pH modulation of Ca^{2+} responses and a Ca^{2+}-dependent K^+ channel in cultured rat hippocampal neurones

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1. The effects of changes in extra- and intracellular pH (pH_o and pH_i, respectively) on depolarization-evoked rises in intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) and the activity of a Ca^{2+}-dependent K^+ channel were investigated in cultured fetal rat hippocampal neurones.

2. In neurones loaded with 2′,7′-bis-(2-carboxyethyl)-5-(and -6)-carboxyfluorescein (BCECF), changes in pH_o evoked changes in pH_i. At room temperature, the ratio $\Delta pH_i : \Delta pH_o$ (the slope of the regression line relating pH_i to pH_o) was 0·37 under HCO_3^-/CO_2-buffered conditions and 0·45 under Hepes-buffered conditions; corresponding values at 37 °C were 0·71 and 0·79, respectively. The measurements of changes in pH_i evoked by changes in pH_o were employed in subsequent experiments to correct for the effects of changes in pH_i on the K_d of fura-2 for Ca^{2+}.

3. In fura-2-loaded neurones, rises in $[Ca^{2+}]_i$ evoked by transient exposure to 50 mM K^+ were reduced and enhanced during perfusion with acidic and alkaline media, respectively, compared with control responses at pH_o 7·3. Fifty percent inhibition of high-$[K^+]_o$-evoked rises in $[Ca^{2+}]_i$ corresponded to pH_o 7·23. In the presence of 10 μM nifedipine, 50% inhibition of high-$[K^+]_o$-evoked responses corresponded to pH_o 7·20, compared with a pH_o of 7·31 for 50% inhibition of $[Ca^{2+}]_i$ transients evoked by *N*-methyl-D-aspartate.

4. Changes in pH_i at a constant pH_o were evoked by exposing neurones to weak acids or bases and quantified in BCECF-loaded cells. Following pH-dependent corrections for the K_d of fura-2 for Ca^{2+}, rises in $[Ca^{2+}]_i$ evoked by high-$[K^+]_o$ in fura-2-loaded cells were found to be affected only marginally by changes in pH_i. When changes in pH_i similar to those observed during the application of weak acids or bases were elicited by changing pH_o, reductions in pH inhibited rises in $[Ca^{2+}]_i$ evoked by 50 mM K^+ whereas increases in pH enhanced them.

5. The effects of changes in pH on the kinetic properties of a BK-type Ca^{2+}-dependent K^+ channel were investigated. In inside-out patches excised from neurones in sister cultures to those used in the microspectrofluorimetric studies, with internal $[Ca^{2+}]$ at 20 μM, channel openings at an internal pH of 6·7 were generally absent whereas at pH 7·3 (or 7·8) the open probability was high. In contrast, channel activity in outside-out patches was not affected by reducing the pH of the bath (external) solution from 7·3 to 6·7. In inside-out patches with internal $[Ca^{2+}]$ at 0·7 μM, a separate protocol was applied to generate transient activation of the channel at a potential of 0 mV following a step from a holding level of −80 mV. In this case open probabilities were 0·81 (at pH 7·8), 0·57 (pH 7·3), 0·19 (pH 7·0) and 0·04 (pH 6·7). Channel conductance was not affected by changes in internal pH.

6. The results indicate that, in fetal rat hippocampal neurones, depolarization-evoked rises in $[Ca^{2+}]_i$ mediated by the influx of Ca^{2+} ions through dihydropyridine-sensitive and -resistant voltage-activated Ca^{2+} channels are modulated by changes in pH_o. The effects of pH_o cannot be accounted for by changes in pH_i consequent upon changes in pH_o. However, changes in pH_i affect the unitary properties of a Ca^{2+}-dependent K^+ channel. The results support the notion that pH_o and/or pH_i transients may serve a modulatory role in neuronal function.

The maintenance of acid–base balance within narrow limits is essential for normal cerebral function. It has often been assumed that the acid–base status of brain tissue remains stable under a wide variety of conditions and that, as a result, changes in pH are of little regulatory significance. Contemporary studies, however, have revealed that marked extra- and intracellular pH (pH_o and pH_i, respectively) shifts occur not only during pathophysiological events such as seizure activity and cerebral ischaemia but also during normal synaptic transmission (reviewed by Chesler & Kaila, 1992; Kaila, 1994). Furthermore, activity-evoked shifts in both pH_o and pH_i might act to modulate the events which initially caused them. For example, the extracellular alkaline transients evoked in hippocampal slices by the activation of glutamate or γ-aminobutyric $acid_A$ ($GABA_A$) receptors can modulate synaptic transmission and augment *N*-methyl-D-aspartate (NMDA) receptor-mediated responses (Voipio *et al.* 1995). Conversely mild extracellular acidosis, which may occur during cerebral ischaemia and convulsive activity, has been suggested to be a factor in limiting the extent of neuronal death following ischaemia and in the self-termination of seizures (see Tombaugh & Sapolsky, 1993 and references therein).

The sensitivities of certain ligand-gated ion channels present in mammalian central neurones, notably the NMDA and $GABA_A$ receptor-operated channels, to changes in both pH_o and pH_i have been established and, indeed, the effects of changes in pH on epileptiform and neurodegenerative phenomena have been interpreted largely in terms of modulation of the activities of these ligand-gated receptor–channel complexes (see Kaila, 1994; McBain & Mayer, 1994). However, modulation of voltage-activated conductances by changes in pH_o and/or pH_i may also play a role. Thus, in rat CA1 hippocampal pyramidal neurones, changes in pH_o affect the magnitude of both Ca^{2+}-dependent depolarizing potentials and hyperpolarizing potentials mediated by Ca^{2+}-activated K^+ conductances ($g_{K(Ca)}$) (Church & McLennan, 1989; Church, 1992) and modulate the activity of voltage-activated Ca^{2+} channels (Tombaugh & Somjen, 1996). However, in many cell types, changes in pH_o evoke changes in pH_i and it remains unclear whether changes in pH_i consequent upon changes in pH_o contribute to the known effects of changes in pH_o on neuronal excitability. For example, although reduced Ca^{2+} influx under low pH_o conditions could account for the inhibition of potentials mediated by $g_{K(Ca)}$ (Church & McLennan, 1989), a role for changes in pH_i either on voltage-activated Ca^{2+} channels themselves (Church, 1992; Tombaugh & Somjen, 1997) and/or on Ca^{2+}-activated K^+ channels cannot be ruled out. In the present study we have examined the effects of changes in pH_o and pH_i on high-$[K^+]_o$-evoked rises in $[Ca^{2+}]_i$ and on the activity of a Ca^{2+}-dependent K^+ channel in cultured fetal rat hippocampal neurones, an experimental preparation in which changes in pH_i can be reliably evoked and quantified.

METHODS

Cell preparation

Primary cultures of fetal rat hippocampal neurones were prepared as described (Baxter & Church, 1996). In brief, 18-day pregnant Wistar rats were anaesthetized with pentobarbitone (50 mg kg^{-1} I.P.) and, following removal of the fetuses, were killed with an intracardiac injection of pentobarbitone. Fetuses were decapitated, hippocampi were removed and, following enzymatic and mechanical dissociation, cells were plated onto glass coverslips coated with poly-D-lysine at a density of 1×10^5 cells cm^{-2}. Cultures were treated with 5-fluorodeoxyuridine to arrest glial cell proliferation and were maintained in a 5% CO_2 atmosphere at 36 °C in serum-free, N2-supplemented Dulbecco's modified Eagle's medium (Life Technologies, Burlington, ON, Canada) containing 22 mM $NaHCO_3$. Neurones were used 8–17 days after plating.

Solutions and chemicals

The standard HCO_3^-/CO_2-free perfusion medium contained (mM): NaCl, 136·5; KCl, 3; $CaCl_2$, 2; NaH_2PO_4, 1·5; $MgSO_4$, 1·5; D-glucose, 10; and Hepes, 10. It was saturated with 100% air and titrated to the appropriate temperature-corrected pH with 10 M NaOH. In standard HCO_3^-/CO_2-containing media, Hepes was isosmotically replaced by NaCl and solutions contained either 26 mM (room temperature, 20–22 °C) or 17·5 mM (37 °C) $NaHCO_3$, by equimolar substitution for NaCl, together with the constituents listed above; pH was 7·3 after equilibration with 95% air–5% CO_2. Low pH HCO_3^-/CO_2-buffered solutions contained either 7 or 10·5 mM $NaHCO_3$ (pH 6·9 after equilibration with 5% CO_2 at 37 °C and at room temperature, respectively); high pH solutions contained either 42·5 or 62·5 mM $NaHCO_3$ (pH 7·7 at 37 °C and at room temperature, respectively). Additional HCO_3^-/CO_2-buffered media for intermediate pH values were formulated according to the empirically derived equations $pH = 6{\cdot}02 + (1{\cdot}04 \times \log[HCO_3^-])$ (37 °C) and $pH = 5{\cdot}83 + (1{\cdot}04 \times \log[HCO_3^-])$ (room temperature); in all cases, changes in $[NaHCO_3]$ were balanced by equimolar changes in [NaCl]. During perfusion with HCO_3^--containing media, the atmosphere in the recording chamber contained 95% air with 5% CO_2. The pH of each solution was re-checked at the appropriate temperature following every experiment.

When external Cl^- was reduced at a constant pH_o, the corresponding methylsulphate salts were substituted. Solutions containing the weak acids sodium propionate (20 or 40 mM, as indicated in the text) or sodium butyrate (20 mM), or the weak base trimethylamine chloride (TMA, 10 mM), were prepared by equimolar substitution for NaCl. For Ca^{2+}-free media, Ca^{2+} was omitted, $[Mg^{2+}]$ was increased to 4 mM and 100 μM EGTA was added. High-$[K^+]$ media contained 50 mM KCl (by substitution for NaCl). In experiments employing NMDA, Mg^{2+} was omitted and 2 μM glycine added. Nifedipine-containing solutions were handled in the manner previously described (Church *et al.* 1994). Perfusion lines were replaced following each experiment.

Compounds were obtained from Sigma Chemical Co. with the exceptions of 2-amino-5-phosphonopentanoic acid (AP5) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), which were from Research Biochemicals International, and fura-2 AM and 2′,7′-bis-(2-carboxyethyl)-5-(and -6)-carboxyfluorescein acetoxymethyl ester (BCECF AM), which were from Molecular Probes, Inc.

Recording techniques

Microspectrofluorimetric studies. Fura-2 and BCECF were employed to estimate $[Ca^{2+}]_i$ and pH_i, respectively. In studies where information was required on the effects of an experimental

manoeuvre on both $[Ca^{2+}]_i$ and pH_i, measurements of $[Ca^{2+}]_i$ and pH_i were performed separately in parallel experiments conducted on sister cultures. Loading medium contained the same elements as the standard pH 7·3 Hepes-buffered solution (see above) with the isosmotic addition of 3 mM $NaHCO_3$ in place of NaCl (see Baxter & Church, 1996). Coverslips plated with neurones were placed in loading medium containing either 5 μM fura-2 AM for 60 min at 35 °C or 2 μM BCECF AM for 30 min at room temperature and then mounted in a temperature-controlled perfusion chamber so as to form the base of the chamber. Neurones were then superfused at a rate of 2 ml min^{-1} for 15 min with the initial experimental solution at the appropriate temperature prior to the start of an experiment. Both NMDA- (20 μM) and high-$[K^+]$-containing solutions were administered in 1 ml aliquots to the inflow of the perfusion chamber and allowed to remain in contact with neurones for 20 s before washout. Except where indicated, experiments were performed at room temperature in the presence of tetrodotoxin (TTX) at 0·5–1 μM.

$[Ca^{2+}]_i$ and pH_i were measured using the dual-excitation ratio method, employing a digital fluorescence microscopy system (Atto Instruments, Inc., Rockville, MD, USA; Carl Zeiss Canada Ltd, Don Mills, ON, Canada). Details of the methods employed have been presented previously (Church *et al.* 1994; Baxter & Church, 1996). In brief, fluorescence emissions from neurones loaded with either fura-2 or BCECF were obtained from multiple neurone somata simultaneously and raw intensity data at each excitation wavelength (334 and 380 nm for fura-2; 488 and 452 nm for BCECF) were corrected for background fluorescence prior to calculation of the ratio. The one-point high-$[K^+]$/nigericin technique was employed to convert background-corrected emission intensity ratios (I_{488}/I_{452}) into pH_i values as described (Baxter & Church, 1996). Analysis was restricted to those neurones able to retain BCECF (as judged by raw emission intensity values) throughout the course of an experiment (see Bevensee *et al.* 1996). Although BCECF is not sensitive to changes in $[Ca^{2+}]$ (Graber *et al.* 1986), the affinity of fura-2 for Ca^{2+} is sensitive to changes in pH such that the K_d increases as pH falls (Martínez-Zaguilán *et al.* 1996). In many experiments employing fura-2, pH_i was often changing and the figures depicting changes in $[Ca^{2+}]_i$ are (with the exceptions of Figs 4*B* and 5*B*) therefore presented as background-corrected I_{334}/I_{380} ratio values to which pH-dependent corrections for the K_d of fura-2 for Ca^{2+} were not applied. However, such corrections were applied prior to the quantitative analysis of data, including the estimation of the −log of the dissociation constant (pK) values for H^+ inhibition of peak $[Ca^{2+}]_i$ transients evoked by high-$[K^+]_o$ or NMDA (Figs 2*B* and 3*B* and *D*). In the latter cases, pH_i values at the peak of evoked $[Ca^{2+}]_i$ transients were estimated from experiments in which changes in pH_i in response to changes in pH_o were measured (see Results) and these pH_i values were employed to correct the K_d of fura-2 for Ca^{2+} according to Martínez-Zaguilán *et al.* (1996).

Single channel recordings. The procedures for recording large conductance Ca^{2+}-dependent K^+ currents (of the BK type and labelled $I_{BK(Ca)}$) have been described (McLarnon & Wang, 1991; McLarnon & Sawyer, 1993; McLarnon, 1995). In brief, patch pipettes were fabricated (Corning 7052 glass) and fire polished prior to the excision of inside-out patches from neurones in sister cultures to those utilized in the microspectrofluorimetric studies. For inside-out patches, the bath (internal) solution was (mM): KCl, 140; NaCl, 5; $CaCl_2$, 0·1; and Hepes, 10 (pH 7·3 with 10 M NaOH). The internal $[Ca^{2+}]$ was then reduced either to 0·7 or 20 μM (as indicated in the text) by the addition of EGTA; free $[Ca^{2+}]$ was maintained at a constant value during changes in pH using correction programs described by Fabiato (1988). The patch pipette solution contained (mM): NaCl, 140; KCl, 5; $CaCl_2$, 1; $MgCl_2$, 1; and Hepes, 10 (pH 7·3 with 10 M NaOH), and the electrodes had final resistances of 6–8 MΩ. $I_{BK(Ca)}$ values were recorded with the patch potential (V) set at either 0 or +20 mV. In some experiments in inside-out patches with 0·7 μM internal $[Ca^{2+}]$, a specific protocol was applied consisting of a 10 s prepulse to $V = -80$ mV prior to V being set at 0 mV. This procedure has previously been shown to lead to activation (at pH 7·3) of $I_{BK(Ca)}$ in inside-out patches excised from cultured fetal rat hippocampal neurones (McLarnon, 1995). Outside-out patches were utilized in some experiments; in these cases, the pipette and bath solutions were reversed.

The amplifier used to record unitary outward $I_{BK(Ca)}$ was an Axopatch-1B (Axon Instruments, Inc.) with a sampling rate of 5 kHz and low-pass filter set at 1 or 2 kHz. Data were stored on computer for subsequent analysis using pCLAMP v. 5.5.1 (Axon Instruments, Inc.). Distributions of amplitudes and open and closed times were collated from the analysis of unitary openings and, except where noted in the text, contained a minimum of 200 events. Channel open probability (P_o) was determined by summing individual open times during the recording period and dividing this sum by the total time at a given patch potential. Experiments were performed at room temperature.

Data analysis

Results are reported as means ± S.E.M. with the accompanying n value referring either to the number of cell populations (i.e. number of coverslips) analysed (microspectrofluorimetric studies) or to the number of patches (single channel studies). The number of neurones from which data were obtained in the former experiments was 1853. Statistical comparisons were performed using Student's two-tailed t test with a 95% confidence limit.

RESULTS

Microspectrofluorimetric studies

In initial experiments we determined the maximum extent to which pH_i changed in response to exposure to HCO_3^-/CO_2- and Hepes-buffered media at a variety of different pH values (Fig. 1). At room temperature, the ratio $\Delta pH_i : \Delta pH_o$ (the slope of the regression line relating pH_i to pH_o) was 0·37 under HCO_3^-/CO_2-buffered conditions ($n = 7$) and 0·45 under Hepes-buffered conditions ($n = 5$). The $\Delta pH_i : \Delta pH_o$ ratio values obtained at 37 °C were greater than those observed at room temperature, being 0·71 under HCO_3^-/CO_2-buffered conditions ($n = 6$) and 0·79 under Hepes-buffered conditions ($n = 4$). The equations relating pH_i to pH_o (see Fig. 1, legend) were employed to estimate changes in pH_i consequent upon changes in pH_o, in order to apply pH-dependent corrections for the K_d of fura-2 for Ca^{2+} in subsequent experiments in which $[Ca^{2+}]_i$ was measured (see Methods).

The effects of changes in pH_o ($n = 4$ in both HCO_3^-/CO_2- and Hepes-buffered media) on rises in I_{334}/I_{380} ratio values evoked by 20 μM NMDA were then examined (Fig. 2*A*). Nifedipine (10 μM) was present to limit the contribution of Ca^{2+} influx via L-type high voltage-activated (HVA) Ca^{2+} channels to the response (see below). No differences were

observed in the effects of changes in pH_o on rises in I_{334}/I_{380} ratio values under the different buffering conditions and the results were therefore pooled after appropriate (i.e. buffering condition-specific) corrections for the pH dependence of the K_d of fura-2 for Ca^{2+} had been applied. Nifedipine (10 μM) reduced NMDA-evoked rises in $[Ca^{2+}]_i$ by 48 ± 2% under control conditions at pH_o 7·3. Compared with responses evoked at pH_o 7·3, NMDA-evoked rises in $[Ca^{2+}]_i$ were reduced by 37 ± 1% at pH_o 6·9 and increased by 46 ± 3% at pH_o 7·7. Figure 2*B* summarizes the pH_o dependence of NMDA-evoked rises in $[Ca^{2+}]_i$; 50% inhibition corresponded to a pH_o of 7·31.

In the neurones employed, rises in $[Ca^{2+}]_i$ evoked by transient application of 50 mM K^+ are mediated primarily by dihydropyridine-sensitive (L-type) HVA Ca^{2+} channels, with smaller contributions from ω-conotoxin GVIA-sensitive (N-type) HVA Ca^{2+} channels and Ca^{2+} channels insensitive to dihydropyridines and ω-conotoxin GVIA but sensitive to crude funnel-web spider venom (Church *et al.* 1994). High $[K^+]_o$-evoked rises in I_{334}/I_{380} ratio values were reduced by 96 ± 1% ($n = 4$) during perfusion with Ca^{2+}-free medium, indicating that the release of Ca^{2+} from internal stores is not involved to an appreciable extent in the high-$[K^+]_o$ response in our experimental preparation. The addition of 40 μM AP5 and 20 μM CNQX to Ca^{2+}-containing perfusion medium reduced K^+-evoked rises in I_{334}/I_{380} ratio values by 6 ± 4% ($n = 6$), indicating that endogenously released glutamate contributes little to the response (see Church *et al.* 1994).

Under both HCO_3^-/CO_2-buffered ($n = 6$) and Hepes-buffered ($n = 6$) conditions, lowering pH_o attenuated rises in I_{334}/I_{380} ratio values evoked by 50 mM K^+ whereas high-$[K^+]_o$-evoked rises in ratio values were enhanced during exposure to high pH media (Fig. 3*A*). After corrections for the pH dependence of the K_d of fura-2 for Ca^{2+} had been applied, perfusion with pH 6·9 and pH 7·7 media were found to reduce and increase, respectively, high-$[K^+]_o$-evoked rises in $[Ca^{2+}]_i$ by 38 ± 4% and 32 ± 2%, compared with control responses evoked at pH_o 7·3. Since the rise in $[Ca^{2+}]_i$ evoked by 50 mM K^+ is dominated by Ca^{2+} influx through L-type Ca^{2+} channels, the results suggest that changes in pH_o modulate the activity of this subtype of HVA Ca^{2+} channel. A 50% inhibition of the high-$[K^+]_o$-evoked rise in $[Ca^{2+}]_i$ corresponded to a pH_o of 7·23 (Fig. 3*B*). Perfusion with methylsulphate-substituted media containing 91 mM Cl^- (the same as in the high-$[HCO_3^-]$, pH 7·7 medium) at pH 7·3 did not affect the magnitude of high-$[K^+]_o$-evoked rises in I_{334}/I_{380} ratio values ($n = 6$; not shown).

To examine the effects of changes in pH_o on rises in $[Ca^{2+}]_i$ mediated by Ca^{2+} influx through dihydropyridine-resistant HVA Ca^{2+} channels, 10 μM nifedipine (a maximally effective concentration in the neurones employed; see Church *et al.* 1994) was added to the perfusate. Addition of

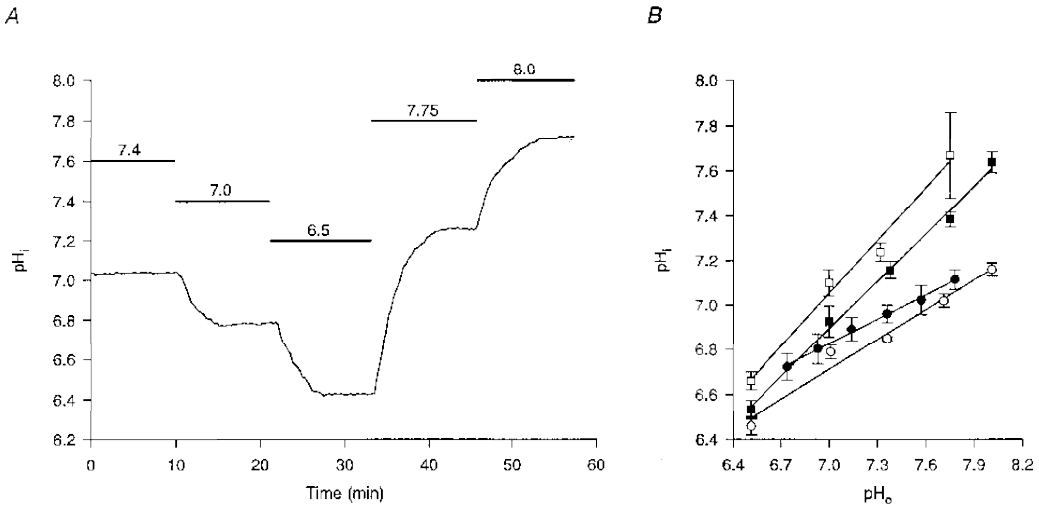

Figure 1. Dependence of pH_i on pH_o

A, in an experiment conducted at 37 °C under HCO_3^-/CO_2-buffered conditions, decreasing or increasing pH_o for the periods indicated by the bars above the trace and to the values shown above the bars, decreased and increased pH_i, respectively. The record is a mean of data obtained from 9 neurones simultaneously. *B*, linear regression analysis of the dependence of pH_i on pH_o at both room temperature (●) and at 37 °C (■) under HCO_3^-/CO_2-buffered conditions and under Hepes-buffered conditions (○ and □, respectively). Each point represents data obtained from a minimum of 4 neuronal populations; error bars are S.E.M. The equations relating pH_i to pH_o at room temperature and at 37 °C under HCO_3^-/CO_2-buffered conditions were, respectively, $pH_i = 4{\cdot}27 + (0{\cdot}37 \times pH_o)$ ($r^2 = 0{\cdot}99$) and $pH_i = 1{\cdot}92 + (0{\cdot}71 \times pH_o)$ ($r^2 = 0{\cdot}99$). The equations relating pH_i to pH_o at room temperature and at 37 °C under Hepes-buffered conditions were, respectively, $pH_i = 3{\cdot}60 + (0{\cdot}45 \times pH_o)$ ($r^2 = 0{\cdot}98$) and $pH_i = 1{\cdot}52 + (0{\cdot}79 \times pH_o)$ ($r^2 = 0{\cdot}99$).

nifedipine (10 μM) to pH 7·3 medium reduced high-$[K^+]_o$-evoked rises in I_{334}/I_{380} ratio values by 79 ± 2% ($n = 7$) and the residual K^+-evoked $[Ca^{2+}]_i$ transients were attenuated during perfusion with low pH media and enhanced during perfusion with high pH media (Fig. 3*C*). After correcting for the pH dependence of the K_d of fura-2 for Ca^{2+}, a 50% inhibition of high-$[K^+]_o$-evoked rises in $[Ca^{2+}]_i$ recorded in the presence of 10 μM nifedipine was found to correspond to pH_o 7·20 (Fig. 3*D*).

In order to determine whether the effects of changes in pH_o on K^+-evoked rises in $[Ca^{2+}]_i$ reflected changes in pH_o *per se* or were secondary to changes in pH_i consequent upon changes in pH_o, weak acids and bases were employed to change pH_i at a constant pH_o (Fig. 4). In five experiments of the type illustrated in Fig. 4*A*, 10 mM TMA evoked a rise in pH_i of 0·26 ± 0·02 pH units measured at 6 min following its introduction, whereas a reduction in pH_i of 0·21 ± 0·03 pH units was observed at 5 min following its washout. In paired experiments performed on sister cultures, high-$[K^+]_o$ was applied such that the peaks of the ensuing rises in $[Ca^{2+}]_i$ occurred at 6 min following the introduction of the weak base and at 5 min following its withdrawal. As shown in Fig. 4*B* (continuous line), the $[Ca^{2+}]_i$ transient appeared to be enhanced when pH_i was raised above resting levels (a 17 ± 6% increase) whereas the response appeared to be reduced when pH_i was below resting levels (a 22 ± 6% decrease), suggesting that changes in pH_i at a constant pH_o modulate K^+-evoked rises in $[Ca^{2+}]_i$. However, when corrections for the effects of pH on the K_d of fura-2 for Ca^{2+} were applied (a 14% decrease in the K_d at the time when pH_i was increased by 0·26 pH units and a 19% increase when pH_i was decreased by 0·21 pH units), it became apparent that changing pH_i at a constant pH_o had only a minor effect on the magnitude of high-$[K^+]_o$-evoked rises in $[Ca^{2+}]_i$. Thus, following the application of pH-dependent corrections for the K_d of fura-2 for Ca^{2+}, K^+-evoked $[Ca^{2+}]_i$ transients increased by 1 ± 6% during exposure to 10 mM TMA and decreased by 2 ± 6% following its washout (Fig. 4*B*).

Experiments analogous to those with TMA were performed with the weak acid propionate ($n = 5$). At 6 min after the start of exposure to 20 mM propionate pH_i decreased by 0·10 ± 0·02 pH units, whereas pH_i increased by 0·10 ± 0·02 pH units at 6 min following its washout. After pH-dependent corrections for the K_d of fura-2 for Ca^{2+} were applied, peak $[Ca^{2+}]_i$ responses were found to be increased by 3 ± 5% at the time at which pH_i was decreased by 0·10 pH units and changed by 0 ± 2% at the time at which

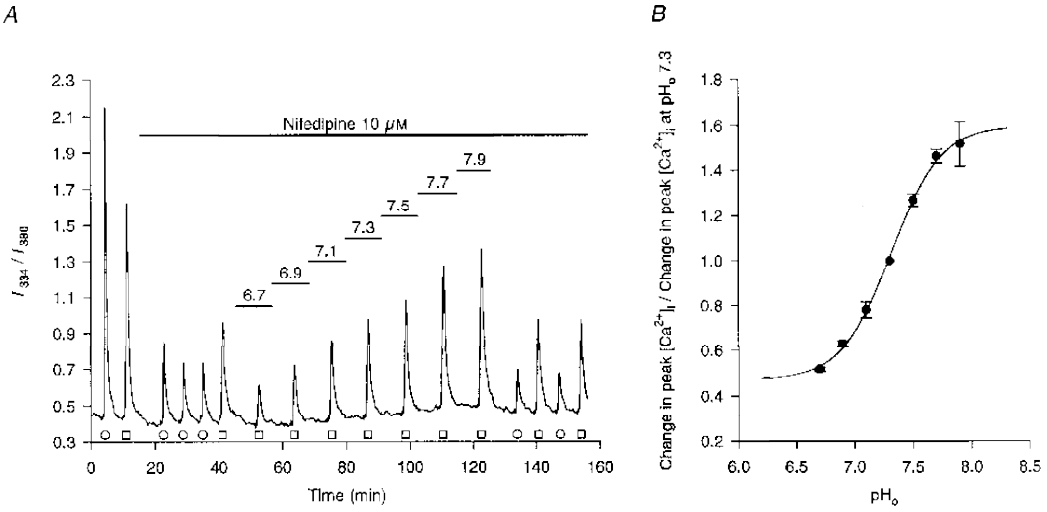

Figure 2. Changes in pH_o modulate NMDA-evoked increases in I_{334}/I_{380} ratio values

A, under control conditions (pH_o 7·3), transient exposures to 50 mM $[K^+]_o$ (○) or 20 μM NMDA (□) evoked rises in the I_{334}/I_{380} ratio value. Subsequent addition of 10 μM nifedipine to the Hepes-buffered perfusion medium reduced high-$[K^+]_o$-evoked increases in the ratio value by 81% (third, fourth and fifth responses) and NMDA-evoked increases in the ratio value by 54% (sixth response). The pH of the perfusion medium was then changed sequentially from 6·7 to 7·9 in 0·2 pH unit increments, for the periods indicated by the bars above the trace, resulting in a gradual increase in NMDA-evoked rises in the I_{334}/I_{380} ratio values. The final four responses shown are K^+- and NMDA-evoked responses upon return to pH 7·3 medium. The record is a mean of data obtained from 10 neurones simultaneously, the experiment being performed at room temperature. *B*, pH-dependent corrections for the K_d of fura-2 for Ca^{2+} (see Methods) were applied to the data shown in *A* and other, similar experiments and a plot was made of pH_o *versus* changes in peak $[Ca^{2+}]_i$ responses evoked by NMDA in the presence of 10 μM nifedipine, normalized to the peak of the $[Ca^{2+}]_i$ response obtained at pH_o 7·3. Each point represents data obtained from a minimum of 3 neuronal populations. The 4-parameter logistic plot ($r^2 = 0·99$) had a pK of 7·31. The extrapolated maximum and minimum values were 160 and 47%, respectively.

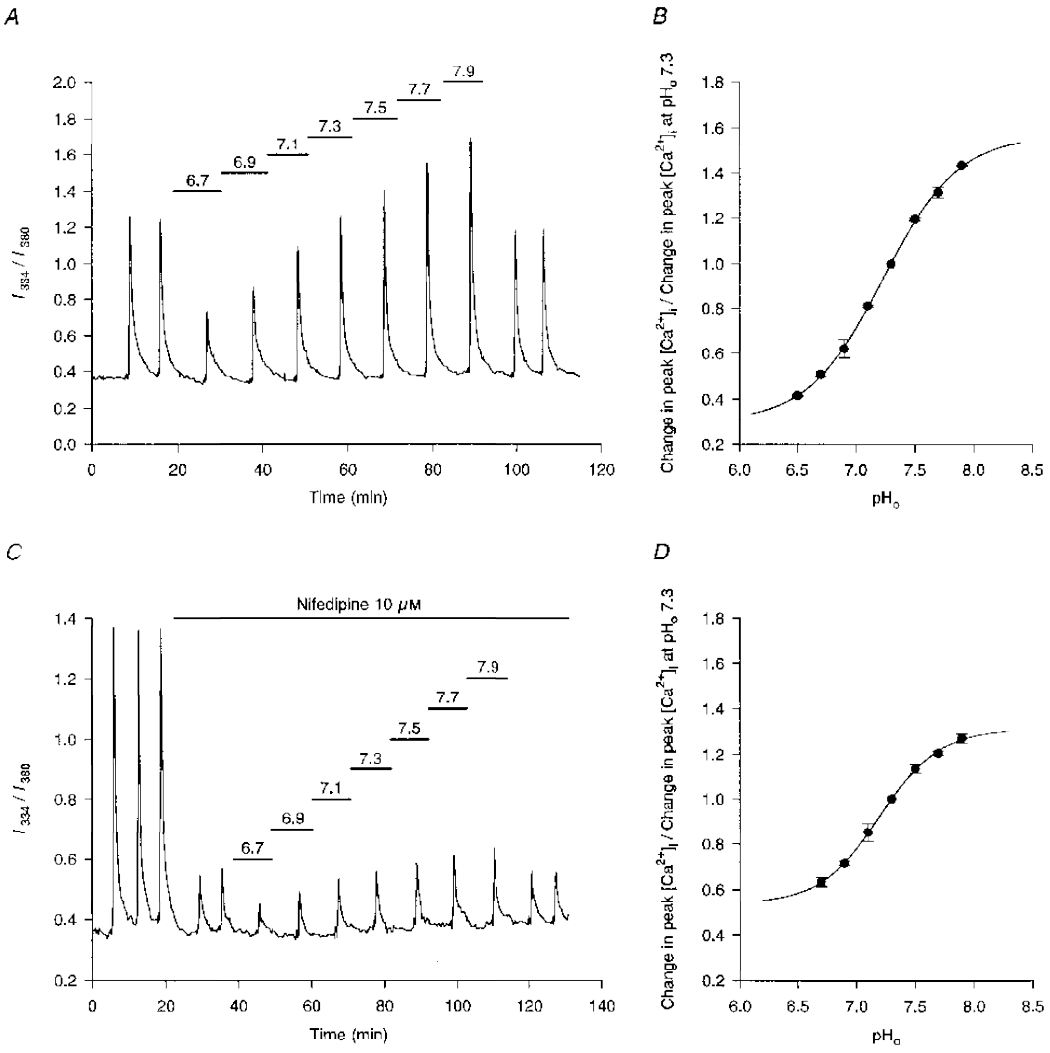

Figure 3. Changes in pH_o modulate high-$[K^+]_o$-evoked increases in I_{334}/I_{380} ratio values

A, under control conditions (pH_o 7·3) two consecutive applications of 50 mM $[K^+]_o$ evoked stable rises in the I_{334}/I_{380} ratio. Subsequent responses to 50 mM K^+ were obtained at the pH_o values indicated by the bars above the trace. The final 2 responses were obtained upon reperfusion with pH 7·3 medium. The record is a mean of data obtained from 16 neurones simultaneously. The experiment was performed at room temperature in HCO_3^-/CO_2-buffered media. *B*, pH-dependent corrections for the K_d of fura-2 for Ca^{2+} (see Methods) were applied to the data shown in *A* and other, similar experiments and a plot was made of pH_o *versus* changes in peak high-$[K^+]_o$-evoked $[Ca^{2+}]_i$ responses, normalized to the peak of the $[Ca^{2+}]_i$ response obtained at pH_o 7·3. Each point represents data obtained from a minimum of 4 neuronal populations. The 4-parameter logistic plot ($r^2 = 0{\cdot}99$) had a pK of 7·23 and extrapolated maximum and minimum values of 158 and 31%, respectively. *C*, under control conditions (pH_o 7·3) three consecutive applications of 50 mM $[K^+]_o$ evoked stable rises in the I_{334}/I_{380} ratio. Addition of 10 μM nifedipine reduced the high-$[K^+]_o$-evoked rises in the I_{334}/I_{380} ratio by ~80% (fourth and fifth responses). Subsequent responses to 50 mM K^+ were obtained at the pH_o values indicated by the bars above the trace. The final two responses were obtained upon reperfusion with pH 7·3 medium. The record is a mean of data obtained from 7 neurones simultaneously. The experiment was performed at room temperature in Hepes-buffered media containing 40 μM AP5 and 20 μM CNQX throughout. *D*, pH-dependent corrections for the K_d of fura-2 for Ca^{2+} were applied to the data shown in *C* and other, similar experiments and a plot was made of pH_o *versus* changes in peak high-$[K^+]_o$-evoked $[Ca^{2+}]_i$ responses, normalized to the peak of the $[Ca^{2+}]_i$ response obtained at pH_o 7·3. Each point represents data obtained from a minimum of 4 neuronal populations. The 4-parameter logistic plot ($r^2 = 0{\cdot}99$) had a pK of 7·20 and extrapolated maximum and minimum values of 131 and 54%, respectively.

pH_i was increased by 0·10 pH units. Similar results were obtained when 20 mM butyrate was employed as the weak acid ($n = 3$; not shown).

In the final series of experiments, high-$[K^+]_o$-induced rises in $[Ca^{2+}]_i$ were evoked initially during perfusion with pH 7·3 medium containing propionate at 40 mM (under which conditions pH_i falls at a constant pH_o) and subsequently during exposure to low pH medium (under which conditions both pH_o and pH_i fall); corresponding pH_i changes were examined in parallel experiments in sister cultures. In three experiments of the type shown in Fig. 5*A*, exposure to 40 mM propionate ($pH_o = 7·3$) evoked a fall in pH_i of 0·15 ± 0·01 pH units measured at 8 min after the start of exposure to the weak acid. Following the washout of propionate and the return of pH_i to normal resting levels, exposure to a pH 6·85 medium evoked a fall in pH_i of 0·17 ± 0·04 pH units measured at 16 min following the start of perfusion. Figure 5*B* illustrates one of three corresponding experiments conducted in fura-2-loaded

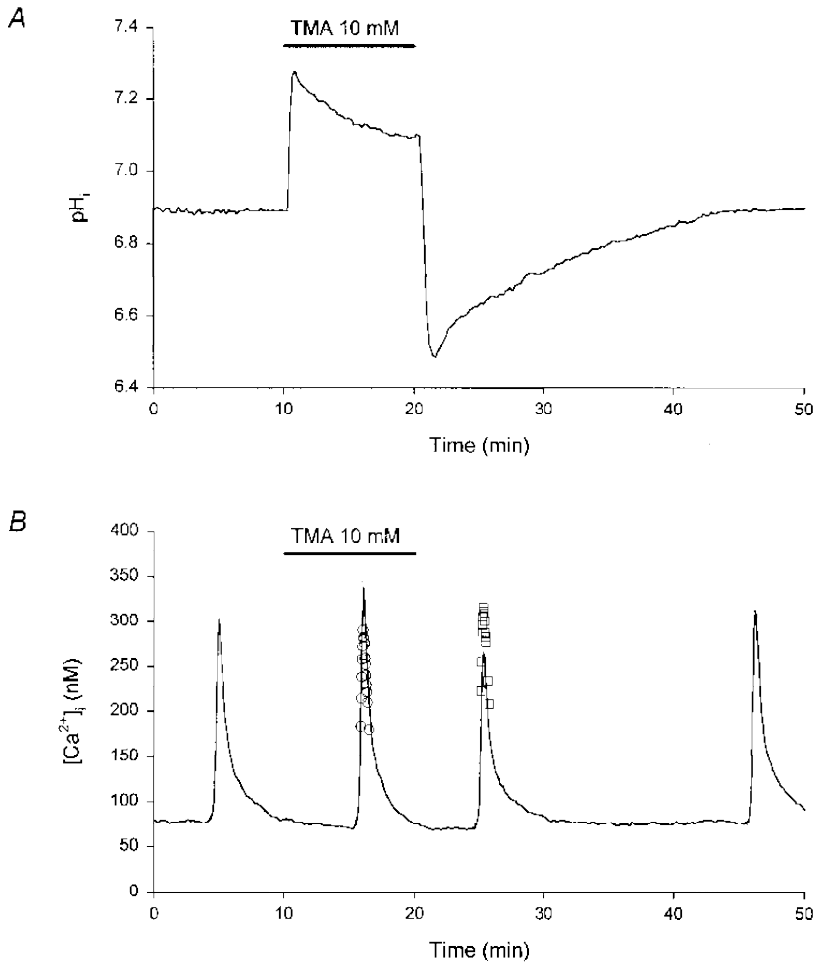

Figure 4. Modulation of high-$[K^+]_o$-evoked increases in $[Ca^{2+}]_i$ by changes in pH_i

A, a 10 min application of the weak base trimethylamine (TMA, 10 mM) evoked a rise in pH_i. Following the withdrawal of TMA, pH_i fell to values below the initial resting level and then recovered gradually. The experiment was performed at room temperature in HCO_3^-/CO_2-buffered media (pH 7·3). The record is a mean of data obtained from 17 neurones simultaneously. *B*, under control conditions (pH_o 7·3) an application of 50 mM $[K^+]_o$ evoked a rise in $[Ca^{2+}]_i$ (first response). Subsequent responses to 50 mM K^+ were obtained at 6 min after the start of a 10 min period of perfusion with a pH 7·3 medium containing 10 mM TMA (second response), and at 5 and 25 min following the withdrawal of TMA (third and fourth responses, respectively). A single K_d value for fura-2 (169·13, corresponding to a resting pH_i of 6·93, measured in 5 experiments of the type illustrated in *A*) was employed to generate the continuous line. The open circles (○) represent the peak of the rise in $[Ca^{2+}]_i$ computed using a K_d 14% lower than that employed for the continuous line, to reflect the rise in pH_i observed at 6 min after the start of application of TMA (see Results). The open squares (□) represent the peak of the rise in $[Ca^{2+}]_i$ computed using a K_d 19% higher than that employed for the continuous line, to reflect the fall in pH_i observed at 5 min following the withdrawal of TMA (see Results). The record is a mean of data obtained from 14 neurones simultaneously. The experiment was performed in a sister culture to that employed in the experiment shown in *A*, under identical conditions.

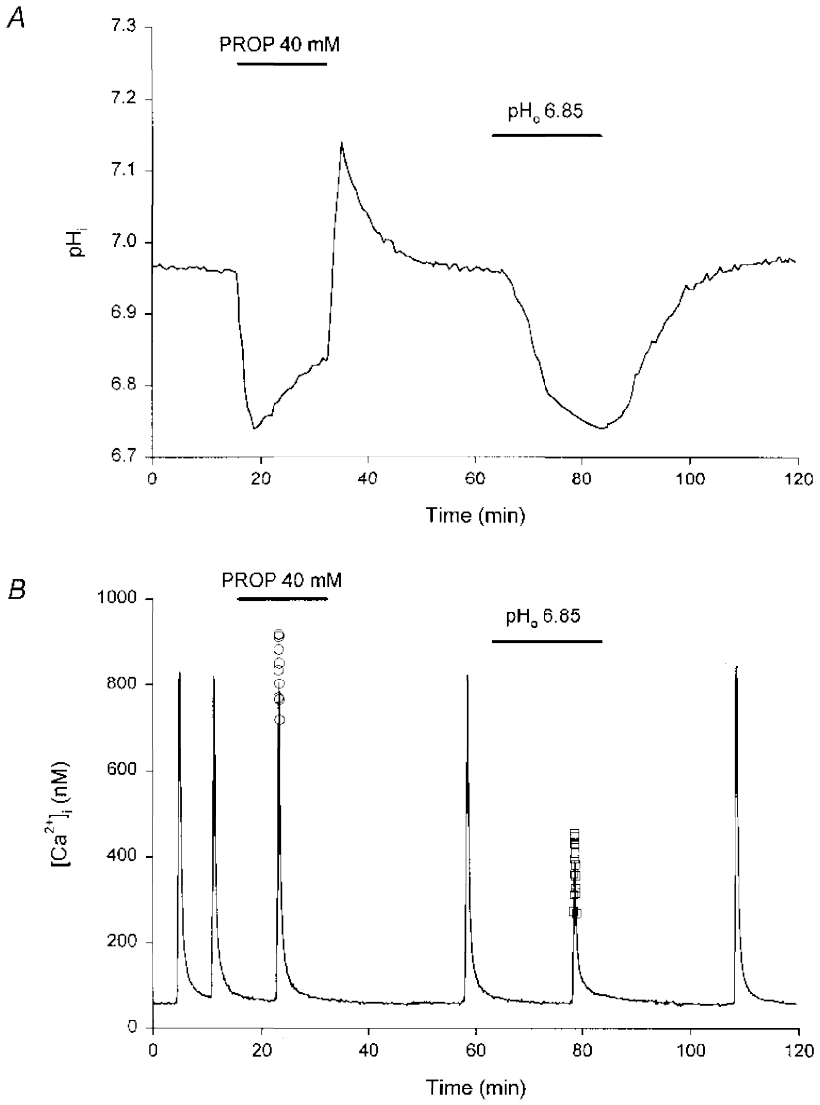

Figure 5. Comparison of the effects of changes in pH_i and pH_o on high-$[K^+]_o$-evoked increases in $[Ca^{2+}]_i$

A, a 16 min application of the weak acid propionate (PROP, 40 mM) evoked a fall in pH_i. Following the recovery of pH_i to the initial resting level, the pH of the perfusate was reduced from 7·3 to 6·85 for 20 min. pH_i fell gradually and recovered to the initial resting level upon re-perfusion with pH 7·3 medium. The experiment was performed at room temperature in HCO_3^-/CO_2-buffered media. The record is a mean of data obtained from 7 neurones simultaneously. *B*, under control conditions (pH_o 7·3) two consecutive applications of 50 mM $[K^+]_o$ evoked stable rises in $[Ca^{2+}]_i$ (first and second responses). The third response was obtained 8 min after the start of a 16 min period of perfusion with a pH 7·3 medium containing 40 mM propionate. The final three responses were obtained immediately prior to perfusion with pH 6·85 medium, at 16 min following the start of a 20 min exposure to pH 6·85 medium, and during a period of reperfusion with pH 7·3 medium. A single K_d value for fura-2 (corresponding to the resting pH_i measured in 3 experiments of the type illustrated in *A*) was employed to generate the continuous line. The open circles (○) represent the peak of the rise in $[Ca^{2+}]_i$ computed using a K_d 14% higher than that employed for the continuous line, to reflect the fall in pH_i observed at 8 min after the start of application of propionate (see Results). The open squares (□) represent the peak of the rise in $[Ca^{2+}]_i$ computed using a K_d 16% higher than that employed for the continuous line, to reflect the fall in pH_i observed at 16 min following the start of exposure to pH 6·85 medium (see Results). The record is a mean of data obtained from 6 neurones simultaneously. The experiment was performed in a sister culture to that employed in the experiment shown in *A*, under identical conditions.

neurones. After correcting for the pH dependence of the K_d of fura-2 for Ca^{2+}, peak $[Ca^{2+}]_i$ responses to 50 mM K^+ were found to be increased by $10 \pm 5\%$ at 8 min following the start of perfusion with 40 mM propionate; in contrast, corrected peak $[Ca^{2+}]_i$ responses to 50 mM K^+ were reduced by $45 \pm 4\%$ at 16 min following the start of perfusion with pH 6·85 medium. Since pH_i values were similar under both conditions, the results indicate that the attenuation of K^+-evoked rises in $[Ca^{2+}]_i$ during exposure to low pH media reflects reductions in pH_o rather than pH_i. Similar paired experiments ($n = 3$) were performed in which cells were exposed initially to pH 7·3 medium containing 10 mM TMA and subsequently to pH 7·85 medium. Measured at 5 min following the start of perfusion with 10 mM TMA, pH_i increased by $0·29 \pm 0·04$ pH units, at which time K^+-evoked rises in $[Ca^{2+}]_i$ (corrected for the pH dependence of the K_d of fura-2 for Ca^{2+}) were found to be increased by $12 \pm 7\%$. The pH_i increase observed at 15 min following the start of exposure of the neurones to pH 7·85 medium was $0·23 \pm 0·02$ pH units, at which time corrected high-$[K^+]_o$-evoked $[Ca^{2+}]_i$ transients were increased by $38 \pm 3\%$.

Single channel recordings

The findings detailed above indicate that changes in pH_i at a constant pH_o exert only a limited effect on the magnitude of rises in $[Ca^{2+}]_i$ mediated by Ca^{2+} influx through HVA Ca^{2+} channels. However, previous studies in hippocampal neurones have indicated that reductions in pH_i at a constant pH_o attenuate both the fast after-hyperpolarization (AHP) observed following a single depolarizing current-evoked action potential and the slow AHP observed after a train of action potentials (Church, 1992). Because these hyperpolarizing potentials are mediated by $g_{K(Ca)}$ (see Storm, 1990), the possibility exists that changes in the magnitudes of potentials mediated by $g_{K(Ca)}$ previously observed in hippocampal neurones during changes in pH_o (Church &

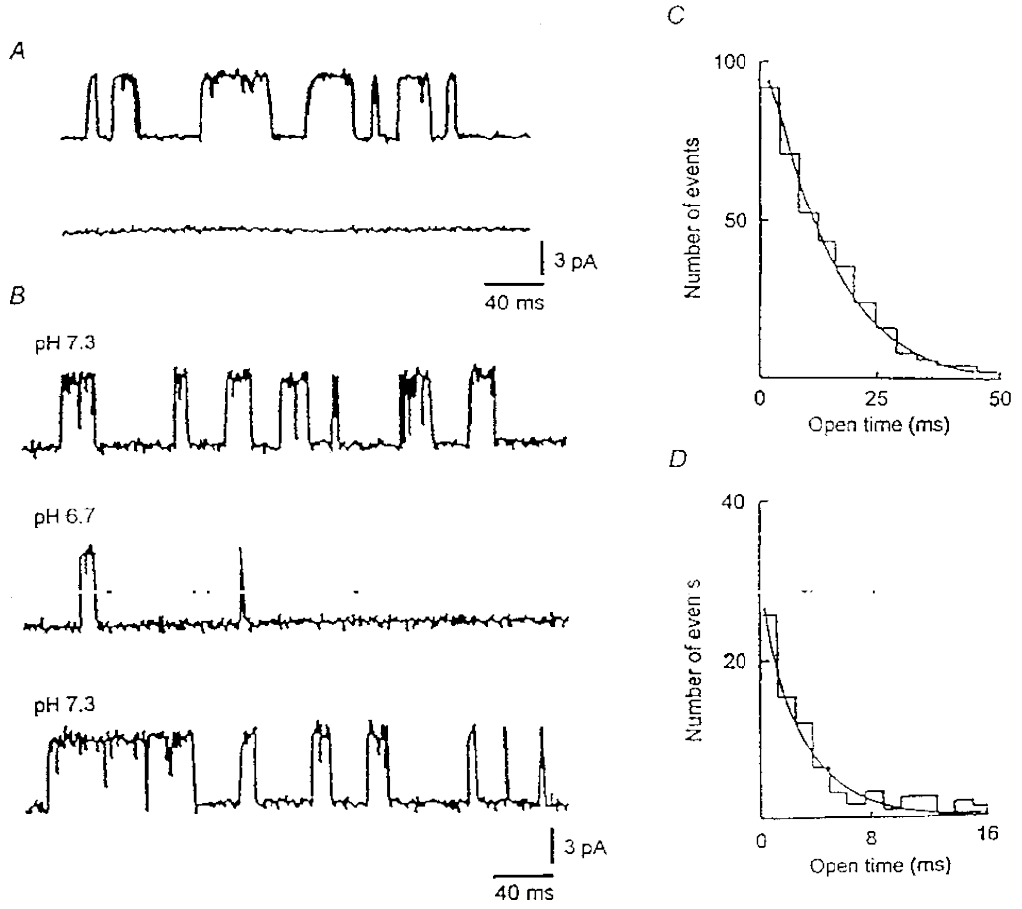

Figure 6. Unitary properties of a Ca^{2+}-dependent K^+ channel

A, single channel current recordings from an inside-out patch with internal (bath) $[Ca^{2+}]$ at 20 μM (upper trace) and lack of activity when internal $[Ca^{2+}]$ was decreased to 0·7 μM (lower trace). In both records, the internal pH was 7·3 and the patch potential was +20 mV. Channel activity was restored upon reperfusion with medium containing 20 μM Ca^{2+} (not shown). *B*, openings from a different inside-out patch with internal $[Ca^{2+}]$ at 20 μM and internal pH values of 7·3 (upper trace), 6·7 (middle trace) and following return to pH 7·3 (lower trace). The patch potential was +20 mV for all records. *C*, distribution of open times (at $V = +20$ mV) at pH 7·3 (collation of 462 events); the mean open time was 19·8 ms. *D*, distribution of open times (at $V = +20$ mV) at pH 6·7 (collation of 107 events); the mean open time was 5·4 ms.

McLennan, 1989) may reflect not only changes in Ca^{2+} influx (consequent upon changes in pH_o) but also direct effects of changes in pH_i on Ca^{2+}-activated K^+ channels. We therefore examined the effects of changes in internal and external pH on the unitary properties of a Ca^{2+}-dependent K^+ channel in, respectively, inside-out and outside-out patches excised from cultured hippocampal neurones. We have characterized previously some of the properties of this BK-type channel in hippocampal neurones, which include a Hill coefficient for activation of ~2, a Mg^{2+}-dependent modulation of kinetic behaviour, block by low concentrations of external TEA and open-channel blockade by class III antiarrhythmic agents (McLarnon & Wang, 1991; McLarnon & Sawyer, 1993; McLarnon, 1995).

Representative unitary recordings of $I_{BK(Ca)}$ from an inside-out patch are shown in Fig. 6*A* (upper trace), where internal pH is 7·3, internal $[Ca^{2+}]$ is 20 μM and V is +20 mV; the internal and external $[K^+]$ were 140 and 5 mM, respectively. An estimate for the unitary (slope) conductance could be derived from measurements of the amplitudes of currents recorded for V over the range 0 to +60 mV (data not shown) and yielded a value of 102 pS, similar to values obtained for BK-type channels in other cell types with physiological-like K^+ across inside-out patches (see Barrett *et al.* 1982). A reduction of internal $[Ca^{2+}]$ to 0·7 μM (lower trace of Fig. 6*A*) led to cessation of channel activity in six out of six inside-out patches examined (also see McLarnon & Sawyer, 1993). The effects on unitary $I_{BK(Ca)}$ of reducing internal pH at a constant internal $[Ca^{2+}]$ (20 μM) are shown in Fig. 6*B*, where the upper trace shows currents recorded at pH 7·3 ($V = +20$ mV). Reducing pH to 6·7 caused a marked decrease in the number of channel openings with activity restored upon reperfusion with pH 7·3 medium. The amplitudes of the currents were unchanged and were not altered by reductions in internal pH at other patch potentials (not shown); hence, unitary conductance was independent of internal pH. In four additional experiments, channel activity evident at pH 7·3 was effectively abolished when internal pH was reduced to pH 6·7 (also see Kume *et al.* 1990). Distributions of open times at pH 7·3 and pH 6·7 ($V = +20$ mV) are presented in Figs 6*C* and *D*, respectively. The distributions were fitted with single exponential functions, as previously employed in studies of BK-type channels (e.g. Christensen & Zeuthen, 1987), and the mean open times were 19·8 ± 0·4 ms (pH 7·3) and 5·4 ± 1·7 ms (pH 6·7). Thus, in inside-out patches, reducing internal pH from 7·3 to 6·7 caused a marked decrease in the number of open events and mean open time ($n = 1$) or a cessation of channel activity ($n = 4$). For the single patch, there were insufficient events at pH 6·7 to permit fitting of a closed time distribution; however, an estimate of a mean closed time was inferred from the occasional events and yielded a value near 300 ms; the corresponding value at pH 7·3 was 4·8 ± 1·8 ms (collation of 760 events). It should be noted that closed time distributions for the BK-type channel in hippocampal neurones require two-component fits (e.g. McLarnon & Sawyer, 1993) and the use of mean values to compare closed times serves only as an approximation for the effects of changes in pH on this quantity.

The channel P_o is the product of mean open time and frequency of channel opening. Overall, for inside-out patches with internal pH of 7·3 and internal $[Ca^{2+}]$ of 20 μM, the channel P_o at $V = +20$ mV was significantly ($P < 0·05$; $n = 5$) reduced from 0·81 ± 0·06 to 0·06 ± 0·03

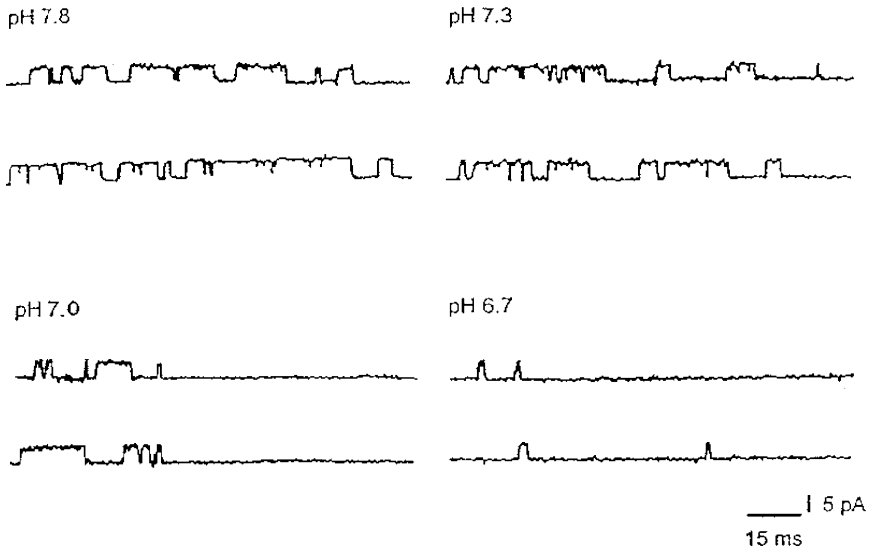

Figure 7. Effects of changes in internal pH on transient activation of $I_{BK(Ca)}$
The protocol was to initially hold the potential of the inside-out patch at −80 mV for 10 s and then to step V to 0 mV. The records shown commence 4 ms following the steps to 0 mV. No further events were evident following the final closures shown in each of the traces, despite a maintained potential of 0 mV (see McLarnon, 1995). P_o values were determined from the analysis of 10 steps, for times of 70 ms following each step, for each of the pH values tested; internal $[Ca^{2+}]$ was 0·7 μM throughout.

when internal pH was lowered from 7·3 to 6·7. The reduction in P_o reflected a decrease in both the frequency of channel opening and the mean duration of the open state, with the effect of acidosis to lower the P_o being greater compared to its effect in reducing mean open time. In essence, reducing internal pH from 7·3 to 6·7 had the most significant effect in increasing closed time. A similar result has been documented for a Ca^{2+}-activated maxi K^+ channel in epithelium where, over the pH range 7·4 to 6·4, acidosis increased closed time by a factor of 10 whereas open time was diminished 2-fold (Christensen & Zeuthen, 1987).

To determine whether a change in external pH might also affect channel activity, we examined the effects of reducing external pH on unitary $I_{BK(Ca)}$ in outside-out patches ($n = 4$). In this configuration the unitary conductance, derived by recording currents at patch potentials of 0 mV and +20 mV, was estimated at 115 pS, a value close to that established for channel conductance using inside-out patches (see above). Reducing the pH of the bath (external) solution from 7·3 to 6·7 had no effect on channel activity in outside-out patches; channel P_o remained unchanged near 0·9 and amplitudes of the unitary currents were unaffected (data not shown). Therefore, over the pH range 7·3 to 6·7, changes in extracellular pH had no effects in altering the unitary properties of $I_{BK(Ca)}$ in hippocampal neurones.

In inside-out patches, increasing internal pH from 7·3 to 7·8 ($n = 2$) had little effect on channel mean open times (18·7 ± 1·6 ms and 20·3 ± 1·3 ms at pH 7·3 and pH 7·8, respectively) or P_o (0·76 ± 0·05 and 0·81 ± 0·06). As previously discussed by Christensen & Zeuthen (1987) and Kume *et al.* (1990), the lack of dependence of P_o on internal pH when pH was > 7·3 could indicate that binding sites for activation of $I_{BK(Ca)}$ were saturated at the internal $[Ca^{2+}]$ of 20 μM. However, when internal $[Ca^{2+}]$ was reduced from 20 to 0·7 μM at an internal pH of 7·3, no activation of $I_{BK(Ca)}$ was evident at maintained potentials of 0 or +20 mV (see Fig. 6*A*; also see Fig. 1*B* in McLarnon & Sawyer (1993) for a plot of P_o for the channel *versus* internal $[Ca^{2+}]$ in cultured fetal rat hippocampal neurones). Therefore, in order to examine possible differences in channel activity between pH 7·3 and pH 7·8, we employed a protocol (McLarnon, 1995) which, at an internal $[Ca^{2+}]$ of 0·7 μM and physiological K^+ across an inside-out patch, leads to transient activation of $I_{BK(Ca)}$. The procedure was to initially hold the excised patch at $V = -80$ mV for 10 s and then step V to 0 mV for 1 s to activate outward unitary $I_{BK(Ca)}$. In Fig. 7, records of $I_{BK(Ca)}$ are shown commencing 4 ms after V had returned to 0 mV from the holding level of −80 mV (to eliminate capacitative artefacts), at four different internal pH values. The protocol was repeated in three additional patches and the overall P_o values were determined by collation of events (for 10 steps at each pH value) over the first 70 ms following the step to 0 mV. Estimated in this manner, the P_o values with internal pH at 6·7, 7·0, 7·3 and 7·8 were 0·04 ± 0·03, 0·19 ± 0·06, 0·57 ± 0·11 and 0·81 ± 0·13, respectively.

DISCUSSION

Effects of changes in pH_o

The sensitivities of voltage-activated Ca^{2+} channels to changes in pH_o have been documented in a variety of cell types (e.g. Klöckner & Isenberg, 1994*b*; Ou-Yang *et al.* 1994). In all cases, falls in pH_o reduce Ca^{2+} currents whereas rises in pH_o increase them. Recently, Tombaugh & Somjen (1996) reported the effects of changes in pH_o on HVA Ca^{2+} currents in acutely dissociated adult rat hippocampal CA1 neurones under voltage clamp; the pK for the effect of pH_o on whole-cell Ca^{2+} current amplitude (7·1) was close to the value (7·2) found in the present study for inhibition of K^+-evoked rises in $[Ca^{2+}]_i$ which, in the fetal neurones employed, are dominated by Ca^{2+} influx through L-type HVA Ca^{2+} channels. We also find that $[Ca^{2+}]_i$ transients dependent upon Ca^{2+} flux through dihydropyridine-resistant HVA Ca^{2+} channels are modulated by changes in pH_o (pK = 7·2), a finding which indicates that changes in neurotransmitter release, in addition to changes in post-synaptic excitability, probably participate in the known effects of changes in pH_o on synaptic transmission in hippocampal slices (Balestrino & Somjen, 1988; Drapeau & Nachshen, 1988; Church & McLennan, 1989).

The pK established in the present study for inhibition of NMDA-evoked rises in $[Ca^{2+}]_i$ (7·3) compares well not only to values found for pH_o modulation of NMDA receptor-mediated currents in neurones and in wild-type $NR1_A/NR2_B$ receptors expressed in *Xenopus* oocytes (McBain & Mayer, 1994; Kashiwagi *et al.* 1996) but also to the pH_o sensitivities of HVA Ca^{2+} channels. Because the application of NMDA (or glutamate) under non-voltage-clamped conditions evokes rises in $[Ca^{2+}]_i$ mediated, at least in part, by voltage-activated Ca^{2+} channels, the present results suggest that the anticonvulsant and neuroprotective effects of mildly reduced pH_o may reflect decreases in Ca^{2+} flux through voltage- as well as ligand-operated Ca^{2+} channels.

Effects of changes in pH_i

Initial studies in neurones indicated that the change in pH_i occasioned by a change in pH_o was relatively small (e.g. Moody, 1984; Tolkovsky & Richards, 1987) but it now appears that the dependence of neuronal pH_i on pH_o is more direct. In the present study, the ratio $\Delta pH_i : \Delta pH_o$ was 0·71 under HCO_3^-/CO_2-buffered conditions at 37 °C, similar to values obtained in rat cortical neurones (0·78; Ou-Yang *et al.* 1993) and synaptosomes (0·68; Sánchez-Armass *et al.* 1994). The basis for the steep dependence of pH_i on pH_o in neurones is unknown although, by analogy with other cell types (see Wakabayashi *et al.* 1997), it may involve the modulation of the activity of pH_i regulating mechanism(s) by changes in pH_o. The dependence of pH_i on pH_o has a number of potential implications, including the possibility that changes in pH_o occurring during pathophysiological events such as cerebral ischaemia may modulate not only ionic conductances and transport mechanisms which are

sensitive to changes in pH_o but also those which are sensitive to changes in pH_i (e.g. Na^+–Ca^{2+} exchange; see below).

In the present study, and in agreement with the findings of Tombaugh & Somjen (1996), changes in pH_i consequent upon changes in pH_o failed to account for the effects of changes in pH_o on K^+-evoked $[Ca^{2+}]_i$ transients (see Fig. 5). Nevertheless, the activities of HVA Ca^{2+} channels in a variety of cell types are modulated by changes in pH_i (e.g. Kaibara & Kameyama, 1988; Mironov & Lux, 1991; Klöckner & Isenberg, 1994*a*). Furthermore, it has been found that changes in pH_i affect HVA Ca^{2+} currents in acutely dissociated adult rat hippocampal neurones under whole-cell voltage clamp, although changes in Ca^{2+} currents were evoked by pH_i changes of unknown magnitude and pK values could not be estimated (Tombaugh & Somjen, 1997). In the present study, however, changes in pH_i at a constant pH_o failed to affect markedly the magnitude of K^+-evoked rises in $[Ca^{2+}]_i$ once corrections for the pH dependence of the K_d of fura-2 for Ca^{2+} had been applied (Fig. 4). A number of explanations may account for this finding. First, as pointed out by Dixon *et al.* (1993), in all cell types studied for which data are available, the pH_i for 50% suppression of HVA Ca^{2+} currents (~6–6·5) is below normal resting pH_i, suggesting in turn that the relatively small perturbations of pH_i from rest employed in the present study may not have been sufficient to affect noticeably responses mediated by Ca^{2+} flux through HVA Ca^{2+} channels. However, in agreement with findings in other cell types (e.g. Moody, 1980), we have observed that reductions in pH_i (at a constant pH_o) of greater magnitude than those employed in the present study act to *increase* K^+-evoked $[Ca^{2+}]_i$ transients in hippocampal neurones (J. Church & K. A. Baxter, unpublished observations). Second, under our experimental conditions (in which outward K^+ currents were not suppressed; cf. Tombaugh & Somjen, 1997), the effects of changes in pH_i on $g_{K(Ca)}$ (and, possibly, other K^+ conductances; see Moody, 1984) may have acted to offset any direct effect of pH_i on Ca^{2+} flux through HVA Ca^{2+} channels. For example, an inhibitory effect of a decrease in pH_i on Ca^{2+} influx may have been attenuated by concomitant blockade of $g_{K(Ca)}$, the result being that changes in pH_i at a constant pH_o have little net effect on the magnitude of depolarization-evoked $[Ca^{2+}]_i$ transients. In this regard, it has been shown in CA3 pyramidal neurones that blockade of $g_{K(Ca)}$ is associated with an increased accumulation of internal Ca^{2+} during repetitive firing (Müller & Connor, 1991; see also Drapeau & Nachshen, 1988). Third, a number of mechanisms which participate in the control of neuronal Ca^{2+} homeostasis, including ATP-dependent Ca^{2+} efflux and forward Na^+–Ca^{2+} exchange, are inhibited by low pH_i (Dipolo & Beaugé, 1982). Falls in pH_i acting on these, and other, mechanisms might also shift the balance between a reduction in Ca^{2+} entry via voltage-activated Ca^{2+} channels and the size of depolarization-evoked $[Ca^{2+}]_i$ transients.

We conclude that K^+-evoked $[Ca^{2+}]_i$ transients in fetal rat hippocampal neurones are more sensitive to modulation by changes in pH_o than changes in pH_i, and that changes in pH_i do not mediate the effects of changes in pH_o on these responses. The results also indicate that pH_i-dependent changes in the activities of HVA Ca^{2+} channels (Tombaugh & Somjen, 1997) do not alone determine the net effect of a change in pH_i on the magnitude of depolarization-evoked $[Ca^{2+}]_i$ transients. The latter will reflect the sum of the effects of pH_i on the various ionic conductances and transport mechanisms involved in intracellular Ca^{2+} homeostasis and which are sensitive to changes in pH_i.

pH modulation of a Ca^{2+}-dependent K^+ channel

Although K^+-evoked $[Ca^{2+}]_i$ transients are more sensitive to changes in pH_o than to changes in pH_i, changes in pH_i (at a constant pH_o) had marked effects on the kinetic properties of a BK-type Ca^{2+}-dependent K^+ channel. The $I_{BK(Ca)}$ examined in the present study probably mediates the fast AHP observed in CA1 pyramidal cells because, in six neurones impaled in hippocampal slices, the class III anti-arrhythmic agent tedisamil inhibited the fast AHP with no effect on the slow AHP (J. Church, unpublished observations); the effect of tedisamil to inhibit the $I_{BK(Ca)}$ examined here has been reported previously (McLarnon & Wang, 1991). Thus, the present results may reflect previous findings that reductions in pH_i at a constant pH_o inhibit fast AHPs in CA1 neurones in the face of only minor reductions in the width of Ca^{2+}-mediated depolarizing potentials (Church, 1992). The results also suggest that changes in the magnitude of fast AHPs evoked by changes in pH_o (Church, 1992) may reflect not only alterations in Ca^{2+} influx (mediated by changes in pH_o) but also changes in the activity of Ca^{2+}-dependent K^+ channels (mediated by changes in pH_i consequent upon changes in pH_o). They also uncover another mechanism for modulation of large conductance Ca^{2+}-activated K^+ channels, in addition to cellular redox potential (DiChiara & Reinhart, 1997; Wang *et al.* 1997), which occurs downstream from Ca^{2+} influx and indicate that changes in pH_i are able to uncouple the activation of BK-type channels from the internal Ca^{2+} load.

Our observation that changes in pH_i modulate a $I_{BK(Ca)}$ in hippocampal neurones is consistent with reports which indicate that protons acting on the cytoplasmic side of the membrane suppress Ca^{2+}-activated K^+ currents in a variety of cell types in an especially sensitive manner (Christensen & Zeuthen, 1987; Kume *et al.* 1990; Copello *et al.* 1991; Laurido *et al.* 1991; Peers & Green, 1991). In all cases in which it has been examined, reductions in internal pH lower P_o and, in the present study, this effect was tentatively ascribed to an increase in the closed time of the channel (see Christensen & Zeuthen, 1987). At an internal $[Ca^{2+}]$ of 20 μM there was little effect on $I_{BK(Ca)}$ when internal pH was increased above 7·3 whereas, when internal $[Ca^{2+}]$ was reduced to 0·7 μM, graded increases in P_o were observed when internal pH was raised from 6·7 to 7·0 to 7·3 to 7·8. These observations suggest that, as in other cell types (e.g. Christensen & Zeuthen, 1987; Kume *et al.* 1990), protons affect the $I_{BK(Ca)}$ in hippocampal

neurones by modifying channel gating rather than by changing single channel conductance, although more detailed studies of channel kinetics are required to determine whether protons compete with Ca^{2+} at regulatory binding sites (e.g. Christensen & Zeuthen, 1987; Copello *et al.* 1991) or whether they exert their actions via an allosteric site on the channel complex (e.g. Laurido *et al.* 1991).

Given the sensitivity of slow AHPs in hippocampal neurones to changes in pH_o and pH_i (Church & McLennan, 1989; Church, 1992), and the fact that SK channels are more sensitive to Ca^{2+} ions than BK channels (Lancaster *et al.* 1991), it will be interesting to determine the effects of changes in pH_i on small conductance (SK-type) Ca^{2+}-activated K^+ channel(s) which underlie slow AHPs. In addition, future studies should assess whether the effects of pH_i in rat hippocampal neurones are relatively selective for Ca^{2+}-activated K^+ currents (as is the case in type I carotid body cells; Peers & Green, 1991) or whether changes in pH_i can also affect Ca^{2+}-independent K^+ currents. Given the lability of neuronal pH_i and the importance of Ca^{2+}-dependent K^+ channels for the regulation of Ca^{2+} influx and neurotransmitter release presynaptically and for the integration of synaptic potentials and neuronal firing behaviour postsynaptically (Storm, 1990), the sensitivity of Ca^{2+}-activated K^+ channels to changes in pH_i may have important implications for neuronal function.

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