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CONCENTRATION OF CARBON DIOXIDE, INTERSTITIAL pH AND SYNAPTIC TRANSMISSION IN HIPPOCAMPAL FORMATION OF THE RAT

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SUMMARY

1. Interstitial pH (pH_0) was measured with ion-selective microelectrodes in the fascia dentata of rats anaesthetized with urethane,while CO_2 levels were controlled by varying pulmonary ventilation and CO_2 content of inspired air. In the CA1 sector of hippocampal tissue slices *in vitro* pH_0 was similarly measured and altered by varying CO_2 in the gas phase, or by adding HCl or NaOH to the artificial cerebrospinal fluid (ACSF) of the bath, or by changing the concentration of HCO_3^-.

2. Orthodromically evoked compound action potentials ('population spikes') were depressed in hypercapnia and increased in hypocapnia. In the fascia dentata of intact brains the population spike of the granule cells varied on average by more than 40% of control amplitude for each 0·1 change of pH_0. In the CA1 zone of tissue slices *in vitro*, the change of population spike amplitude was approximately 30% per pH change of 0·1 caused by altered CO_2 or HCO_3^- concentration, but only about 15% per pH change of 0·1 when HCl or NaOH were administered.

3. In anaesthetized rats the focal synaptic potential (FEPSP) evoked by a given stimulus intensity was weakly influenced by varying $[CO_2]$; in tissue slices weak effects on FEPSP were inconsistent. In hippocampus both *in situ* and *in vitro* the population spike triggered by a given magnitude of FEPSP increased in hypocapnia and decreased in hypercapnia. This suggests that the main effect of CO_2 is on the electric excitability of postsynaptic cells, with minor or no effect on transmitter release and on the interaction of the transmitter with its receptors.

4. Hypercapnia of anaesthetized rats was usually associated with a slight increase of $[K^+]_0$ in the fascia dentata. Tissue $[Ca^{2+}]_0$ changed little and not consistently. Neither of these two ions, nor concomitant changes of blood pressure or tissue partial pressure of oxygen, (P_{t,O_2}), could account for the effects of pH on neuronal excitability.

5. The results show that increasing the extracellular concentration of H^+ ions has a moderately depressant effect on the firing threshold of hippocampal neurones. The more powerful effects of elevated $[CO_2]$ and of lowered $[HCO_3^-]$ may probably be explained by a direct effect on the neuronal membrane. The brain, by regulating breathing, controls its own excitability.

INTRODUCTION

It has been known for many years that respiratory acidosis and alkalosis have profound effects on cerebral function. Foerster, for example, reported in 1924 that hyperventilation can provoke seizures in epileptic patients and Lennox, Gibbs & Gibbs in 1936 that elevation of the inspired CO_2 concentration can suppress them. Foerster (1924) also noted that in surgical patients hyperventilation lowered the threshold to electric stimulation of the exposed motor cortex. DeFinis (1932) and Brody & Dusser de Barenne (1932) have shown the same in experimental animals. Other investigators found that hypercapnia of experimental animals usually results in depression of synaptic transmission and of neuronal firing, although biphasic and excitatory effects have also been reported, especially for neurones involved in respiratory control (Kirstein, 1951; Krnjević, Randić & Siesjö, 1965; Cohen 1968; Papajewski, Klee & Wagner, 1969; Caspers & Speckmann, 1974; Carpenter, Hubbard, Humphrey, Thompson & Marshall, 1974).

Variations in the partial pressure of alveolar CO_2 could influence cerebral function in several ways. Molecular CO_2 itself, since it is soluble in the nerve membrane, could act as a 'narcotic' gas. Changes in both extracellular and intracellular proton concentration ($[H^+]_o$ and $[H^+]_i$) could be responsible for the neuronal effects if, as it has been believed, CO_2 influences pH on both sides of cell membranes (Waddell & Bates, 1969), at least during acute exposure (Siesjö & Messeter, 1971). More indirectly, CO_2 (or tissue pH) could influence neuronal function by altering cerebral blood flow (Kety & Schmidt, 1948; Brown, 1953; Gotoh, Meyer & Takagi, 1965) and thus altering the partial pressure of oxygen in the tissue (P_{t, O_2}) (Clark, Misrahi & Fox, 1958; Sugioka & Davis, 1960; Kreisman, Sick, LaManna & Rosenthal 1981). Finally, possible pH-related changes in tissue ion levels, especially Ca^{2+}, have been much discussed (György & Vollmer, 1923, Foerster, 1924; Barner and Greaves, 1936), then discredited but not disproven (Cumings & Carmichael, 1937; McCance & Watchorn, 1937; Brown 1953). Experiments on nerve tissues from cold-blooded animals *in vitro* (Dettbarn & Stämpfli, 1957; Brown & Walker, 1970; Zidek & Lehmenkühler, 1978; Zidek, Lange-Asschenfeldt, Lehmenkühler & Caspers, 1979; Gillette, 1983; Moody, 1983, 1984) did not solve these questions, not only because of possible species differences but also because in many studies CO_2 and pH were outside the range that would be tolerated by an intact mammal.

With ion-selective microelectrodes it is now possible to record tissue pH and other ion concentrations together with electric responses. Using such electrodes we examined quantitatively the correlation of moderate changes of tissue pH with synaptic transmission in both intact brains and isolated tissue slices. In hippocampal formation *in vitro* we compared the effect of changing P_{CO_2} to that of adding small amounts of strong acid or base to the bathing solution. We also determined the phase of synaptic transmission that was influenced by the acidity of the tissue. Parts of the data have already been reported in preliminary form (Somjen, 1985; Somjen & Balestrino, 1986; Balestrino & Somjen, 1987; Somjen, Allen, Balestrino & Aitken, 1987).

METHODS

Anaesthetized animals

Twenty adult male Sprague–Dawley rats of 350–450 g body weight were used. They were anaesthetized with urethane (1·5 g/kg body wt I.P.). The trachea was cannulated. Femoral arterial pressure was recorded in some animals by a strain gauge. Intravenous injections were given by a catheter in the femoral vein.

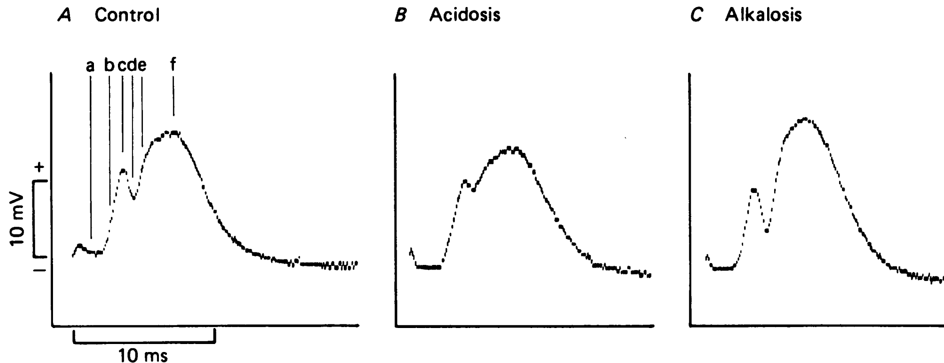

Fig. 1. Averaged potential waves evoked in fascia dentata; *A*: while rat was breathing room air; *B*: while it was ventilated with 10% CO_2 in 90% O_2; *C*: during forced hyperventilation with 100% O_2. Vertical lines in *A* are cursors positioned for computer measurements: a: cursor on baseline; b: cursor pointing at maximal rate of rise of focal postsynaptic potential (FEPSP); c and e: bases of population spike; d: peak of population spike; f: maximum amplitude of FEPSP.

Bipolar stimulating electrodes of 0·25 mm interpolar distance, 0·1 mm diameter, were inserted through a small hole drilled in the skull to stimulate the perforant path in the angular bundle. The stimulating electrode was tilted in the antero-posterior plane at an angle of 14 deg from the vertical to touch the brain 8·7 mm posterior to bregma and 4·2 mm lateral to the mid-line. It was lowered approximately 2·5 mm into the brain, its final depth being adjusted to produce maximal evoked potentials in the fascia dentata.

Double-barrelled ion-selective electrodes were used to record both pH_o and electric responses (Lothman, LaManna, Cordingley, Rosenthal & Somjen, 1975; Somjen, 1984) from the granule cell layer of the fascia dentata of the dorsal hippocampal formation. The recording electrode was inserted vertically 4·0 mm posterior to bregma and 2·1–2·3 mm lateral from the mid-line to a depth of 3·5–4·0 mm, the final depth being adjusted to record maximal positive focal postsynaptic potentials (FEPSPs) and negative compound action potentials (population spikes) evoked by stimulation of the angular bundle (see Fig. 1; and Somjen, Aitken, Giacchino & McNamara, 1985).

In some experiments two ion-selective electrodes were used, one for pH and the other either for interstitial potassium ($[K^+]_o$) or for calcium ($[Ca^{2+}]_o$), held so that the two tips touched the brain surface simultaneously at a distance of 0·3–0·5 mm in the plane parallel to the axis of the hippocampal formation. The evoked potentials recorded from the reference barrels of the two electrodes were similar in waveform but different in amplitude.

In some experiments a polarographic O_2 microelectrode (Diamond Electro-Tech) of about 1 μm tip diameter was inserted in the hemisphere opposite to that used for pH recording. The polarographic electrode was lowered 1·5–2·0 mm into the brain.

During initial control recordings the rats were breathing room air. They were then paralysed with gallamine triethoiodide (Flaxedil) and artificially ventilated with a Harvard respirator, initially with about 4·5–5·5 ml nominal tidal volume at 50–55 strokes/min. Actual tidal volume was less because of compression in the tubing. The respirator, together with recording equipment, was

placed in an adjacent room at about 2 m distance from the rat to avoid artifacts in the ion tracings caused by movement of personnel.

Ion-selective electrodes were made from double-barrelled glass tubing as described earlier (Lothman *et al.* 1975; Somjen, 1981, 1984). Combined tip diameter of the two barrels was 5·0–8·0 μm. Ligands used were WPI IE-010 for pH measurement, WPI IE-190 for K^+ and WPI IE-202 for Ca^{2+} ions. Connection of FET-input stage amplifiers was by means of chlorided silver wire. Electrodes were calibrated both before and after each experiment. Extracellular electric responses were recorded from the reference barrel with respect to earth. The animal was connected to earth by a chloride-coated silver wire inserted under the skin of the neck.

TABLE 1. Artificial cerebrospinal fluid (ACSF) solutions

		pH	Osmolarity (mosmol/l)	$[Ca^{2+}]$ (mM)
Control		7·45 ± 0·07	306 ± 5	1·03 ± 0·07
Acid	10% CO_2	7·13 ± 0·06		1·05 ± 0·02
	HCl	7·24 ± 0·05	308 ± 4	1·08 ± 0·03
	Low HCO_3^-	7·10 ± 0·02		1·13 ± 0·01
Alkaline	2% CO_2	8·08 ± 0·03		0·95 ± 0·04
	NaOH	7·71 ± 0·09	325 ± 7	0·94 ± 0·02

Values determined by standard laboratory bench instruments. Each entry shows the mean value ± standard deviation. $[Ca^{2+}]$ refers to free ionized calcium concentration.

A feed-back controlled circuit provided the polarographic current for tissue O_2 pressure (P_{t,O_2}) measurements (Schiff & Somjen, 1985). The output voltage was kept at −0·700 V. The polarographic current was recorded as the voltage drop across the 150 MΩ source resistance.

Electrode potentials and blood pressure were registered by a Grass P7 polygraph and also by a Vetter instrumentation tape-recorder. The evoked potential waves were analysed with a Tecmar analog-to-digital converter and an IBM PC.

The semi-automated program of Aitken (1985) was used to analyse evoked potentials, as illustrated in Fig. 1. Four responses evoked at 2 s intervals were averaged and on each record the following were measured: the initial maximal rate of rise of the FEPSP, the maximal amplitude of the FEPSP, and the amplitude of the population spike. Stimulus–response functions (input–output curves) were computed from the averaged responses to varying stimulus intensities, usually from 0·05 or 0·1 mA and 0·1 ms to 1·5 mA and 0·5 ms in six steps. The same sets of stimulus intensities were used for all input–output curves in any one experiment. Usually stimulus sequences were applied at 6 min intervals (about 1·5 min stimulation and 4·5 min rest between sequences). Six input–output curves were usually obtained in the initial control period while the rat was breathing room air, and then four input–output curves in each experimental condition while it was artificially ventilated.

Hippocampal tissue slices

Rat hippocampal slices of 400 μm thickness were prepared according to standard methods (Dingledine, 1984) as adapted in this laboratory (Aitken, 1985). The slices were maintained at 35 °C in an 'interface' chamber. The pH of the tissue slice was continuously monitored with an ion-selective microelectrode as described in the preceding section. A tungsten microcathode was used to stimulate the Schaffer collateral–commisural fibre bundle. Evoked potentials were recorded in both stratum radiatum and stratum pyramidale of the CA1 zone, using the reference barrel of a pH microelectrode in one and a NaCl-filled micropipette in the other, variably in different experiments. Methods of recording and computing evoked potentials were similar to those used in intact rats (see above) and as described by Aitken (1985). Two to six evoked potentials were averaged for each set of measurements.

The artificial cerebrospinal fluid (ACSF), perfusing the chamber at a rate of 3·5 ml/min, had the following composition (mM): NaCl, 130; KCl, 3·5; NaH_2PO_4, 1·25; $NaHCO_3$, 24; $CaCl_2$, 1·2;

$MgSO_4$, 1·2; glucose, 10. In the control state the ACSF was saturated with 90% O_2, 5% CO_2 and 5% N_2 and the same gas mixture flowed through the airspace of the chamber. One or more of the following treatments were tested on each slice: (1) CO_2 concentration was either raised to 10% or reduced to 2% in both gas phase and ACSF, while O_2 was kept constant at 90% and N_2 was altered as needed; (2) $NaHCO_3$ concentration was lowered to 12 mM, while maintaining osmolarity constant by raising NaCl to 142 mM; (3) HCl (20 mM) or NaOH (12 mM) were added. The pH, osmolarity and free [Ca^{2+}] in these solutions were measured with standard bench instruments, and are shown in Table 1. In most slices pH was changed both in the alkaline and in the acid direction, often by more than one method of treatment, always with a return to control solution between changes.

At the beginning of each experiment, stimulation intensity was adjusted to evoke a half-maximal population spike. This stimulus was then delivered once every 10 s while changing pH, and the population spikes were recorded at varying pH levels. In addition, input–output (or strength–response) curves were constructed from responses evoked by varying stimulus intensities under control conditions, when a new steady pH was reached, and after recovery, (Balestrino, Aitken & Somjen, 1986). Data from slices in which the population spike did not return at least part of the way toward control amplitude upon wash-out were discarded.

Input–output curves were analysed as described by Balestrino *et al.* (1986). The afferent compound action potential ('presynaptic volley') was plotted as a function of stimulus intensity (Fig. 10*A*); the initial rate of rise (slope) of the FEPSP recorded in stratum radiatum was plotted as a function of presynaptic volley (Fig. 10*B*), while the population spike amplitude recorded in stratum pyramidale was plotted as functions of both the FEPSP recorded in stratum pyramidale and of the presynaptic volley recorded in stratum radiatum (Fig. 10*C* and *D*). Linear regression was computed for the relation of FEPSP and presynaptic volley and the regression coefficient ('slope') used for quantitative comparisons. The 'area under the curve' was computed for the other input–output functions, always using the same range of abscissal values for quantitative comparisons within experiments (Balestrino *et al.* 1986).

RESULTS

Anaesthetized rats

The initial pH of the cerebral tissue could not always be determined reliably because electrodes tended to acquire spurious voltages when transferred from the calibrating solution into the brain. When readings were close to what may be expected (Katzman & Pappius, 1973), the initial pH in the hippocampal formation appeared to be between 7·15 and 7·35. This is consistent with reports that the interstitial fluid (ISF) of the brain is more acid than the cerebrospinal fluid (CSF) (Cragg, Patterson & Purves, 1977). Changing the conditions of ventilation resulted in consistent responses of the pH electrode (Fig. 2). We will report all pH readings in intact brains as departures from the initial level.

To induce respiratory alkalosis, the rate of artificial respiration was doubled. Tidal volume was unaltered except in early experiments when it was increased by 20–30%. Raising tidal volume tended to impair the circulation and was therefore abandoned. During forced overbreathing the rats were usually given 100% oxygen. To achieve acidosis, the animals were ventilated either with 5% or with 10% CO_2 in 95 or 90% O_2, either at a normal rate or during hyperventilation (e.g. Fig. 3). The sequence of respiratory changes was varied from rat to rat, and between periods of alkalinization and acidification the tissue pH (pH_o) was returned to near its control value.

As expected, the pH_o of the fascia dentata became alkaline during hyperventilation and acid during CO_2 administration. The potential of the hippocampal formation

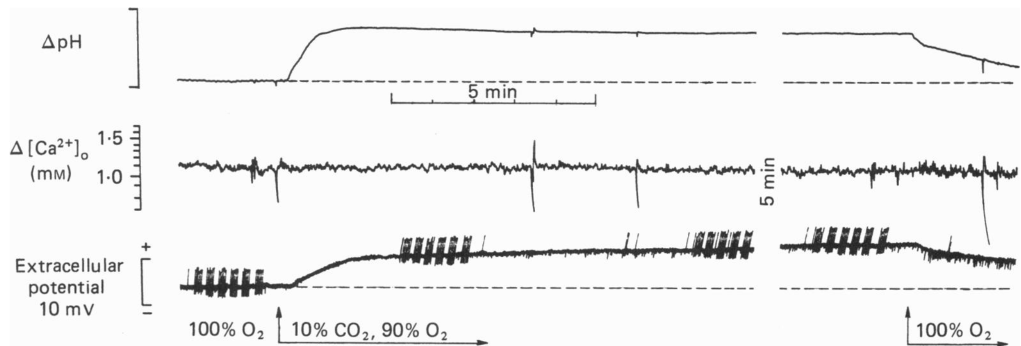

Fig. 2. Polygraph records of potential of pH microelectrode, Ca^{2+}-selective microelectrode, and reference potential of pH electrode. Calibration for uppermost trace: 0·5 pH; positive (upward) shift of pH potential signals increase of $[H^+]_o$, i.e. decrease of pH. Sharp transients on $[Ca^{2+}]$ trace are artifacts. Vertical strokes of pen on electric potential (lowest) trace correspond to evoked potentials (as in Fig. 1) on a compressed time scale. Arrows mark changes in inspired gas mixture.

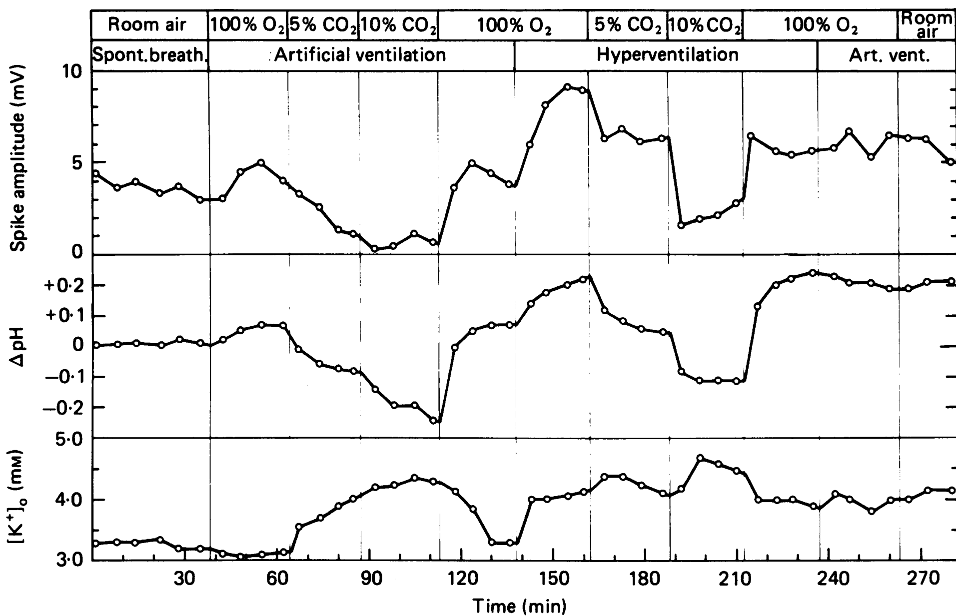

Fig. 3. Population spike amplitude (evoked by 1·2 mA, 0·1 ms stimuli); changes of interstitial pH; and changes of interstitial potassium concentration, $[K^+]_o$, recorded in one experiment. Abscissa shows time from start of recordings; changes in pulmonary ventilation are shown above graphs. Spike amplitudes are averages of four responses.

relative to the (earthed) body potential shifted in the positive direction during hypercapnia (Fig. 2) and negatively during hypocapnia. This is similar to the shifts of the electric potential of the CSF in the ventricles and of the cortical surface reported by others (Tschirgi & Taylor, 1958; Besson, Woody & Marshall, 1971; Loeschke, 1971).

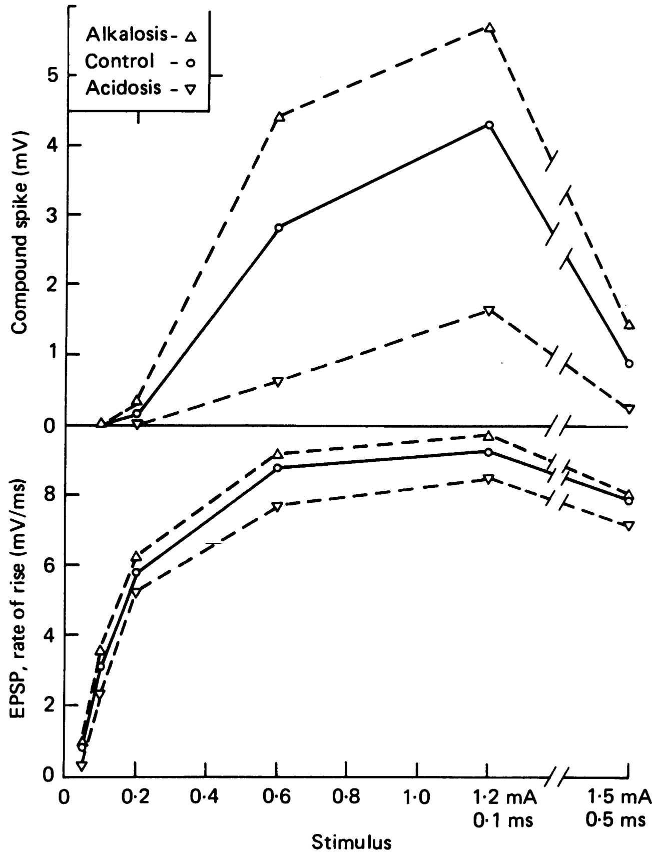

Fig. 4. Strength–response ('input–output') curves for population spike amplitude and for maximal rate of rise of FEPSPs from one experiment. Control: ΔpH −0·05 to +0·07 relative to initial level; data are the mean of twelve series of recordings (four each from beginning, middle and end of recording period). Acidosis: ΔpH −0·31 to −0·36; rat ventilated with 10% CO_2. Alkalosis: ΔpH +0·13 to +0·18, during hyperventilation with 100% O_2. Duration of stimulus pulses 0·1 ms, except for pulses of 1·5 mA, which were of 0·5 ms duration.

The population spike evoked by stimulating the angular bundle became larger whenever the brain became more alkaline and smaller whenever the reaction became more acid (Figs 1 and 3). The change was consistent at all stimulus intensities (Fig. 4). It will, incidentally, be noticed that in the strength–response curves of Fig. 4 the pulses of 1·5 mA and 0·5 ms evoked smaller responses than those of 1·2 mA and 0·1 ms pulses. It seems that the strongest pulse recruited afferent fibres of inhibitory function. This phenomenon was seen in several but not in all rats.

In some rats no population spike could be evoked, even though FEPSPs were of large amplitude. In two the population spike increased throughout the experiment, as though subject to long-term potentiation. In ten rats a consistent and statistically significant ($P < 0·01$) positive linear correlation was found between the amplitude of the population spike evoked by a given stimulus, and the pH of the interstitial fluid

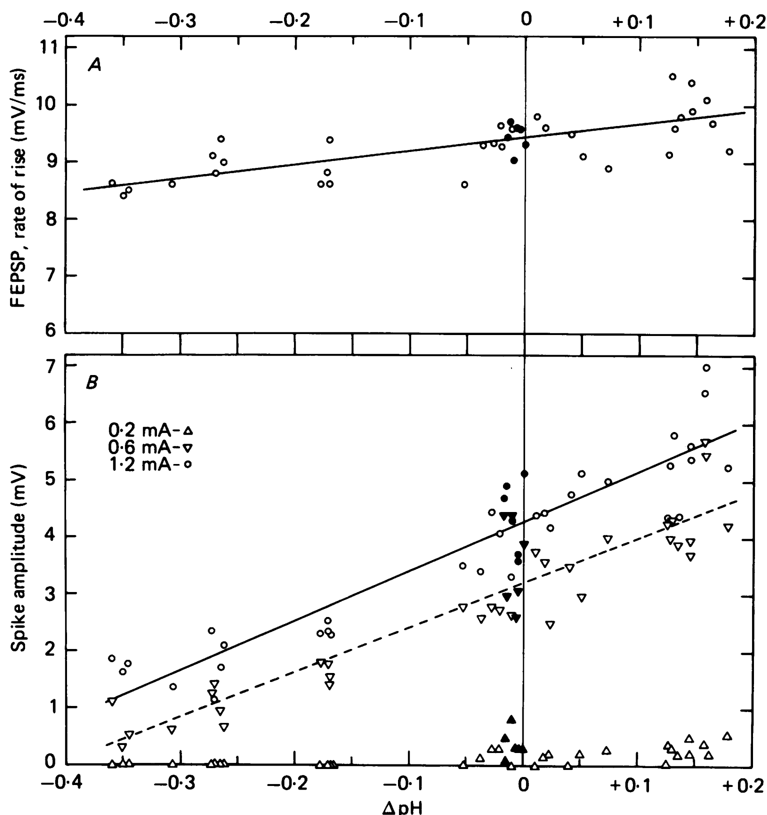

Fig. 5. Statistical regression of the maximal rate of rise of FEPSP (*A*), and of the population spike amplitude (*B*), on interstitial pH. Each point the average of four consecutive responses. Data from one experiment (same as Fig. 4). Filled symbols during initial control period of spontaneous respiration in room air, open symbols during numerous changes of artificial ventilation. Stimulus intensities indicated on inset; all pulses 0·1 ms duration.

(Fig. 5). In fifteen sets of measurements in the ten rats (obtained by using more than one stimulus intensity in some rats), the population spike changed on the average by more than 40 % of its control amplitude with each 0·1 pH change of tissue acidity. There was, however, considerable variation between rats: the least change of population spike was 19·6 % and the greatest was more than 100 % per pH change of 0·1. Although smaller population spikes generally tended to be relatively more affected than large ones (Fig. 5), this factor alone could not account for all the variability.

The FEPSP also appeared to change with tissue pH (Figs 4 and 5), but less so than the population spike. In three experiments the correlation between the FEPSP and pH was not statistically significant; in eight others it was ($P < 0.05$ to $P < 0.001$). The level of significance was about the same, whether the initial rate of rise or the maximal amplitude of the FEPSP was used in these calculations. When the correlation was significant, the FEPSP changed by an average of 5·3 % (range: 2·0–13·3 %) of its control value for each 0·1 pH unit change of acidity.

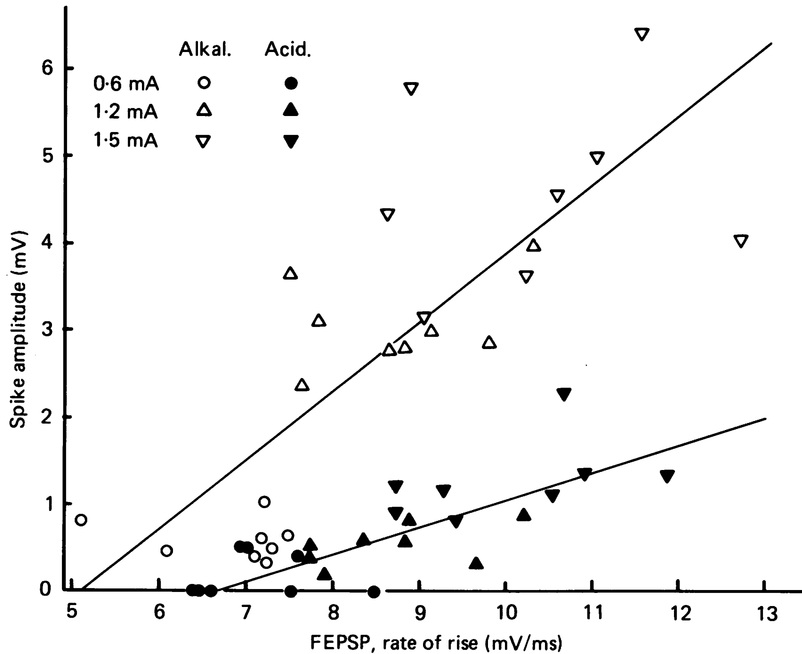

Fig. 6. The statistical regression of population spike amplitude on FEPSP during hyperventilation with 100% O_2 (open symbols) and with 10% CO_2 in 90% O_2 (filled symbols). Inset shows stimulus intensities; pulse duration 0·1 ms for 0·6 and 1·2 mA stimuli and 0·5 ms for 1·5 mA stimuli.

Since both the FEPSP and the population spike varied with pH, the apparent influence of pH on the spike could have been mediated by the change of the synaptic drive. Small changes of EPSPs could, theoretically, cause large changes in the number of postsynaptic neurones that are discharged. By plotting population spike amplitude against FEPSP it became clear, however, that the number of granule cells firing in response to a given FEPSP was much greater when the tissue was alkalotic than when it was acidotic (Fig. 6). This indicates a change in the electrical excitability of the postsynaptic neurones.

We had to consider whether cerebral hypoxia induced during hyperventilation (Sugioka & Davis, 1960) could have influenced the excitability of the neurones. For this reason changes in the partial pressure of O_2 ion the tissue of the brain were recorded in three rats. For reasons discussed in detail by others (e.g. Clark *et al.* 1958; Grunewald, 1969; Klinowski & Winlove, 1980) actual P_{t,O_2} could not reliably be determined and we prefer the term 'relative oxygen availability' as defined by Clark *et al.* (1958). The relative units shown in Fig. 7 correspond to the readings (mV) across the 150 MΩ source resistance of the polarographic amplifier.

In agreement with observations reported by others (Clark *et al.* 1958; Kreisman *et al.* 1981), when the inspired gas was changed from room air to 100% O_2 the P_{t,O_2} in the brain increased, but not nearly as much as when CO_2 was added to the O_2. During hyperventilation P_{t,O_2} decreased, but 100% O_2 kept the P_{t,O_2} at or above the control level recorded when the rat was spontaneously breathing room air (Fig. 7). It should be noted that in the last 30 min of the experiment of Fig. 7 cardiovascular function

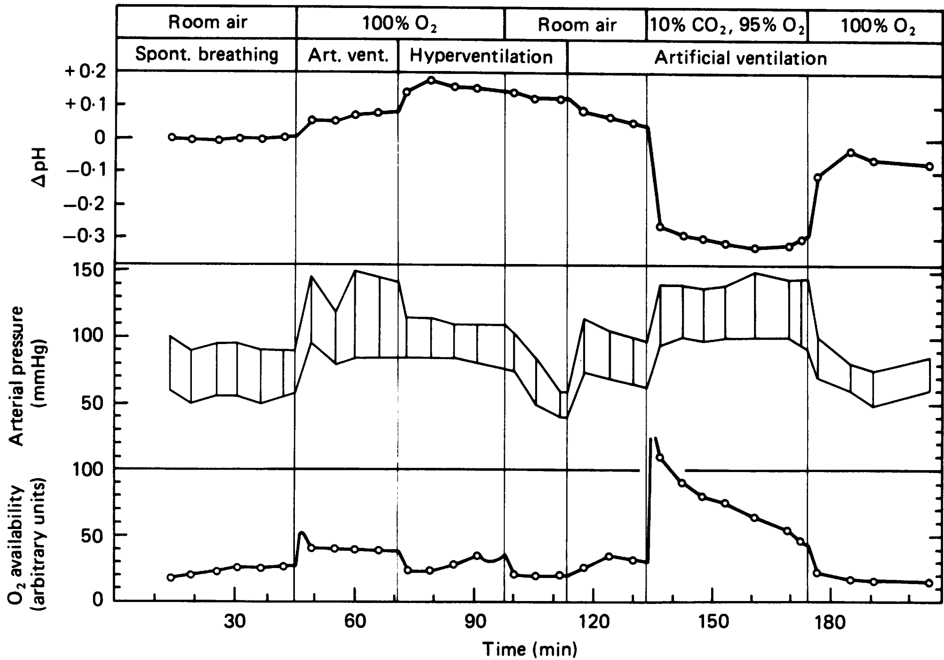

Fig. 7. Changes of interstitial pH in fascia dentata, systolic and diastolic pressures in the femoral artery, and polarographic current recorded in cortex of opposite hemisphere in one rat. Time in minutes from completion of preparation. Changes of pulmonary ventilation are indicated above graphs.

seemed impaired and, correspondingly, both P_{t,O_2} and the pH of the brain were lowered, in spite of the administration of O_2. Measurements from input–output curves of the experiment of Fig. 7 were therefore not included in the averages reported above. It should also be noted that 5 or 10% CO_2 was administered sometimes by normal artificial ventilation and sometimes by forced overbreathing. Synaptic transmission was affected in each case as expected from the change of cerebral pH, indicating that mechanical obstruction of cardiac output by positive pressure hyperventilation did not influence the results.

In some cases excessive hyperventilation with room air led to a spreading depression-like response, in which the electric potential of the fascia dentata suddenly shifted in the negative direction, $[K^+]_o$ rose sharply, $[Ca^{2+}]_o$ dropped and all evoked potentials disappeared. Such a response was not seen during hyperventilation with O_2 or with CO_2–O_2 mixtures. It was usually preceded by depression of the arterial pressure and an acid shift of cerebral pH that occurred in spite of continued overbreathing and suggested lactacidosis. Two rats died in this condition. Data obtained following the occurrence of spreading depression were disregarded, even if responses appeared to recover.

In three experiments the interstitial concentration of K^+ ions was measured, together with the interstitial pH. As illustrated in Fig. 3, acidification of the tissue was usually associated with an increase of $[K^+]_o$, alkalinization with a decrease. An exception is seen near the 140 min mark of Fig. 3 when $[K^+]_o$ seemed to rise during

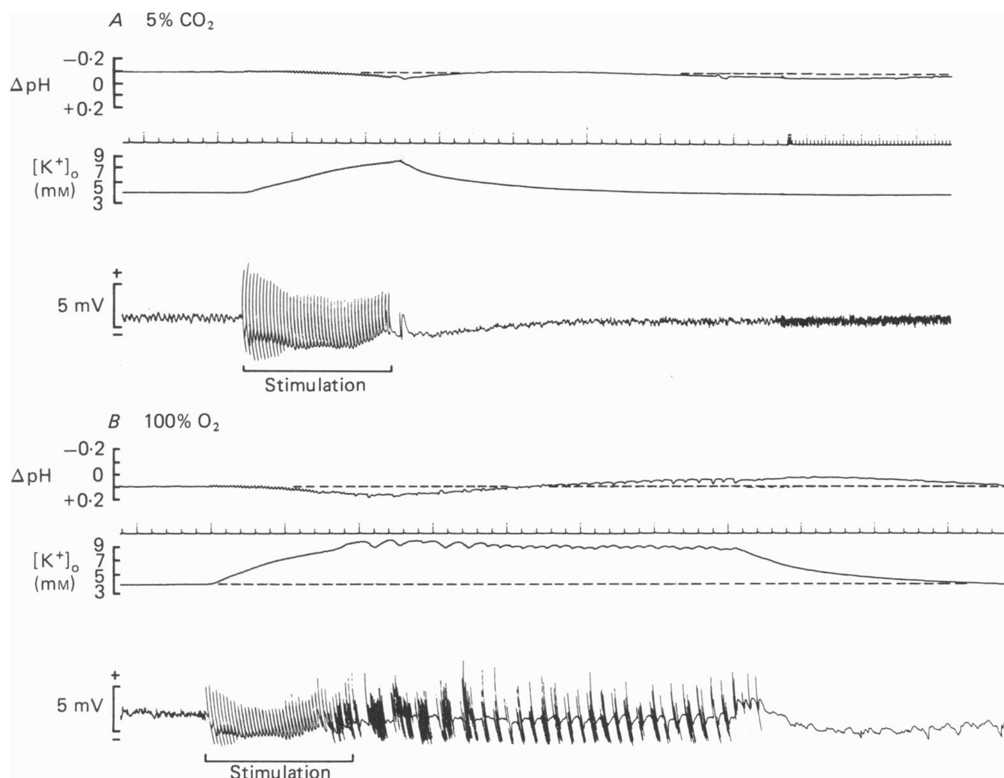

Fig. 8. Paroxysmal after-discharge in hypocapnic brain. Polygraph recording of interstitial pH, $[K^+]_o$ and electric responses in fascia dentata to repetitive stimulation, while rat was ventilated with 5% CO_2 in 95% O_2 (*A*) and hyperventilated with 100% O_2 (*B*). Stimulation of angular bundle at 4 pulses/s for 10·5 s, with 0·6 mA, 0·1 ms pulses in both cases. Time in seconds.

hyperventilation-induced alkalinization. During twenty-one changes of ventilation or of inspired gas in the three rats, the pH and $[K^+]_o$ changed in the opposite sense in eighteen cases and in the same sense in three. Data are insufficient for quantitative estimation of the magnitude of the influence of $[H^+]_o$ on $[K^+]_o$.

In two rats $[Ca^{2+}]_o$ was measured concurrently with pH. In one $[Ca^{2+}]_o$ did not change detectably (Fig. 2); in the other there were small shifts of $[Ca^{2+}]_o$ but these were not consistent. At the end of these two experiments spreading depression was deliberately provoked by high-frequency stimulus trains to verify that the ion-selective electrode recorded the expected drop of $[Ca^{2+}]_o$.

In five other experiments stimulus trains of increasingly high frequency were delivered to estimate the minimal level of stimulation that provoked paroxysmal firing (cf. Somjen *et al.* 1985). As expected, paroxysmal firing was always more readily provoked in alkalotic than in acidotic brains (Fig. 8). In some cases high-frequency stimulation provoked spreading depression. This never occurred during the administration of 10% CO_2.

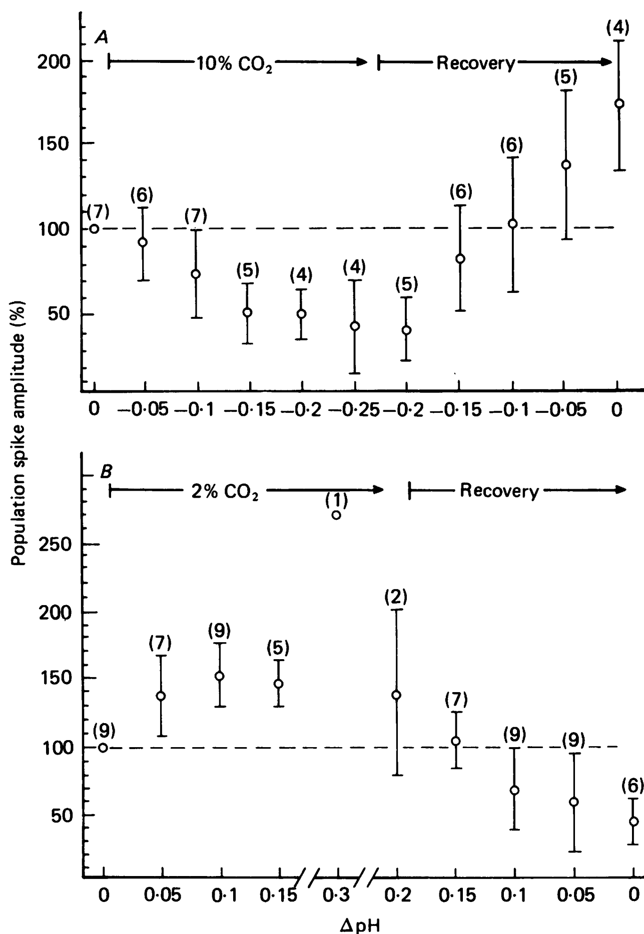

Fig. 9. Mean changes of population spike amplitude of hippocampal tissue slices induced by increasing (*A*) or decreasing (*B*) CO_2 concentration. In brackets the number of observations. Error bars show standard deviation (except for $n = 2$ the limits are shown). Time sequence from left to right, but rate of change of pH varied, hence abscissal intervals indicate different time intervals for different slices. All slices exposed to both high and low $[CO_2]$, but order of exposure varied. Ordinates normalized to spike amplitude measured before exposure.

Hippocampal tissue slices

The pH of the interstitial fluid (pH_0) of hippocampal tissue slices was always more acid than that of the bathing fluid. The difference was determined by pushing the ion-selective microelectrode through the slice into the bathing fluid at the end of each experiment. In control medium of pH 7·45 (S.D. ± 0·04; see Table 1) the pH_0 about 100 μm beneath the surface of the slice was 7·18 ± 0·10. A similar difference has been noted earlier (Schiff & Somjen, 1985) in slices that were submerged in artificial CSF, not placed at a gas–ACSF interface as in this study.

The pH_0 of twenty-four tissue slices was changed by varying $[CO_2]$ in both the gas and in the bathing fluid. Fifteen were exposed to both elevated and lowered $[CO_2]$;

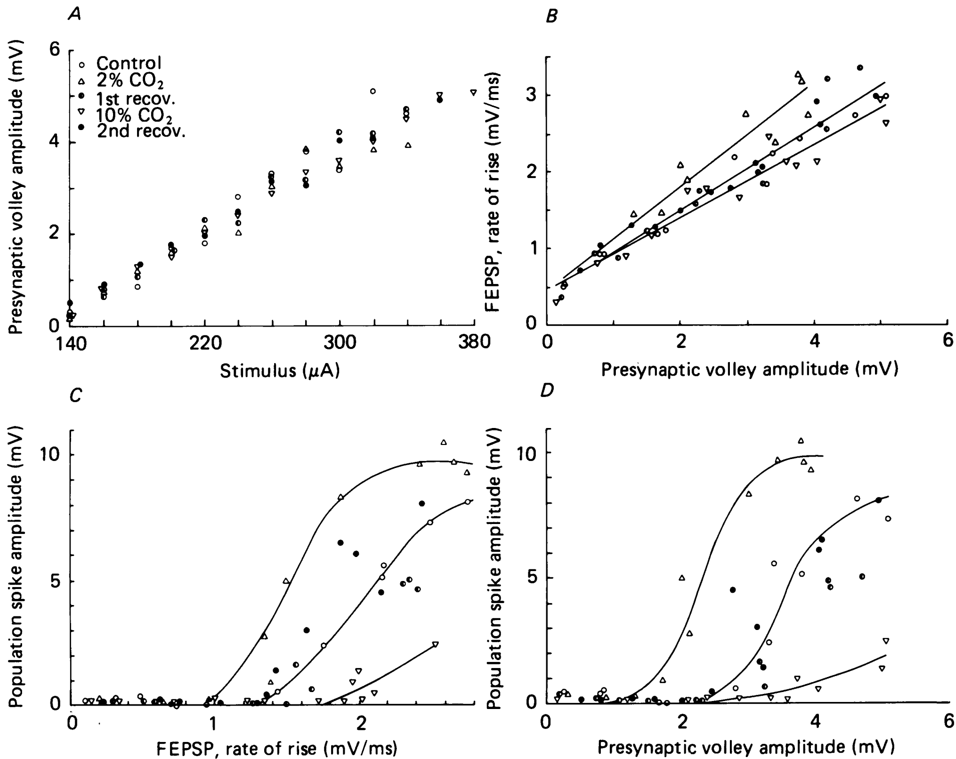

Fig. 10. Input–output curves recorded in CA1 region in 2, 5 (= control) and 10% CO_2. All data from a single slice. Points show averaged potentials of four consecutive sweeps. *A*: presynaptic volley amplitude as function of stimulus intensity; *B*: FEPSP magnitude (initial slope) as function of presynaptic volley amplitude; *C*: orthodromic population spike amplitude as function of FEPSP magnitude; *D*: population spike amplitude as function of presynaptic volley. In 10% CO_2 pH was 0·2 unit more acid; in 2% it was 0·26 unit more alkaline than in control solution.

in five others the effect of raising [CO_2] was compared to that of lowering [HCO_3^-], and four others were tested with elevated [CO_2] only.

When the [CO_2] was lowered from 5 to 2% the amplitude of the population spike of CA1 pyramidal cells reversibly increased; when [CO_2] was increased to 10% the population spike was reversibly depressed. Following hypercapnia lasting about 5–10 min, the final spike amplitude was close to the initial control level. Following more extended exposure to an altered [CO_2], lasting up to 20 min, the spike amplitude over- or undershot the original level (Fig. 9). In these experiments the recording of input–output curves (Fig. 10) made the longer exposure necessary.

A significant ($P < 0·05$) linear regression of population spike upon pH_o was found in all but two out of twenty-four experiments in which [CO_2] levels were altered (Fig. 11). In the eight experiments in which the exposure to altered [CO_2] was short and the population spike recovered to near its control size the regression was computed using data during hyper- and hypocapnia as well as during recovery. In the experiments in which the spike amplitude overshot the control value (Fig. 9) the

measurements obtained during recovery were not included. The mean coefficients ('slope') of the regressions of these two groups of experiments were almost identical. The population spike changed by an average of 32 % of its control amplitude for each 0·1 unit change in pH_0, as estimated from all twenty-four experiments. This is less than the average change of the population spike of dentate granule cells in anaesthetized rats, but the difference is not statistically significant.

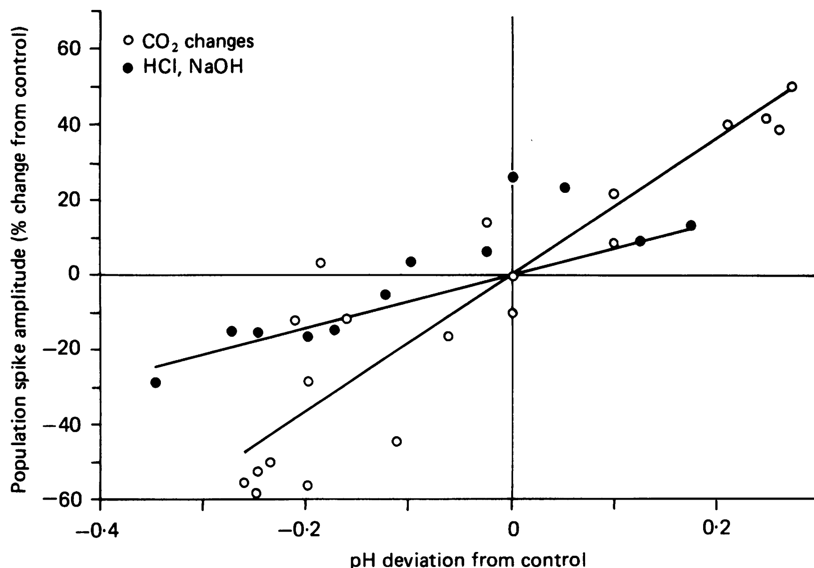

Fig. 11. The regression of the orthodromic population spike on pH_0. All data from one tissue slice exposed first to high, then to low $[CO_2]$, then to HCl followed by NaOH. Each point from the mean of four consecutive sweeps. Stimulation by constant pulses, set to evoke 50 % of maximal spike amplitude in the initial control state. Coefficient of correlation, $r = 0·68$ for $[CO_2]$ changes and $r = 0·77$ for HCl and NaOH addition; $P < 0·02$ for both.

Changes of successive stages of synaptic transmission were analysed by plotting input–output curves between components of the evoked potential waves (Balestrino *et al.* 1986). There was no detectable change of the afferent fibre volley evoked by a given stimulus pulse when $[CO_2]$ was changed (Fig. 10*A* and Table 2). In some but not all experiments exposure to 10 % CO_2 slightly depressed the FEPSP corresponding to a given input volley, and exposure to 2 % CO_2 enhanced it (Fig. 10*B*). This effect was so inconsistent that it disappeared when results from all experiments were pooled (Table 2). The curve expressing postsynaptic population spike amplitude as a function of FEPSP magnitude shifted consistently to the right during 10 % CO_2 administration, and to the left under the influence of 2 % CO_2 (Fig. 10*C* and Table 2). The same changes were seen in the relationship between input (presynaptic volley) and output (population spike) of the synaptic pathway (Fig. 10*D* and Table 2).

Halving $[HCO_3^-]$ lowered the pH_0 and depressed the population spike comparably to doubling $[CO_2]$. The change of the spike was 34·1 % of the control amplitude per

0·1 unit change of pH (S.D. among experiments: ±18·1; *n*: 5; S.E.M.: ±8·1). This is statistically not different from the 31·9% amplitude change per 0·1 pH unit achieved by changing [CO_2] (S.D.: ±18·9; *n*: 24; S.E.M.: ±3·9). By contrast, adding either HCl or NaOH to the bathing fluid always had, for a given pH_o, much weaker effects than changing [CO_2] tested on the same slice (Fig. 11). In two slices out of ten trials the

TABLE 2. Quantification of the changes in experimental input–output curves

Function	10% CO_2	2% CO_2
Stimulation–presynaptic volley*	+3±16% (n.s.) $n = 11$	−2±16% (n.s.) $n = 13$
Presynaptic volley–FEPSP†	−7±15% (n.s.) $n = 10$	−4±12% (n.s.) $n = 12$
FEPSP–population spike*	−27±35% ($P < 0·05$) $n = 11$	+55±55% ($P < 0·01$) $n = 11$
Presynaptic volley–population spike*	−53±32% ($P < 0·01$) $n = 12$	+91±118% ($P < 0·05$) $n = 13$

All entries are the mean (±S.D.) percentage difference between the experimental condition and the mean of control and recovered values. Significance calculated by *t*-test.

* Area under the curve (see Balestrino *et al.* 1986).

† Coefficient ('slope') of linear regression.

regression of the population spike on HCl- and NaOH-induced pH changes was not significant. The mean change of spike amplitude upon pH_ochanges induced by strong acid or base was only 15·2% (S.D.:±18·5; *n*: 10; S.E.M.:±5·85; including the two experiments in which regressions were not significant). The difference between the pooled regressions of spike amplitude on pH computed for changing [CO_2] and for adding HCl or NaOH was significant ($P < 0·02$). Raymond & Bokesch (1983) found a similar difference when they compared the effect of [CO_2] to that of adjusting HEPES buffer on the excitability of frog nerve *in vitro*.

It is to be noted that slices were exposed to HCl and NaOH longer (about 30 min) than to changed [CO_2] because of the slower penetration of the strong acid and base into the tissue. Administration of HCl or NaOH was always continued until the pH_o reached a steady level, suggesting uniform distribution throughout the tissue. Treatment with low HCO_3^- took, however, just as long as that with strong acid and base, and therefore the slower onset of the pH change could not explain the different response of the tissue to HCl and NaOH.

DISCUSSION

In the light of the literature cited in the Introduction it was no surprise that inhaled CO_2 depressed neuronal firing, and that hyperventilation enhanced it. The very great sensitivity of hippocampal neurones was, however, unexpected. Small departures from normal pH influenced the transmission of signals so that excitability appeared to be a continuous function of tissue acidity, without discontinuity or threshold. The applied pH values were well within the range found clinically in humans, and the alterations of excitability could explain, at least in part, the disturbances of cerebral function seen in acidotic and alkalotic patients.

Our goal was to assess the consequences of variations in P_{t, CO_2} and tissue acidity

independently from those of cerebral oxygenation and other concomitant changes that accompany clinical conditions (Brown, 1953; Meyer & Gotoh, 1960; Kreisman *et al.* 1981). The regression describing the dependence of the neurone population discharge on pH changes induced by variation of P_{CO_2} was similar for tissue *in situ* and *in vitro*. There may, of course be a small but real difference, masked by experimental variability. After all, two different zones of the hippocampal formation (fascia dentata and CA1) are being compared, and other factors such as the age of the rats (younger for slices) and the presence of the anaesthetic could have played a part. It must also be stressed that inclusion of data from preparations with small population spikes may have exaggerated the steepness of the average regression calculated for intact brains.

Since P_{O_2} was controlled in the environment of the tissue slices *in vitro*, cerebrovascular factors and P_{t, O_2} could not have had a major influence. This conclusion is reinforced by the fact that the population spike amplitudes in intact brains were, at a constant pH, the same whether the rat was ventilated with room air or with 100% O_2.

Cameron (1971) reported that the $[K^+]$ of CSF rose during respiratory acidosis and Lehmenkühler, Caspers & Kersting (1985) noted an increase of $[K^+]_o$ in cerebral cortex when rats were given 20% CO_2 to breathe. Using more moderate increases in inspired CO_2 we confirmed this observation. The excess K^+ may have come from blood plasma where its concentration is higher than in cerebral ICF, especially if the blood–brain barrier was breached by acidosis, as suggested by Cameron, Davson & Segal (1970). However, Cameron *et al.* (1970) used 18% CO_2 to induce acidosis. With 10% CO_2 in inspired air Cameron (1971) found no increase in the transfer of radioactively labelled K^+ from blood into brain. Therefore the variation of $[K^+]_o$ with tissue pH is more likely to be the consequence of transmembrane redistribution of K^+ in neurones and/or glial cells. However, redistribution of K^+ could not have contributed to the changes in neuronal excitability. Within the moderate range of $[K^+]_o$ changes seen in these experiments elevation of $[K^+]_o$ enhances and its decrease depresses the excitability of hippocampal neurones (Balestrino *et al.* 1986). The opposite changes were seen when $[K^+]_o$ was elevated in acidosis and depressed in alkalosis. The smaller effect of $[K^+]_o$ was apparently overridden by the larger effect of P_{CO_2}.

The role of Ca^{2+} in respiratory alkalosis and acidosis has long been debated (see Introduction and review by Brown, 1953 and by Somjen *et al.* 1987). Recently Lehmenkühler *et al.* (1985) reported that administration of 20% inspired CO_2 caused the $[Ca^{2+}]_o$ in rat's neocortex first to rise and then to fall below its resting level. Using 10% inspired CO_2 we found that $[Ca^{2+}]_o$ in cat's neocortex increased slightly (Allen, 1983; Somjen *et al.* 1987) during acidification. In rat hippocampus changes of $[Ca^{2+}]_o$ either did not occur or they remained below the limit of detection. Even if $[Ca^{2+}]_o$ had changed, it could not have accounted for the effect of changing P_{CO_2}. Unlike very low and very high $[Ca^{2+}]_o$, moderate changes in the concentration of this ion influence the amplitude of the FEPSP in hippocampal formation but only very slightly the electric excitability of pyramidal cells (Dingledine & Somjen, 1981; Balestrino *et al.* 1986). By contrast, changing the pH influenced mainly the electric excitability of the postsynaptic neurones. The small changes of FEPSP were,

moreover, not in the expected direction since the FEPSPs decreased in acidosis (when $[Ca^{2+}]_o$ may rise), and increased in alkalosis (when $[Ca^{2+}]_o$ may decline).

How much of the effect of CO_2 could be attributed to changing pH_o? We used NaOH and HCl to answer this question, because they are unlikely to have pharmacological actions other than through changing pH_o. We could not expect to control cerebral pH_o of intact brain by infusing strong acid or base into the circulation (Katzman & Pappius, 1973; Nattie, 1983), but we could do so in isolated tissue slices. Adding H^+ (as HCl) does not materially change the concentration of dissolved molecular CO_2 in a solution at equilibrium with a gas of constant P_{CO_2}. Since changes of $[CO_2]$ acted more powerfully than equivalent amounts of HCl or NaOH, pH_o could not be the only factor operating. The question, then, is whether pH_i could be the added factor? Adding strong acid or base to extracellular fluid is said to influence intracellular pH only weakly or not at all (Waddell & Bates, 1969; Thomas, 1984; Moody, 1984). It had been thought that CO_2 penetrates cell membranes and therefore strongly affects pH_i (Waddell & Bates, 1969; Lipton & Korol, 1981), at least in the initial 45 min of exposure (Siesjö & Messeter, 1971). More recently, however, it was found that raising $[CO_2]_o$ has a biphasic effect on neurones and glial cells of invertebrates, causing rapid acidification initially, from which the cell recovers, sometimes partially and often completely (DeWeer, 1978; Thomas, 1984; Schlue & Thomas, 1985). Sick & Balestrino (1987) measured pH_i with the intracellular indicator dye neutral red and also with the creatine kinase technique. Raising $[CO_2]$, adding HCl, or lowering $[HCO_3^-]$, in the same range as used in the experiments reported here, caused a much smaller acidification of cells than the acid shift of the interstitial milieu. Therefore the depression of excitability caused by high $[CO_2]$ or by low $[HCO_3^-]$ is not likely to be due to intracellular acidosis. More probably the mechanism must be sought within the membrane itself.

While cerebral pH does not markedly change with systemic 'metabolic' acidosis and alkalosis (Katzman & Pappius, 1973; Nattie, 1983), it can do so with cerebral metabolic processes. Seizure discharges, for example, are followed by acidification of the brain (Dusser de Barenne, Marshall, McCulloch & Nims, 1938; Jasper & Erickson, 1941; Meyer, Gotoh & Tazaki 1961; Caspers & Speckmann, 1972; Somjen, 1984; Siesjö, von Hanwehr, Nergelius, Nevander & Ingvar, 1985). Comparing the magnitude of the pH changes measured in hippocampal formation after paroxysmal discharges (Somjen, 1984) with those imposed on it in this study confirms that post-ictal acidification may well be a factor in the post-ictal depression of neurones. However, since cerebral acidosis may result from the accumulation of lactic acid more than of CO_2 (Siesjö *et al.* 1985), specific effects of that compound could complicate the picture.

Thus the acid products of cerebral metabolism, especially CO_2, act back to inhibit the very tissue that produced them. Superimposed on this negative feed-back loop there is the, as yet ill understood, excitant effect of CO_2 on respiration. Since the brain regulates respiration and, through respiration, the pH and CO_2 content of its own fluids, the brain also exercises control over its own excitability.

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