Journal of Physiology (1991), **442**, *pp.* 431–446
With 10 figures
Printed in Great Britain

EXTRACELLULAR ALKALINIZATION EVOKED BY GABA AND ITS RELATIONSHIP TO ACTIVITY-DEPENDENT pH SHIFTS IN TURTLE CEREBELLUM

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(*Received 3 January 1991*)

SUMMARY

1. The effect of γ-aminobutyric acid (GABA) on extracellular pH (pH_o) was investigated in the turtle cerebellum, *in vitro*, using double-barrelled, H^+-selective microelectrodes. Responses evoked by GABA were compared with pH_o shifts evoked by repetitive stimulation of the parallel fibres.

2. In media buffered with 35 mM-HCO_3^- and 5% CO_2, superfusion of GABA (1 mM) elicited an abrupt alkaline shift in the molecular layer, which averaged $0{\cdot}05 \pm 0{\cdot}02$ pH units ($\pm$S.D., range 0·02–0·12 pH units). pH_o often recovered in the continued presence of GABA, and displayed a rebound acidification upon wash-out.

3. The GABA-evoked alkaline shift was blocked by picrotoxin and was mimicked by the $GABA_A$ agonists isoguvacine and muscimol. The $GABA_B$ agonist baclofen did not elicit an alkaline shift. Alkaline shifts evoked by stimulation of the parallel fibres were unaffected by picrotoxin.

4. In nominally HCO_3^--free solutions, buffered with 35 mM-HEPES, superfusion of GABA caused either no pH_o change or a slow acid shift. In contrast, the alkaline shift evoked by stimulation of the parallel fibres became enhanced in HEPES-buffered media.

5. The alkaline shift evoked by GABA was accompanied by an increase in extracellular K^+ ($[K^+]_o$) which averaged 1·7 mM above baseline. Experimental elevation of $[K^+]_o$ to a comparable level always caused a pure acid shift in the extracellular space.

6. The GABA-evoked alkaline shift persisted when synaptic transmission was blocked using 4 mM-kynurenic acid or saline prepared with nominally zero Ca^{2+} and 10 mM-Mg^{2+}. The alkaline shift evoked by repetitive stimulation of the parallel fibres was completely abolished in these media.

7. Although the GABA-evoked alkaline shift was blocked in nominally HCO_3^--free media, substitution of 35 mM-formate for HCO_3^- restored the GABA response. Superfusion of 1 mM-GABA in formate saline produced an alkaline shift of $0{\cdot}040 \pm 0{\cdot}034$ pH units.

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8. These results indicate that gating of $GABA_A$ channels in the vertebrate CNS gives rise to an HCO_3^- efflux which can significantly increase the pH of the brain microenvironment. However, this mechanism cannot account for the extracellular alkalinization caused by parallel fibre stimulation. Extracellular alkaline shifts capable of modulating local synaptic operations may therefore be a consequence of either excitatory or inhibitory synaptic transmission.

INTRODUCTION

An increase in extracellular pH (pH_o) has often been observed at the onset of neuronal activity (Chesler, 1990). These alkalinizations have a rapid onset and can reach magnitudes sufficient to modulate the activity of ligand and voltage-gated channels (Konnerth, Lux & Morad, 1987; Tang, Dichter & Morad, 1990; Traynelis & Cull-Candy, 1990). It has been suggested that extracellular alkaline shifts arise due to the influx of acid (e.g. hydrogen ions) or efflux of base (e.g. hydroxyl or bicarbonate ions) across nerve cell membranes (Chesler & Chan, 1988). Whether such fluxes are mediated via a particular channel or class of channels has yet to be established.

In the central nervous system, extracellular alkaline shifts have typically been evoked by synchronous synaptic activation of a neuronal population (Kraig, Ferreira-Filho & Nicholson, 1983; Somjen, 1984; Carlini & Ransom, 1986; Chesler & Chan, 1988). Similar pH_o changes have been elicited by direct application of neurotransmitters (Endres, Ballanyi, Serve & Grafe, 1986; Jarolimek, Misgeld & Lux, 1989; Chesler & Rice, 1991), suggesting involvement of ligand-gated channels. In this regard, the role of $GABA_A$ receptors is of interest, since the GABA-gated anion channel has a significant permeability to HCO_3^- (Bormann, Hamill & Sakmann, 1987; Kaila & Voipio, 1987; Kaila, Pasternack, Saarikoski & Voipio, 1989; Kaila, Saarikoski & Voipio, 1990), and the electrochemical gradient for HCO_3^- is outward. Thus, in crayfish muscle, superfusion of GABA evoked a picrotoxin-sensitive HCO_3^--dependent fall in intracellular pH, accompanied by a rise of surface pH (Kaila & Voipio, 1987).

Although $GABA_A$ channels of vertebrate neurones are permeable to HCO_3^- (Bormann *et al.* 1987), it is unclear whether GABA-evoked HCO_3^- fluxes account for synaptically generated alkaline shifts, or whether brain pH can be significantly modulated via this pathway. We have therefore investigated the effect of GABA on brain pH_o and its relationship to stimulus-evoked alkaline shifts. Experiments were conducted on the isolated turtle cerebellum, as this preparation survives exceptionally well *in vitro*, and has been studied extensively with respect to its electrophysiological responses (Hounsgaard & Nicholson, 1990) and associated extracellular ion shifts (Chesler & Chan, 1988; Rice & Nicholson, 1990; Chesler & Rice, 1991).

Our results demonstrate that application of GABA causes a HCO_3^--dependent alkalinization of the brain extracellular space generated directly by the gating of $GABA_A$ anion channels. By contrast, synaptically-evoked alkaline shifts are found to be generated by a process that is insensitive to picrotoxin and is HCO_3^--independent. Portions of these results have appeared in preliminary communications (Chen & Chesler, 1990; Chen, Chesler & Rice, 1990).

METHODS

Turtles of species *Pseudemys scripta elegans* with plastron lengths of 12–15 cm (Kons Scientific, Germantown, WI, USA) were decapitated, and cerebella removed into a standard turtle saline solution (see below). Following dissection of the pial membrane from the dorsal surface, the cerebellum was placed dorsal-side-up in a recording chamber. Gravity-fed, room-temperature (22–25 °C) saline entered below the preparation and was removed by suction adjacent to the dorsal surface, thereby superfusing the tissue from all sides. To insure against alkaline transients of the bath due to egress of CO_2 from inactive solution lines, the flow system was constructed from CO_2-impermeable Saran tubing (Clarkson Controls & Equipment, 12059 Woodbine St., Detroit, MI 48239, USA) and lines were routinely flushed before solution changes.

Standard turtle saline had the following composition (in mM): NaCl, 105; KCl, 5; $NaHCO_3$, 35; glucose, 20; $CaCl_2$, 2; $MgCl_2$, 3 (gassed with 95% O_2, 5% CO_2, pH 7·5–7·6). In HEPES-buffered saline, $NaHCO_3$ was replaced by 35 mM-HEPES, titrated to pH 7·55–7·8 with NaOH and gassed with 100% O_2. To take account of the added NaOH, the NaCl content was adjusted to maintain a sodium concentration of 140 mM. Formate-HEPES solutions contained 35 mM-sodium formate and 35 mM-HEPES, titrated to pH 7·8 with NaOH, with NaCl reduced accordingly. GABA, baclofen, kynurenic acid, and muscimol were purchased from Sigma. Isoguvacine was obtained from Research Biochemicals Inc., Natick, MA, USA. In the concentrations employed in this study, these agents did not significantly affect the pH of solutions.

Double-barrelled ion-selective microelectrodes were fabricated from thin-walled borosilicate glass capillaries (AM Systems 6170, Stone Everett WA, USA), which were bound with heat-shrink tubing, twisted, and pulled on a vertical pipette puller (Narishige PE-2). Electrodes were broken to a tip diameter of 2–4 μm, silanized (using 4% trimethylchlorosilane in xylene) and filled with ion exchangers via the tips. pH-sensitive barrels contained a H^+ cocktail (Fluka 82500), followed by 150 mM-NaCl buffered with phosphate (pH 7·4). K^+-sensitive barrels contained a valinomycin-based cocktail (Fluka 60031) followed by 0·1 M-KCl. Reference barrels were filled with 150 mM-NaCl. pH electrodes, calibrated before and after experiments in standard phosphate buffers, had slope responses greater than 50 mV per decade. Responsiveness of the pH electrodes was unaffected by the drugs used in this study. K^+ electrodes, calibrated in 2·5, 5, 10, and 20 mM-KCl in 150 mM-NaCl, had typical slopes of 55–58 mV per decade. Absolute pH and K^+ activity in the tissue were calculated with respect to the superfusate.

Input stages for ion and DC reference signals used high-impedance ($> 10^{13}\Omega$) operational amplifiers (Analog Devices 515L) wired for unity gain. Ion signals were obtained by on-line subtraction of DC reference signals. Fast field potentials recorded from the reference barrel were filtered at 10 kHz. The slow ion and reference potentials were filtered at 5 Hz. Records were monitored on a strip-chart recorder, digitized (Neuro-Data DR-484) and stored on videotape.

Constant-current, sub-maximal stimuli (50 μs, 1·5–2·5 mA) were delivered to the parallel fibres using a twisted pair of 50 μm, teflon-coated, platinum–iridium wires placed on the dorsal surface. Ion-selective microelectrodes were inserted into the molecular layer along an activated beam of parallel fibres and advanced until the parallel fibre volley was maximized, typically at a depth of 100–200 μm below the dorsal surface. pH recordings from the granular layer were obtained by lowering electrodes an additional 500 μm. Simultaneous K^+ and pH records from the molecular layer were obtained using a dual electrode manipulator (Narishige MD-4R) with electrode tips spaced approximately 50 μm apart.

RESULTS

Superfusion of GABA in HCO_3^--buffered media

The effect of GABA on the parallel fibre-evoked field potentials provided a convenient means of following the influx and wash-out of GABA from the cerebellum. These fields consist of a rapid parallel fibre volley, followed by a slower negativity (arrow, Fig. 1), attributed to postsynaptic activation of the Purkinje cell dendrites (Llinas, 1981). Superfusion of GABA has been shown to abolish the postsynaptic component in a number of cerebella (Hackett, 1974; Malenka & Kocsis, 1982; Strauss, 1986). In frog cerebellum, GABA was also reported to diminish the

amplitude of the initial parallel fibre volley (Strauss, 1986). These effects have required concentrations of the order of 1 mM, consistent with effective uptake of GABA from the extracellular space of intact, isolated tissues (Brown, Collins & Galvan, 1980).

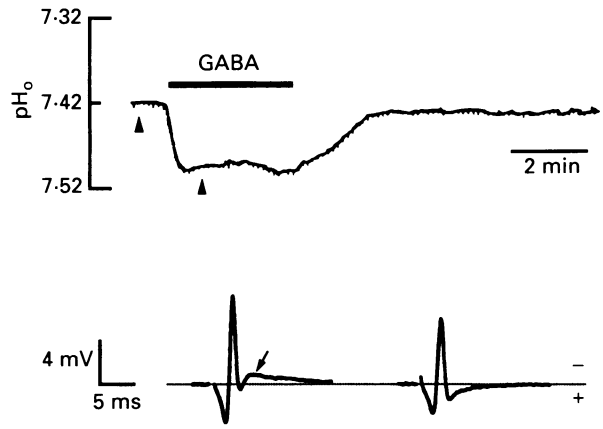

Fig. 1. Effect of GABA on pH_0 and evoked field potentials in the molecular layer. Superfusion of GABA (1 mM) in HCO_3^--buffered saline caused a reversible alkaline shift (downward deflection). Bottom traces illustrate field potentials evoked by single shocks to the parallel fibres, before and during application of GABA (time of evoked field potentials indicated by arrow-heads). GABA reversibly depressed the parallel fibre volley and blocked the late synaptic negativity (arrow, bottom trace). Small, sharp deflections on the pH_0 trace are stimulus artifacts.

In the turtle cerebellum, superfusion of 1 mM-GABA caused a decrease in the amplitude of the parallel fibre volley and completely abolished the postsynaptic component of the field potential (Fig. 1). A reversible negative DC shift of approximately 0·5 mV was also consistently observed (not shown). Coincident with the onset of these effects, an abrupt rise in pH_0 occurred, as shown in Fig. 1. From a baseline pH_0 of $7{\cdot}36 \pm 0{\cdot}08$ (ranging from 7·24 to 7·49), the mean response to 1 mM-GABA was an alkaline shift of $0{\cdot}05 \pm 0{\cdot}02$ pH units ($\pm$S.D., $n = 71$ trials in thirty-two preparations; range 0·02–0·12 pH units), with an initial rate of alkalinization of $0{\cdot}13 \pm 0{\cdot}16$ pH units min^{-1}. In the granular layer, application of 1 mM-GABA elicited similar alkaline shifts (Fig. 2*A*). Lower concentrations of GABA (10^{-5}–10^{-4} M) usually failed to elicit a response in either layer; however, small alkalinizations were sometimes seen. Changes in pH were never observed in control trials in which the electrode was placed in the superfusate.

GABA-evoked alkaline shifts in the molecular layer could be elicited repetitively at 10–15 minute intervals without decrement. Shorter intervals were not attempted. In ten preparations, during continued exposure to 1 mM-GABA, the initial alkaline shift was followed by a slow acidification towards baseline ($0{\cdot}018 \pm 0{\cdot}001$ pH units min^{-1} in thirty-six trials). In these preparations, wash-out of GABA often resulted in a rebound acidification ($0{\cdot}034 \pm 0{\cdot}026$ pH units in thirty-three trials, Fig. 2*B*), which required 5–10 min to recover to baseline.

Pharmacological basis of GABA-evoked alkaline shifts

Both $GABA_A$ and $GABA_B$ receptors are found in the molecular layer of the turtle cerebellum (Albin, Richfield, Reiner, Young & Penney, 1989). To determine whether the GABA-evoked alkaline shift could be attributed to a single GABA receptor subtype, specific agonists and antagonists were employed.

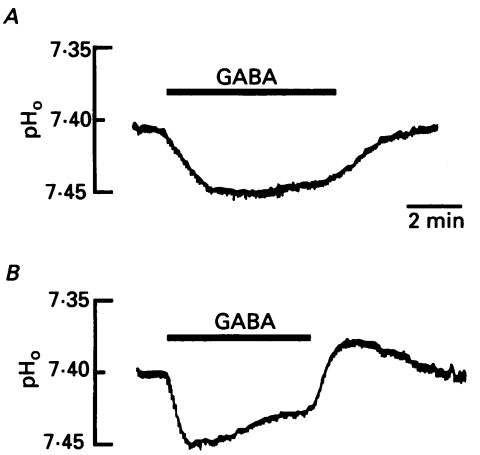

Fig. 2. GABA-evoked pH_o shifts in granular and molecular layers. *A*, effect of GABA on pH_o in the granular layer. Superfusion of GABA (1 mM) in HCO_3^--buffered saline caused a reversible alkaline shift in the granular layer, similar to responses observed in the molecular layer. *B*, during application of GABA (1 mM) in the molecular layer, the alkaline shift often displayed a slow recovery. Upon wash-out of GABA an acid rebound was observed.

In paired trials on seven preparations, the $GABA_A$ antagonist picrotoxin (1 mM) consistently blocked the GABA-evoked alkaline shift (Fig. 3*A*). Picrotoxin alone had no effect on baseline pH_o. The mean response to GABA (1 mM) in the presence of picrotoxin was an alkaline shift of $0{\cdot}005 \pm 0{\cdot}009$ pH units ($n = 19$ trials), compared with an average control response of $0{\cdot}046 \pm 0{\cdot}030$ pH units.

Use of the $GABA_A$ agonists isoguvacine and muscimol provided further evidence that the GABA-evoked alkaline shift was mediated by $GABA_A$ receptors. As shown in Fig. 3*B*, superfusion of isoguvacine (1 mM) gave rise to alkalinizations similar to those evoked by GABA. The average alkalinization evoked by 1 mM-isoguvacine was $0{\cdot}045 \pm 0{\cdot}019$ pH units (fifteen trials in five preparations). Qualitatively similar results were obtained with muscimol in a smaller number of trials. Unlike GABA, low concentrations of isoguvacine (10^{-5}–10^{-4} M) always gave rise to a small alkaline shift. This observation appears consistent with the inefficient removal of isoguvacine by GABA uptake systems (Krogsgaard-Larsen, Johnston, Lodge & Curtis, 1977).

In the *in vitro* frog cerebellum, the $GABA_B$ agonist baclofen blocked the postsynaptic component of the parallel fibre-evoked field potential, requiring 0·10–1·0 mM for complete inhibition of the response (Strauss, 1986). In the isolated turtle cerebellum, superfusion of baclofen (1 mM) also blocked the postsynaptic component of the field potential (not shown), but had little immediate effect on pH_o.

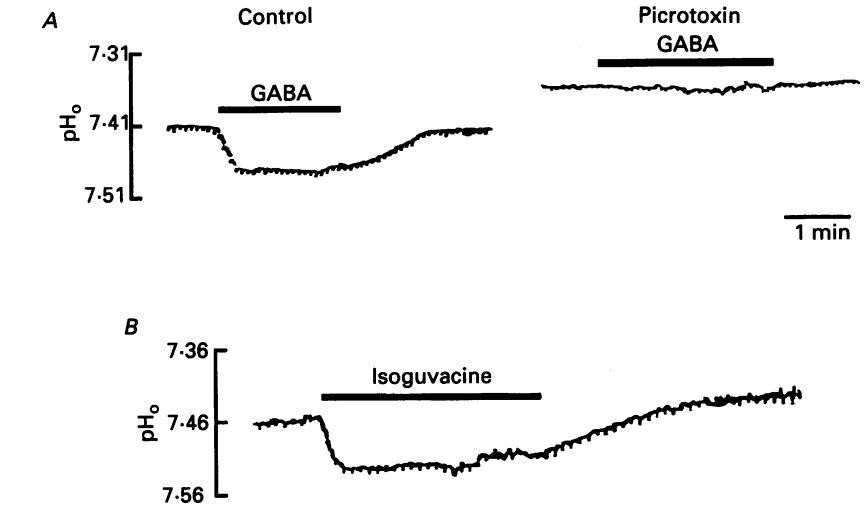

Fig. 3. GABA-evoked alkaline shift is mediated by $GABA_A$ receptors. *A*, in paired trials, the alkalinization evoked by GABA (1 mM) was blocked by picrotoxin. *B*, superfusion of the $GABA_A$ agonist isoguvacine (1 mM) mimicked the GABA-evoked alkaline shift.

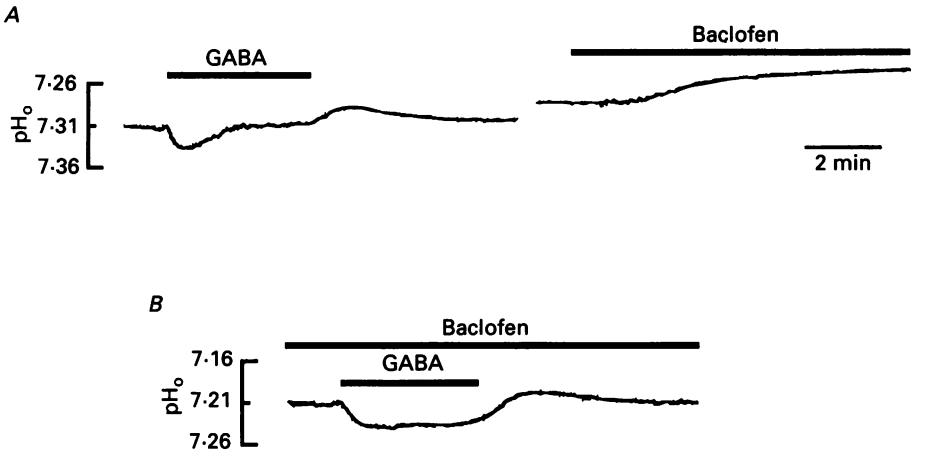

Fig. 4. $GABA_B$ receptors do not mediate the GABA-evoked alkaline shift. *A*, in paired trials, superfusion of baclofen (1 mM) was never associated with a rapid alkaline shift. Small, slow pH_o shifts with long latency were sometimes noted. *B*, exposure to GABA (1 mM) in the presence of baclofen (1 mM) gave rise to alkaline shifts similar to controls. Records in *A* and *B* are consecutive responses from the same preparation.

After several minutes, a slow acid or alkaline response was sometimes observed (Fig. 4*A*). The average pH_o shift immediately after application of baclofen was an extremely small alkalinization ($< 0{\cdot}01$ pH units). In addition, in the presence of 1 mM-baclofen, application of GABA (1 mM) still evoked an immediate alkaline shift ($0{\cdot}031 \pm 0{\cdot}009$ pH units, $n = 8$ trials in four preparations; Fig. 4*B*), further indicating that $GABA_B$ receptors were not involved in the generation of the response.

Superfusion of GABA in HCO_3^--free media

To test the HCO_3^--dependence of the GABA-evoked alkaline shift, experiments were conducted in nominally HCO_3^--free media, buffered with 35 mM-HEPES. Because of a prominent extracellular acidosis in the molecular layer (Chesler & Chan,

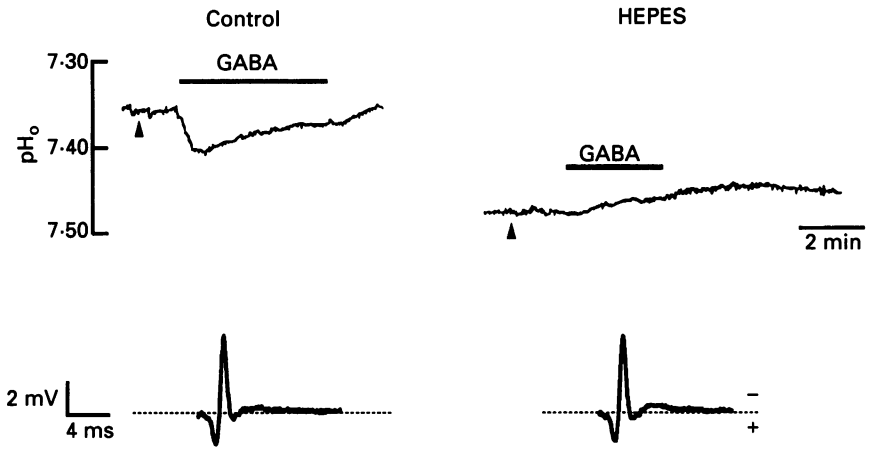

Fig. 5. Bicarbonate-dependence of GABA-evoked alkaline shift. In paired trials of 35 mM-HCO_3^- *versus* 35 mM-HEPES-buffered saline, the GABA-evoked alkalinization was consistently abolished in HEPES media. The response recovered upon return to HCO_3^--buffered saline (not shown). In HEPES-buffered media, a slow acid shift was often noted. Field potentials (lower traces) evoked by single shocks to the parallel fibres (arrow-heads in top traces) were not significantly altered in the absence of HCO_3^-.

1988), it was difficult to attain the same baseline pH_o in both solutions. In most cases, the HEPES saline was titrated to a more alkaline pH to compensate for its lower buffering power. This sometimes resulted in a slightly more alkaline pH_o in HEPES media.

Experiments were begun with control applications of GABA (1 mM) in normal, 35 mM-HCO_3^--buffered solution. HEPES-buffered saline (35 mM) was then superfused for at least twenty minutes prior to the second application of GABA. Throughout these experiments the parallel fibres were stimulated at 0·2 Hz to monitor the evoked field potentials. The fields were not significantly altered in HEPES-buffered media (Fig. 5) as reported previously (Chesler & Chan, 1988).

In ten paired trials in nine preparations, the GABA-evoked alkaline shift was reversibly blocked by transition to HCO_3^--free solution (Fig. 5). Control alkalinization in HCO_3^- media averaged $0{\cdot}039 \pm 0{\cdot}014$, while the average initial response in HEPES-buffered solution was a pH_o rise less than 0·01. Baseline pH_o in the HEPES-buffered media ranged from 7·33 to 7·60. Typically, a slow acid shift (Figs 5 and 9) was the only pH_o response. Upon wash-out of GABA, a rebound acidification was not observed.

The effect of GABA on extracellular K^+

In a number of preparations, application of GABA has been shown to cause a rise in $[K^+]_o$ (Deschenes & Feltz, 1976; Kudo & Fukuda, 1976; Sykova, 1979). In the turtle cerebellum, a similar increase in $[K^+]_o$ was noted during superfusion of GABA.

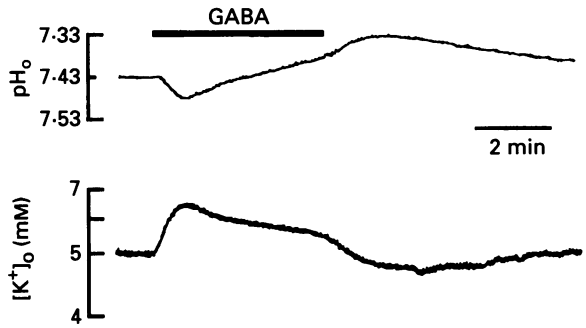

Fig. 6. Simultaneous recording of pH_o and K^+. Superfusion of GABA (1 mM) consistently caused a rise in extracellular $[K^+]_o$ with an onset and peak similar to that of the alkaline shift. Wash-out of GABA was associated with a prolonged $[K^+]_o$ undershoot with a time course similar to the acid rebound.

The rise in $[K^+]_o$ had an onset similar to that of the extracellular alkaline shift (Fig. 6) and an average peak of $1{\cdot}70 \pm 0{\cdot}39$ mM above baseline ($n = 14$ trials in six animals). Upon wash-out of GABA, a prolonged undershoot of $[K^+]_o$ was typically observed.

Experimental increases in $[K^+]_o$ have been shown to cause both acid and alkaline transients in the brain extracellular space (Kraig *et al.* 1983). To determine whether an increase of $[K^+]_o$ alone could be responsible for the GABA-evoked alkaline shift, the concentration of K^+ in the superfusate was raised to 10 mM. Elevation of $[K^+]_o$ in this manner always caused an acidification of the extracellular space (Fig. 7). When applying saline containing 10 mM-K^+, the acid shift sometimes began before a detectable increase in $[K^+]_o$. Such effects may have been circuit mediated, arising from transient, non-uniform elevation of $[K^+]_o$ throughout the preparation.

The effect of synaptic blockade on the GABA-evoked alkaline shift

Because superfusion of GABA will increase $[K^+]_o$ and influence circuits throughout the cerebellum, indirect, synaptically-mediated pH shifts could occur. To eliminate this possibility, synaptic transmission was blocked using saline prepared with nominally zero Ca^{2+} plus 10 mM-Mg^{2+} or 4 mM-kynurenic acid. Kynurenate has been shown to abolish synaptic transmission from parallel fibres to Purkinje cells in this preparation (Chesler & Chan, 1988). In either saline, the postsynaptic component of the field potential was blocked. In contrast, the alkaline shift evoked by GABA was insensitive to these treatments (Fig. 8). In a total of seven trials in these media, carried out in four preparations, superfusion of 1 mM-GABA produced an average alkaline shift of $0{\cdot}051 \pm 0{\cdot}017$ pH units.

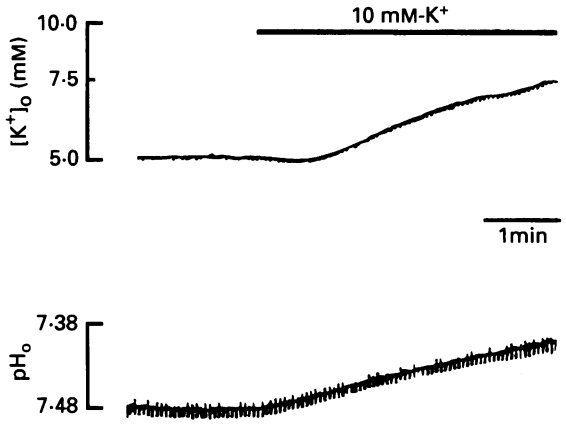

Fig. 7. Effect of elevated $[K^+]_o$ on pH_o. Superfusion of HCO_3^--buffered saline containing 10 mM-K^+ was consistently associated with acidification of the extracellular space. Disparity in the onset of the acid shift and the rise in $[K^+]_o$ responses was sometimes noted. Sharp deflections on the pH_o trace are stimulus artifacts.

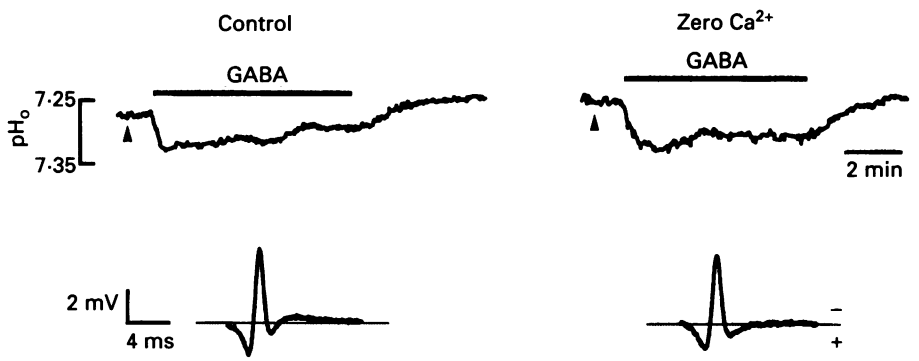

Fig. 8. Effect of synaptic blockade on GABA-evoked alkaline shift. In paired trials, exposure to saline containing zero Ca^{2+} and 10 mM-Mg^{2+} failed to abolish the alkaline shift evoked by GABA (1 mM). In contrast, the postsynaptic components of the field potentials (bottom traces) evoked prior to application of GABA (arrow-heads, top traces) were abolished in this solution.

Effect of formate substitution in HCO_3^--free media

Among the weak bases which can pass through the $GABA_A$ receptor–channel complex, HCO_3^- and formate have the largest permeabilities (Inomata, Oomura, Akaike & Edwards, 1986; Bormann *et al.* 1987; Kaila and Voipio, 1987; Kaila *et al.* 1989; Mason, Mattsson, Pasternack, Voipio & Kaila, 1990). At physiological pH, formate is in equilibrium with a small concentration of formic acid which, being neutral, can permeate cell membranes. Thus, formic acid will accumulate within cells until its intracellular and extracellular concentrations are equal. Under these circumstances the equilibrium potentials for formate and H^+ are identical (Roos &

Boron, 1981). Accordingly, the electrochemical gradient for formate will be outward, and addition of GABA would be expected to cause a formate efflux and an extracellular alkaline shift (Mason *et al.* 1990).

Experiments in formate-containing media were performed on five preparations. Control exposures to 1 mM-GABA were conducted using nominally HCO_3^--free,

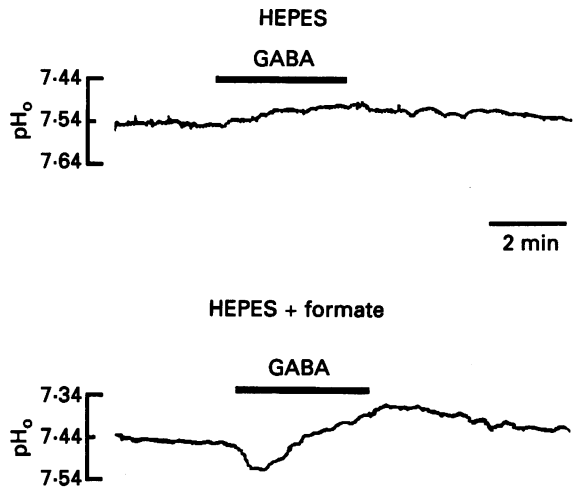

Fig. 9. Formate supports a GABA-evoked alkaline shift. In control trials, in 35 mM-HEPES-buffered saline, superfusion of GABA (1 mM) failed to elicit an alkaline shift. In HEPES media containing 35 mM-formate plus 4 mM-kynurenate, superfusion of GABA (1 mM) gave rise to an immediate alkaline shift, followed by a slow acidification. Top and bottom traces are consecutive traces from the same experiment.

35 mM-HEPES-buffered saline, in which GABA-evoked alkaline shifts do not normally occur (Figs 5 and 9). HEPES-buffered saline containing 35 mM-sodium formate was then superfused for at least 30 min before a second exposure to GABA. To guard against the possibility of synaptically generated alkaline shifts, the formate media were prepared with either 4 mM-kynurenate, or zero Ca^{2+} plus 10 mM-Mg^{2+}. Under these conditions, superfusion of 1 mM-GABA gave rise to alkaline shifts followed by slow acidifications (Fig. 9), which resembled the responses in HCO_3^- saline. In four of five preparations, an immediate alkalinization was observed which averaged $0{\cdot}040 \pm 0{\cdot}034$ pH units (n = fifteen trials in four preparations).

Comparison of GABA and parallel fibre-evoked alkaline shifts

Stimulation of the parallel fibres causes a rapid extracellular alkaline shift (Kraig *et al.* 1983; Chesler & Chan, 1988). Since the parallel fibres activate GABAergic stellate cells, which in turn inhibit the Purkinje cell dendrites (Llinas, 1981), all or part of the parallel fibre response could be GABA mediated. We therefore compared the pharmacological sensitivity and HCO_3^--dependence of the parallel fibre and GABA-evoked alkaline shifts.

The parallel fibre-evoked alkaline shift was completely abolished in zero-Ca^{2+}, high-Mg^{2+} saline (Fig. 10*A*). The parallel fibre response was also blocked in media

containing 4 mM-kynurenate (not shown), as demonstrated previously (Chesler & Chan, 1988). These observations are consistent with an earlier report which demonstrated that the parallel fibre response was dependent on synaptic transmission (Kraig *et al.* 1983). In the present context, these results further demonstrated

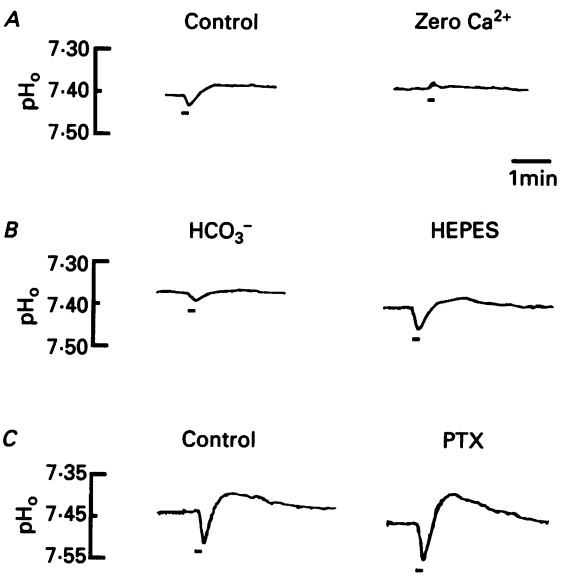

Fig. 10. Characteristics of the parallel fibre-evoked alkaline shift. *A*, the parallel fibre-evoked response (5 Hz for 10 s; horizontal bar) was blocked in HCO_3^--saline containing zero Ca^{2+} and 10 mM-Mg^{2+}. *B*, equimolar substitution of 35 mM-HCO_3^- with HEPES amplified the parallel fibre-evoked response (5 Hz for 10 s). *C*, in 35 mM-HCO_3^--saline, exposure to picrotoxin (1 mM) failed to inhibit the parallel fibre-evoked alkaline shift (10 Hz for 10 s).

that the GABA-evoked response, which persisted in these media (Fig. 8), could not have had a synaptic component.

In a previous study, replacement of 35 mM-HCO_3^- buffer with lower concentrations of HEPES (10–15 mM) caused a four- to fivefold increase in the parallel fibre-evoked alkaline shift (Chesler & Chan, 1988). In the present study, equimolar substitution of HEPES for HCO_3^- caused a two- to threefold enhancement of the response. These observations indicate that the parallel fibre-evoked response is not HCO_3^- dependent. However, the issue of whether the parallel fibre response may have had a GABA-mediated component could not be adequately addressed using HEPES-buffered Ringer, since replacement of HCO_3^- with a non-volatile weak acid affects the extracellular buffering power. In a closed system, a solution buffered with HEPES has, at most, a quarter of the buffering power of an equimolar HCO_3^--buffered solution in equilibrium with CO_2 (Roos & Boron, 1981). Thus, amplification of a HCO_3^--independent pH_o shift, caused by a decrease in buffering power, would be likely to obscure the inhibition of a HCO_3^--dependent response.

To more directly address whether the parallel fibre-evoked alkaline shift had a GABA-mediated component, we tested the sensitivity of the response to picrotoxin.

In three preparations, the parallel fibres were repetitively stimulated in 35 mM-HCO_3^--buffered media, before and after application of 1 mM-picrotoxin. In contrast to the GABA-evoked alkaline shift, the parallel fibre-generated alkalinization appeared unaffected or slightly enhanced in the presence of picrotoxin (Fig. 10*C*).

DISCUSSION

The nature of the GABA-evoked alkaline shift

A number of studies have demonstrated that neuronal activity can cause a significant rise in pH_o (Chesler, 1990). It has been suggested that these events may be channel-mediated, arising through the downhill flux of acid equivalents across ligand- or voltage-gated channels (Chesler & Chan, 1988). A channel-mediated flux of acid-equivalents has been well described in muscle fibres of the crayfish, where a GABA-gated efflux of HCO_3^- contributes significantly to current flow (Kaila *et al.* 1989) and therefore affects extracellular and intracellular pH (Kaila & Voipio, 1987; Kaila *et al.* 1990).

Our present data indicate that pH_o in the vertebrate brain can be similarly modulated by a GABA-gated HCO_3^- efflux. This conclusion is based on the following evidence: (1) the GABA-evoked alkaline shift was blocked by picrotoxin and was mimicked by isoguvacine, but was never elicited by baclofen, indicating that it was mediated via $GABA_A$ receptors; (2) the response was consistently blocked in the nominal absence of HCO_3^-, whereas evoked field potentials were not significantly altered; (3) the alkaline shift persisted when synaptic transmission was abolished in nominally zero-Ca^{2+}, high-Mg^{2+} media, indicating that it was a direct consequence of the gating of $GABA_A$ receptor channels; (4) formate ions, which can permeate the $GABA_A$ receptor channel (Bormann *et al.* 1987; Mason *et al.* 1990) were able to substitute for HCO_3^-, giving rise to a GABA-evoked alkaline shift in HEPES-buffered media.

Alternatively, an alkaline shift could arise due to a decrease in extracellular acid production. Because the extracellular space of the turtle cerebellum is acidic with respect to the bathing media (Chesler & Chan, 1988; Hounsgaard & Nicholson, 1990), a rise in pH_o might occur if GABA decreased the rate of metabolic acid production, perhaps due to its inhibitory effect on Purkinje cells (Hackett, 1974; Malenka & Kocsis, 1982). However, an alkaline shift of this kind would not be expected to be HCO_3^- dependent, and should be diminished in the absence of synaptic transmission. Furthermore, such a pH_o shift would be enhanced in HCO_3^--free solutions (due to lower buffering power), whereas the GABA-evoked response was abolished in these media.

Although the GABA-gated anion channel has a significant permeability to HCO_3^-, desensitization of the $GABA_A$ receptor might severely limit the magnitude of GABA-evoked pH_o shifts. In crayfish muscle, the $GABA_A$ receptors display little desensitization and can therefore give rise to prolonged HCO_3^- fluxes (Kaila & Voipio, 1987; Kaila *et al.* 1990). However, in most vertebrate preparations, these receptors desensitize within seconds. Consequently, the steady-state conductance in the presence of agonist may be only 10 or 20% of the transient peak conductance (Numann & Wong, 1984; Bormann & Clapham, 1985). Despite this rapid

desensitization, we estimate that the HCO_3^- efflux would be sufficient to generate the observed alkaline shifts.

The maximum alkaline shift evoked by GABA was approximately 0·10 pH units. Assuming a pH_o of 7·40 and a constant CO_2 tension of 40 torr, an extracellular HCO_3^- increase of 7·2 mM would be required to raise pH_o to 7·50. To generate the mean observed alkaline shift (0·05 pH units), a HCO_3^- increase of 3·4 mM would be needed. If a population of cells (with an average volume of 10^{-12} l) occupied 40% of the tissue volume, and the extracellular volume fraction was 20%, we calculate that a 3 mM rise in extracellular HCO_3^- would result from an average HCO_3^- current of 30 pA per cell over 5 s.

Bicarbonate currents of this magnitude appear reasonable despite receptor desensitization. In Purkinje cells, peak GABA-evoked chloride currents exceeded 1000 pA, under a driving potential of 60 mV (Llano, Marty, Johnson, Ascher & Gähwiler, 1988). Since the electrochemical gradient for HCO_3^- is of similar magnitude, and the HCO_3^- permeability is approximately one fifth that of chloride (Bormann *et al.* 1987; Kaila *et al.* 1989), associated HCO_3^- currents might reasonably exceed several hundred pA, decaying to tens of pA after receptor desensitization.

GABA-evoked acid shifts

In the continued presence of GABA, the evoked alkalinization was often associated with a slow recovery towards baseline pH. Receptor desensitization, while probably responsible for limiting the magnitude of the response, is too rapid a process to account for the slow recovery of pH. A purely diffusive dissipation also appears unlikely, since the wash-out of GABA was typically followed by an acid rebound. This observation suggested that the alkaline shift was partly masked, and later superseded, by a slow acidifying process.

In crayfish muscle, similar surface acidifications were attributed to active acid extrusion. In these muscle fibres, acid extrusion was stimulated by intracellular acidification resulting from the GABA-evoked HCO_3^- efflux (Kaila *et al.* 1990). In the turtle cerebellum, the associated rise of $[K^+]_o$ may have served as an additional acidifying influence. For example, in the nominal absence of HCO_3^-, superfusion of GABA still gave rise to an acid shift, which was associated with an increase in $[K^+]_o$ (not shown). In addition, experimental increases in $[K^+]_o$ always caused a pure extracellular acidification. These acid shifts are likely to reflect metabolic responses to elevated $[K^+]_o$ (Kraig *et al.* 1983). Indeed, upon wash-out of GABA, the acid rebound was associated with an undershoot of extracellular K^+ (Fig. 6) suggesting increased Na–K-ATPase activity (Heinemann & Lux, 1975).

A dual mechanism of extracellular alkalinization

In turtle cerebellum, extracellular alkaline shifts can also be evoked by stimulation of the parallel fibres (Chesler & Chan, 1988), or by direct application of excitatory amino acids (Chesler & Rice, 1991). Our results indicate that these alkalinizations are not mediated by GABAergic inhibitory transmission, since they persisted in HCO_3^--free solutions and were insensitive to picrotoxin. The mechanism underlying these 'excitatory' alkaline shifts remains unknown. A channel-mediated flux of acid-

equivalents remains a plausible hypothesis; however, HCO_3^- does not appear to play a role.

In the molecular layer of the cerebellum, application of GABA is known to have a potent inhibitory effect on the Purkinje cells (Hackett, 1974; Malenka & Kocsis, 1982). It is therefore unlikely that the GABA-associated alkaline shift could have had an unrecognized excitatory synaptic component. In fact, application of GABA was found to block the parallel fibre-evoked alkalinization (Chesler & Rice, 1989), consistent with its inhibitory action. In addition, whereas the parallel fibre response was blocked in the absence of synaptic transmission (Kraig *et al.* 1983; Chesler & Chan, 1988), the GABA effect was not diminished.

It is significant that both excitatory and inhibitory neurotransmitters evoke a rapid alkalinization of the extracellular space through different mechanisms. Slow excitatory synaptic transmission may be enhanced by an associated rise in pH_o (Tang *et al.* 1990; Traynelis & Cull-Candy, 1990) while $GABA_A$-dependent inhibition can be diminished by alkalosis (Gallagher, Nakamura & Shinnick-Gallagher, 1983). Thus, both pH-mediated effects would promote a general increase in excitability, consistent with the augmentation of central synaptic transmission (Balestrino & Somjen, 1988) and the induction of seizure (Aram & Lodge, 1987) that have been associated with extracellular alkalosis.

In view of the significant extracellular alkalinizations that can accompany activation of $GABA_A$ receptors, pH-mediated interactions should be considered when evaluating the effects of GABA. NMDA responses, for example, could be particularly enhanced by GABA-evoked alkaline shifts (Tang *et al.* 1990; Traynelis & Cull-Candy, 1990). Nevertheless, it remains unclear whether GABAergic modulation of pH_o occurs during inhibitory synaptic transmission. While the exposure of $GABA_A$ receptors to agonist is normally brief, the associated HCO_3^- fluxes are also extremely localized, and within the confines of the subsynaptic space, significant alkaline shifts might occur. Thus, from the standpoint of pH-mediated interactions, the placement of inhibitory and excitatory synaptic contacts could be an important parameter in local synaptic operations.

We wish to thank Charles Nicholson and Margaret Rice for critical review of the manuscript. Ms Susan Lim and Ms Janet Ng provided technical assistance. Supported by NIH grant NS27011.

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