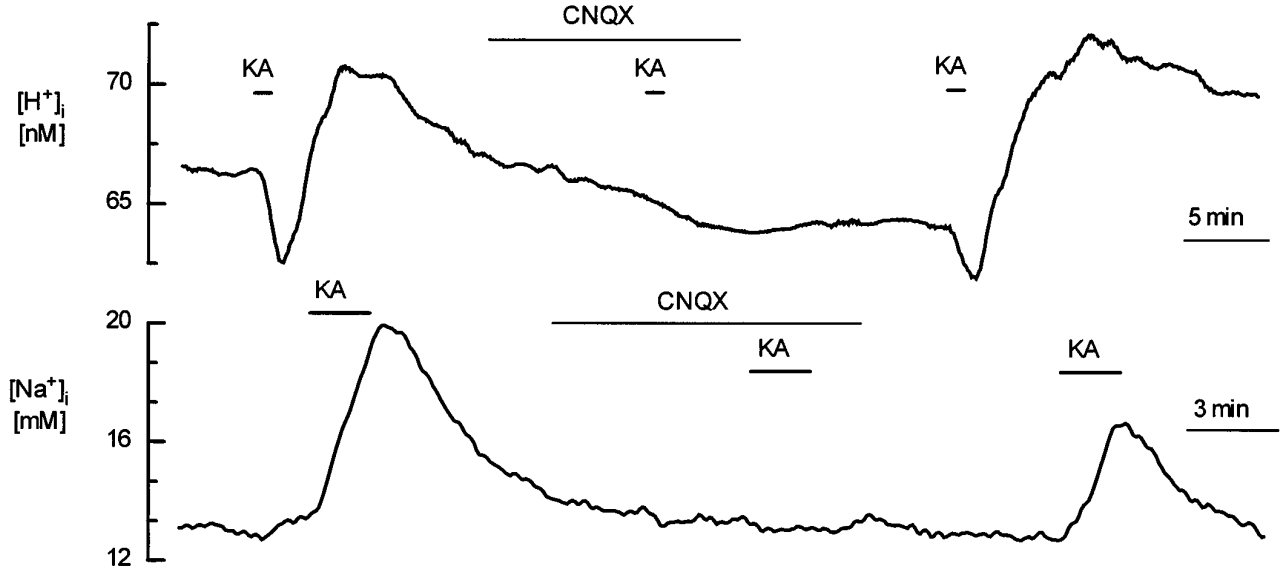

Figure 9. CNQX blocked kainate-induced $[H^+]_i$ and $[Na^+]_i$ transients. KA-induced $[H^+]_i$ and $[Na^+]_i$ transients are shown in standard CO_2/HCO_3^--buffered saline and during application of the ionotropic, non-NMDA receptor blocker CNQX (25 μM) (*solid bars*). KA (1 mM) was applied for 1 min (*upper trace*) or 2 min (*lower trace*, indicated by *short bars*). The recordings were obtained from two different cells; note the different time scales. CNQX completely blocked both the KA-induced $[H^+]_i$ and $[Na^+]_i$ transients.

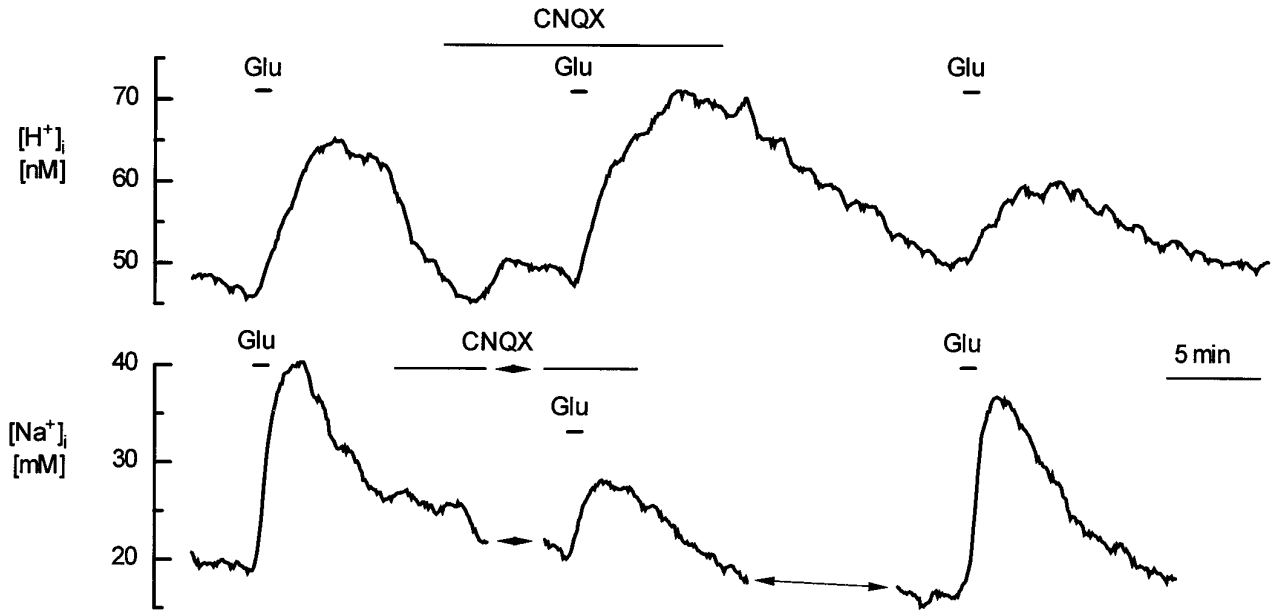

Figure 10. CNQX reduced the increase in $[Na^+]_i$ induced by Glu, but not the increase in $[H^+]_i$. Glu-induced $[H^+]_i$ and $[Na^+]_i$ transients are shown in standard CO_2/HCO_3^--buffered saline and during application of the ionotropic, non-NMDA receptor blocker CNQX (25 μM) (*solid bars*). Glu (1 mM) was applied for 1 min (indicated by *short bars*). The recordings were obtained from two different cells, and two segments were deleted from the *lower trace* (*arrows*) to enable better comparison between the traces.

Na^+, and OH^- is 1:1:2:1 (Bouvier et al., 1992) (see Fig. 1*C*). Using our data, the D-Asp-induced increase in $[H^+]_i$ was slightly higher than expected from the $[Na^+]_i$ increase and this stoichiometry. Assuming an intraglial buffering power of about 60 mM (Chesler, 1990; Deitmer, 1995) and a baseline $[H^+]_i$ of 55 nM (Table 1), the D-Asp-induced $[H^+]_i$ increase of 10.4 nM (Table 1) would correspond to a total increase in intracellular acid by 4.2 mM, implying a $[Na^+]_i$ increase of twice this, or 8.4 mM; the average observed $[Na^+]_i$ increase, however, was only 6.1 mM (Table 1). Several factors could account for this discrepancy. The actual inward transport of Na^+ might be higher than suggested by the net concentration increases because of removal of Na^+ by Na^+,K^+-ATPase activity. Moreover, the buffering capacity of the cells could be lower than 60 mM or the transporter might not be the only source for the increase in $[H^+]_i$.

Other sources of Glu/D-Asp-induced acidification could include increased Glu metabolism by the ATP-dependent enzyme glutamine synthetase (Sonnewald et al., 1993) or Na^+,K^+-ATPase activation (Thompson and Prince, 1986; Erecinska, 1989; Pellerin and Magistretti, 1994), because in both cases H^+ is released with

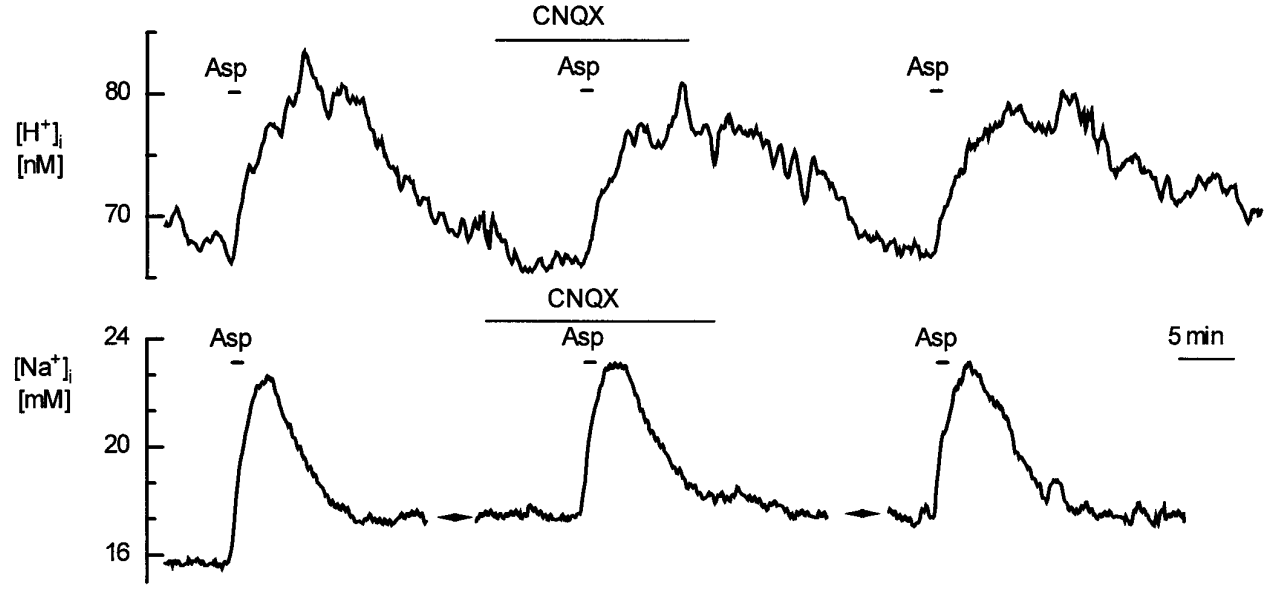

Figure 11. CNQX had only minor effects on aspartate-induced $[H^+]_i$ and $[Na^+]_i$ transients. $[H^+]_i$ and $[Na^+]_i$ transients were induced by D-Asp (*Asp*) in standard CO_2/HCO_3^--buffered saline and during application of CNQX (25 μM) (*solid bars*). D-Asp (1 mM) was applied for 1 min (*short solid bars*). The recordings were obtained from two different cells, and two segments were deleted from the *lower trace* (*arrowheads*) to enable better comparison between the traces.

ATP breakdown (Hochachka and Mommsen, 1983). Outwardly directed Na^+/HCO_3^- cotransport also might be activated as discussed below. In any case, an intraglial accumulation of Glu or D-Asp by 4–5 mM in our study (calculated from the $[H^+]_i$ increase) corresponds reasonably well to Glu concentrations reached in the brain (~10 mM) (Erecinska and Silver, 1990).

The Glu-induced $[H^+]_i$ increase was 1.5 times larger than the D-Asp-induced acidification, but had a similar time course. This difference reflects the substrate specificity of Glu uptake; D-Asp produced smaller effects than Glu in terms of membrane current (Brew and Attwell, 1987; Barbour et al., 1993) and pH changes (Bouvier et al., 1992; Brune and Deitmer, 1995). Another reason for this difference might be astrocytic Glu-uptake sites which do not accept D-Asp as substrate (Flott and Seifert, 1991).

Origin of KA-evoked $[H^+]_i$ changes

In CO_2/HCO_3^--free saline, where baseline $[H^+]_i$ is primarily regulated by Na^+/H^+-exchange (Pappas and Ransom, 1993), KA induced either no $[H^+]_i$ changes or a small acidification. At the same time, KA-induced $[Na^+]_i$ increases reduced the driving force for Na^+/H^+ exchange from 24:1 to 8:1 (see also Aronson, 1985) (for ion concentrations, see Materials and Methods and Table 1). This reduced driving force was apparently sufficient to keep $[H^+]_i$ more or less constant, suggesting that $[Na^+]_i$ increases do not cause obligatory $[H^+]_i$ increases in hippocampal astrocytes in CO_2/HCO_3^--free saline. They could, however, slow recovery from an acid load, as indicated by the delayed $[H^+]_i$ recovery compared with $[Na^+]_i$ recovery (compare Fig. 8*A*).

In CO_2/HCO_3^--buffered saline, in contrast, KA evoked alkaline-acid shifts. The ionic dependence and pharmacology of these transients suggested that they were primarily caused by transmembrane HCO_3^- fluxes mediated by electrogenic Na^+/HCO_3^- cotransport (O'Connor et al., 1994; Pappas and Ransom, 1994). They were blocked by CNQX and, therefore, dependent on activation of ionotropic non-NMDA receptors. The $[H^+]_i$ transients were independent of extracellular Ca^{2+}, making the contribution of a plasma membrane Ca^{2+}/H^+ ATPase unlikely (Schwiening et al., 1993; Paalasmaa et al., 1994).

The KA-induced transients still were seen in Cl^--free saline, although the alkaline shift was considerably reduced in amplitude, suggesting that they were not dependent on Cl^-, but possibly influenced by the transmembrane H^+ gradient. Electroneutral Cl^-/HCO_3^- exchange and Na^+-dependent Cl^-/HCO_3^- exchange were not likely, therefore, to contribute significantly to the KA-induced $[H^+]_i$ changes. Removal of $[Na^+]_o$ or application of DIDS diminished the KA-induced alkalinization by about 80% and blocked the acidification, indicating that these $[H^+]_i$ changes were attributable to Na^+-dependent anion transport. In addition, a Na^+-independent mechanism might be responsible for a small percentage of the KA-induced alkalinization (Grichtchenko and Chesler, 1994). Hippocampal neurons, which do not express Na^+/HCO_3^- cotransport (Schwiening and Boron, 1994), only showed an acidification upon KA application.

To understand how KA could alter Na^+/HCO_3^- cotransport activity in a biphasic manner, one has to consider that this transporter is electrogenic and, therefore, influenced by membrane potential. The stoichiometry of Na^+/HCO_3^- cotransport is 1:2 in hippocampal astrocytes (O'Connor et al., 1994). With the steady-state ion concentrations given in the present study ($[Na^+]_i$ = 15 mM; $[Na^+]_o$ = 140 mM; $[HCO_3^-]_i$ = 17.3 mM) (see Chesler, 1990); $[HCO_3^-]_o$ = 23 mM), its reversal potential (E_{rev}) is ~−75 mV, which is close to the baseline membrane potential of hippocampal astrocytes (O'Connor et al., 1994; Steinhauser et al., 1994). Depolarization of the membrane above −75 mV should, therefore, lead to inward Na^+/HCO_3^- cotransport.

KA depolarizes astrocytes by 5 mV/sec (Backus et al., 1989); the total depolarization can reach 20–25 mV (Bowman and Kimelberg, 1984; Kettenmann and Schachner, 1985). The similar time courses of KA-induced alkalinization and depolarization (see Bowman and Kimelberg, 1984; Kettenmann and Schachner, 1985; Backus et al., 1989) strongly suggest that the alkalinization resulted from accelerated inward Na^+/HCO_3^- cotransport during KA application (see above). KA also caused a $[Na^+]_i$ increase, with a slightly slower time course than the depolarization (Fig. 12*A*,*B*). This KA-induced $[Na^+]_i$ increase causes a progressive increase in the E_{rev} of the transporter to about −50 mV. Because the KA-induced membrane depolarization is faster than the $[Na^+]_i$ increase, the membrane potential initially is more positive than E_{rev}, favoring influx of Na^+/HCO_3^- causing intracellular alkalinization (Fig. 12*B*).

Further consideration of the relationship between membrane potential and E_{rev} can account for the KA-induced acidification. Because of activation of Na^+,K^+-ATPase secondary to increased $[Na^+]_i$, Glu or Glu agonists induce rapid repolarization and long-lasting hyperpolarization after their removal (Ransom et al., 1975; Bowman and Kimelberg, 1984; Thompson and Prince, 1986; Erecinska, 1989). This produces a discrepancy between membrane potential and E_{rev} that is opposite to what is seen initially, leading to reversal of Na^+/HCO_3^- cotransport and acidification (Deitmer and Schneider, 1995) during periods when E_m is more negative than E_{rev} (Fig. 12*B*). Na^+/HCO_3^- cotransport, therefore, links membrane potential with $[H^+]_i$ and $[Na^+]_i$ homeostasis in astrocytes.

Glu and D-Asp also depolarize astrocytes (Bowman and Kimelberg, 1984; Kettenmann and Schachner, 1985) and, therefore, also might activate inward Na^+/HCO_3^- cotransport. Indeed, a CO_2/HCO_3^--dependent alkalinization was seen in about 20% of the cells during perfusion with Glu. In the majority of cells, however, the acidification attributable to Glu uptake apparently obscured this alkalinizing influence. The enhancement of the Glu-induced acid shift in CO_2/HCO_3^--free solution and after CNQX, which would reduce depolarization, may be partly attributable to removal of a superimposed alkalinization process mediated by Na^+/HCO_3^- cotransport.

Origin of the $[Na^+]_i$ transients

Glu and its agonists evoked large $[Na^+]_i$ increases in hippocampal astrocytes, as reported earlier for leech glial cells (Ballanyi et al., 1989), cultured cerebral astrocytes (Kimelberg et al., 1989), and oligodendrocytes (Ballanyi and Kettenmann, 1990). The different amplitudes of $[Na^+]_i$ increase, and CNQX sensitivity of the tested substances mirror their substrate characteristics vis a vis Glu receptors and Glu uptake.

The KA-induced $[Na^+]_i$ increase was dependent on activation of ionotropic non-NMDA receptors, because it was blocked by CNQX. It was caused, therefore, by influx of Na^+ through receptor-coupled cation channels (Sontheimer et al., 1988). Another cause of $[Na^+]_i$ increase would be inwardly directed Na^+/HCO_3^- cotransport in these cells (see above). This was indicated by the KA-induced alkalinization and the smaller amplitude of the $[Na^+]_i$ increase in CO_2/HCO_3^--free, compared with CO_2/HCO_3^--buffered, saline (Table 1).

In addition to receptor-mediated Na^+ influx, our data suggest that 40–50% of the Glu-induced $[Na^+]_i$ increase was caused by

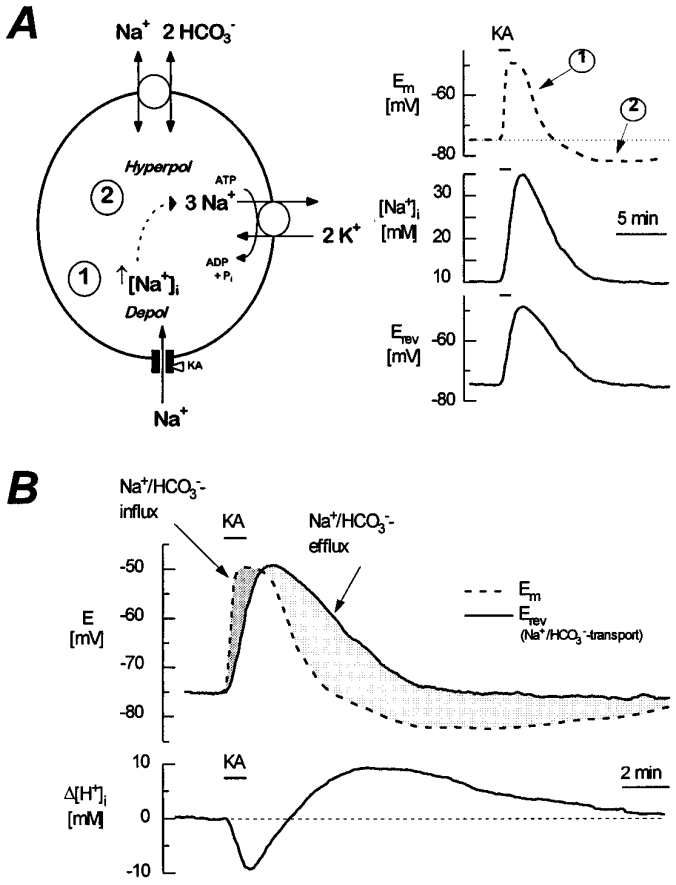

Figure 12. Proposed voltage shifts and transmembrane Na^+/HCO_3^- movements in astrocytes during kainate application in CO_2/HCO_3^--buffered saline. *A*, Model of KA-induced changes in membrane potential, $[Na^+]_i$, and Na^+/HCO_3^--cotransporter reversal potential in astrocytes. KA causes depolarization and increases $[Na^+]_i$ (*1*). This is followed by Na^+ pump stimulation leading to hyperpolarization and normalization of $[Na^+]_i$ (*2*). The reversal potential of Na^+/HCO_3^- cotransport is altered by the changes in $[Na^+]_i$, illustrated by the hypothetical curves to the *right*. The *upper trace* shows the presumed changes in membrane potential (E_m) resulting from KA application (1 mM for 1 min) (indicated by *bars*) (see also Bowman and Kimelberg, 1984; Kettenmann and Schachner, 1985; Backus et al., 1989). The *middle trace* shows the averaged KA-induced $[Na^+]_i$ change of six representative cells. The *lower trace* shows changes in the reversal potential of Na^+/HCO_3^- cotransport (E_{rev}), calculated from the average $[Na^+]_i$ change in the *middle trace* (see Discussion). *B*, Proposed mechanism of the alkaline-acid transients seen in astrocytes resulting from KA application (1 mM for 1 min) (indicated by *bars*). The *upper trace* shows changes in the E_{rev} (*solid line*). Superimposed on the E_{rev} trace is the presumed change in E_m induced by KA (*dashed line*) (see above). During the fast, KA-induced membrane depolarization, E_m is more positive than E_{rev}, favoring influx of Na^+/HCO_3^- and, therefore, intracellular alkalinization. During repolarization and hyperpolarization of the membrane, E_m is more negative than E_{rev}, because of the relatively slower recovery of $[Na^+]_i$, favoring efflux of Na^+ and HCO_3^- and intracellular acidification. The *lower trace* shows the KA-induced biphasic alkaline-acid shift in $[H^+]_i$ averaged from six representative cells.

Na^+ transport via Glu uptake, because it persisted in CNQX (Fig. 10). The D-Asp-induced $[Na^+]_i$ increases, on the other hand, were primarily attributable to Glu uptake, because they were only slightly altered by CNQX (Fig. 11).

Conclusions

Our study indicates that $[Na^+]_i$ increases are not primarily responsible for Glu- or KA-induced acidifications in hippocampal astrocytes. Instead, our results imply that the $[H^+]_i$ transients are primarily caused by Glu uptake, resulting in acidification, and by electrogenic Na^+/HCO_3^- cotransport activity, resulting in biphasic alkaline-acid shifts. In the brain, the relative influence of these two mechanisms and, therefore, the direction of glial $[H^+]_i$ changes might vary depending on the physiological situation. During normal neuronal activity, glial cells alkalinize after activation of inward Na^+/HCO_3^- cotransport, and only blocking this transporter unmasks an acidification, which probably results from neurotransmitter uptake (Chesler and Kraig, 1989; Rose and Deitmer, 1995). The glial uptake of base promotes extracellular acidification, thereby dampening neuronal excitability in a feedback-like manner (Ransom, 1992). During pathological situations such as stroke or epilepsy, in contrast, extracellular Glu concentrations are increased for longer time periods, and glial cells strongly acidify (Kraig and Chesler, 1990), probably because of maximal Glu uptake obscuring any alkalinization.

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