Pathophysiology of pH and Ca^{2+} in bloodstream and brain[1]

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The highlights of the literature and our work on tetany and hyperventilation are reviewed. Our studies concern the following: (1) the changes of $[Ca^{2+}]$ in circulating plasma caused by respiratory and "metabolic" acidosis and alkalosis; (2) critical plasma $[Ca^{2+}]$ levels associated with signs of tetany and neuromuscular blockade; (3) changes in cerebral $[Ca^{2+}]_o$ caused by hypo- and hyper-calcaemia, and the changes in cerebral $[Ca^{2+}]_o$ and pH_o caused by acute systemic acidosis and alkalosis; and (4) effects of changing $[Ca^{2+}]_o$ and pH_o levels on synaptic transmission in hippocampal formation. Our main conclusions are (1) changes of plasma $[Ca^{2+}]$ caused by "metabolic" pH changes are greater than those associated with varying CO_2 concentration; (2) acute systemic $[Ca^{2+}]$ changes are associated with small cerebral $[Ca^{2+}]_o$ changes; (3) the decreases in systemic and cerebral $[Ca^{2+}]_o$ caused by hyperventilation are too small to account for the signs and symptoms of hypocapnic tetany; (4) moderate decrease of $[Ca^{2+}]_o$ depresses and its increase enhances synaptic transmission in hippocampal formation; and (5) H^+ ions in extracellular fluid have a weak depressant effect on neuronal excitability. CO_2 is a strong depressant, which is only partly explained by the acidity of its solution. CO_2 concentration is a significant factor in controlling cerebral function.

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Les points saillants de la littérature et de nos études sur la tétanie et l'hyperventilation sont ici revues. Nos études concernent (1) les changements plasmatiques de $[Ca^{2+}]$ produit par l'acidose et l'alkalose "métaboliques"; (2) les niveaux critiques de $[Ca^{2+}]$ associés aux signes de tétanie et de blocage neuromusculaire; (3) les changements de $[Ca^{2+}]_e$ cérébral causes par l'hypo- et hyper-calcémie, ainsi que par l'acidose ou l'alkalose aigue systemique; (4) les effets des modifications de $[Ca^{2+}]_e$ et de pH_e sur la transmission synaptique dans l'hippocampe. Nos principales conclusions sont (1) les changements de $[Ca^{2+}]$ dans le plasma causes par les variations "métabolique" de pH sont plus importants que ceux associés aux modifications des niveaux de CO_2; (2) les changements aigus de $[Ca^{2+}]$ dans la grande circulation sont associés à de petites variations dans le $[Ca^{2+}]_e$ cérébral; (3) les diminutions de $[Ca^{2+}]$ dans la circulation systémique et l'interstice cérébrale produites par l'hyperventilation sont trop petites pour rendre compte des signes et symptomes de la tétanie hypocapnique; (4) une réduction moderée du $[Ca^{2+}]_e$ entraine une attenuation de la transmission synaptique dans l'hippocampe, alors qu'une élévation augmente celle-ci; (4) les ions H^+ dans le fluide extracellulaire ont un faible effet deprimant sur l'excitabilité neuronale. Toutefois le puissant effet deprimant du CO_2 ne peut être complètement expliqué par la baisse de pH entraînée par ce gaz en solution. La concentration de CO_2 est donc importante en soi dans le contrôle de la fonction cérébrale.

Introduction

One hundred years ago Ringer (1886) discovered that a small amount of calcium calms the spontaneous twitching of isolated frog muscle immersed in saline, and somewhat later Sabbatini (1901) found that direct application of citrate or oxalate increases, whereas calcium decreases, the excitability of the exposed cerebral cortex. The insight that the tetany of rickets and of hypoparathyroidism are a consequence of calcium deficiency followed rapidly (Bogen 1908; MacCallum and Voegtlin 1909; Howland and Marriott 1918). Partial muscle contractures and morbid exaggeration of reflexes were already well known to be among the prodromes of "grand" hysterical attacks (Richer 1879). Tingling and cramps were then noticed among the consequences of hyperventilation (Vernon 1909), and the connection between the hyperventilation and the similar cramps of emotionally disturbed patients was thereafter recognized (Henderson 1909). As the spasms typical of hyperventilation are similar to those seen in parathyroid deficiency, it was natural to suspect that hypocalcaemia causes both. But in fact, serum calcium levels of deliberately hyperventilating subjects increased slightly (Grant and Goldman 1920). Even earlier, Rona and Takahashi (1913) proposed that only part of the calcium in blood plasma is ionized, the remainder being bound mainly to protein and to a lesser degree to carbonate and bicarbonate. They also suggested that plasma pH regulates the ratio of ionized/bound fractions. This led to the theory that the tetany of alkalosis is caused by the increased binding of calcium, at the expense of the free fraction (György and Vollmer 1923).

Since cerebrospinal fluid (CSF) contains almost no protein, and in CSF almost all of the calcium is free, it has been thought that CSF calcium changes in proportion to plasma calcium (McLean and Hastings 1935). This notion gained importance when Foerster (1924) reported that hyperventilation can precipitate seizures in epileptic patients while Lennox et al. (1936) showed that elevated inspired CO_2 can suppress seizures. Yet when calcium levels were measured in the CSF of hyperventilating volunteers, a decrease again could not be demonstrated (McCance and Watchorn 1937). (For additional bibliography of the earlier literature, see Brown (1953) and Jesserer (1958)).

Until relatively recently there was no good method to measure "free" calcium directly. After attempts at using frog hearts as bioassay (McLean and Hastings 1935), finally Pedersen in 1970 (using Raaflaub's (1951) murexide method) succeeded in reliably determining ionized plasma calcium concentration as a function of plasma pH. Calcium-selective electrodes were introduced into biology around this time (Ross 1967), and ionized calcium was added to total serum calcium among the routine measurements in clinical diagnostic laboratories. With calcium-selective electrodes the dependence of ionized calcium on pH could be confirmed (Siggaard-Andersen et al. 1980).

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Still, the interrelations of $[Ca^{2+}]$, pH, and neuromuscular and central nervous excitability remained unresolved. First, it was not clear whether the lowering of ionized calcium level in alkalosis was sufficient to explain the tetany of hyperventilation. Second, there were reasons to believe that hydrogen ions and (or) molecular CO_2 also exert a direct influence on excitable membranes (Krnjević et al. 1965; Caspers and Speckmann 1974; Carpenter et al. 1974). The uncertain state of knowledge was reflected in the different points of view adopted in recent medical writings (compare for example p. 51 of Riley (1982), p. 544 of Andreoli (1985), and p. 236 of Levinsky (1983)).

It was against this background that we undertook the research we are about to describe. Our first goal was to determine the effect of changing blood pH on ionized calcium $[Ca^{2+}]$ in circulating arterial blood. This was necessary because measurements in vitro could not be accepted without reservation, since H^+ and other ions behave in whole blood differently from plasma and, more importantly, homeostatic regulation or passive interchange with extravascular compartments could influence the concentration of calcium in vivo. Next we determined the critical level of circulating $[Ca^{2+}]$ at which neuromuscular function is impaired while $[Ca^{2+}]$ was lowered by i.v. infusion of calcium chelators. We then determined the influence of $[Ca^{2+}]$ and pH of circulating plasma on $[Ca^{2+}]_o$ and pH_o in the interstitial fluid of the cerebral cortex. Finally we investigated the relation of cerebral synaptic function to $[Ca^{2+}]_o$, CO_2 concentration, and pH_o.

While this research program is not yet complete, some conclusions are already clear. This paper is a concise review of our findings so far. Preliminary accounts of some of our observations have appeared (Allen and Somjen 1983, 1985*a*, 1985*b*; Allen et al. 1985; Somjen and Allen 1983; Balestrino et al. 1984; Balestrino and Somjen 1987; Somjen 1985). Detailed reports are now appearing (Balestrino et al. 1986) or are in preparation.

Methods

Ionized calcium and pH were measured in arterial blood and in cerebral cortex of cats anaesthetized with chloralose and urethane. An extracorporeal arterioarterial loop, including a flow cell housing ion-selective and reference electrodes, was inserted in a common carotid artery. The amplified voltages of the ion-selective electrodes were recorded by polygraph. Details have been described by Allen and Somjen (1985*a*). CO_2 concentration in exhaled air and arterial blood pressure were continuously monitored. Arterial Po_2, Pco_2, $[Ca^{2+}]$, and pH were also measured with standard bench instruments in blood samples drawn at intervals to check flow-cell electrode performance. In some experiments the contractions of extensor digitorum longus (EDL) muscle, evoked by motor nerve stimulation, were recorded with a force displacement transducer. EMG was recorded with needle electrodes in the same and selected other muscles (Allen et al. 1985).

Interstitial ion concentrations were recorded in the brain of cats and rats with double-barreled glass capillary microelectrodes as described by Walker (1971), and Somjen (1981, 1984).

Evoked potentials and interstitial ion concentrations were recorded in fascia dentata of rats anaesthetized with urethane as described by Somjen (1984; also Somjen et al. 1985).

Hippocampal tissue slices were prepared and maintained at 35–36°C in an "interface" chamber according to standard methods (Dingledine 1984). The artificial cerebrospinal fluid (ACSF) contained 3.5 m*M* K^+, 1.2 m*M* total Ca^{2+}, and 1.2 m*M* Mg^{2+} and had a mean pH of 7.47, except when modified as noted. The bundle of Schaffer collateral fibres was stimulated, extracellular potential responses in stratum (st.) radiatum and st. pyramidale of CA1 sector were recorded and input–output curves were plotted and analyzed as described by Aitken (1985) and Balestrino et al. (1986).

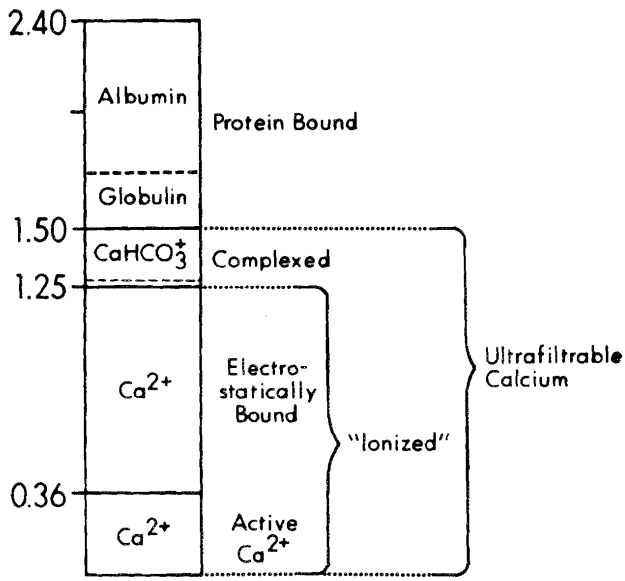

FIG. 1. Diagrammatic representation of calcium fractions in blood plasma. The values are in mmol/L. Adapted from Siggaard-Andersen et al. (1980).

Clarification of some terms

Figure 1 illustrates the various states in which calcium exists in blood plasma. The ultrafiltrable fraction is determined by the plasma protein concentration while the ionized fraction depends on proteins, certain anions, as well as pH. The activity coefficient is a function mainly of total ionic strength (cf. Siggaard-Andersen et al. 1980; Toffaletti et al. 1976). In protein-free fluid such as CSF or ACSF some calcium is weakly bound by bicarbonate, phosphate, and certain other anions. Thus in our ACSF only about 1.0 m*M* of the 1.2 m*M* dissolved calcium is ionized, as we confirmed using a standard Ca^{2+}-meter. It should be noted that, according to Toffaletti and Bowers (1979), the Ca^{2+} that is weakly bound to HCO_3^- may be available for biophysical reactions.

Even though ion-selective electrodes respond to ion activity, we report our values as free ionized concentration, because it is this value that is exactly known in calibrating solutions. This conversion is valid because total ionic strength was virtually identical in all experimental conditions, and thus the activity coefficient may be assumed to have been constant.

The terms, hypo- and hyper-calcia, refer to tissue calcium concentration, as also used in bone pathology.

Results

Ionized calcium and pH of circulating arterial plasma

These experiments were performed on anaesthetized, paralyzed, and artificially ventilated cats. Alkalosis was induced by hyperventilation (with room air or oxygen) or by i.v. infusion of NaOH; acidosis was induced by adding 5 or 10% CO_2 to inspired O_2 (with or without hyperventilation) or by infusion of HCl.

As expected, acidification of the blood raised arterial plasma $[Ca^{2+}]$ and alkalinization decreased it (Fig. 2). These changes were more marked with the infusion of strong acid or base ("metabolic" acidosis and alkalosis) than when CO_2 levels were varied ("respiratory" pH changes). Average $[Ca^{2+}]$ changed by 0.031 m*M* per 0.1 pH change ($N = 21$; coefficient correlation, $r = 0.85$) in induced respiratory states and 0.066 m*M* per 0.1 pH ($N = 10$; $r = 0.84$) in metabolic acid–base shifts, as determined from statistical regression (Allen 1983). The two regression coefficients are significantly different ($p < 10^{-6}$). A similar difference was also noted in rats by Oberleithner et al. (1982), who attributed it to bicarbonate: increasing CO_2 raises HCO_3^- in

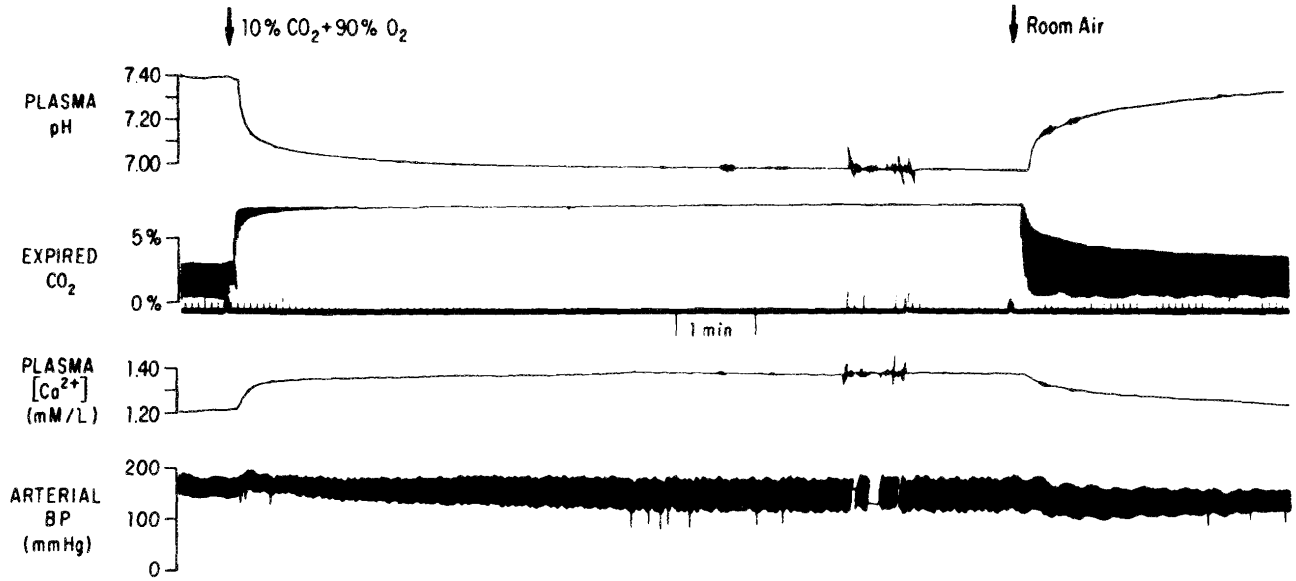

FIG. 2. Polygraph tracings of amplified output of ion-selective electrodes in flow cell, of capnograph, and of arterial pressure transducer. The anaesthetized paralyzed cat was ventilated by positive pressure either with room air or (between arrows) with 10% CO_2 and 90% O_2.

circulating blood, partially binding the Ca^{2+} that has been released from protein; lowering P_{CO_2} has the opposite effect.

The pulse pressure regularly increased during acidosis and decreased during alkalosis. This could not be attributed to the intrathoracic pressure exerted by the respirator, since it occurred even when respiratory rate and volume were kept constant as in the example of Fig. 2. It could be an effect on cardiac contractility of changing levels of $[Ca^{2+}]$, pH, or both.

We then asked whether the hypocalcaemia induced by respiratory alkalosis was sufficient to explain neuromuscular tetany of hyperventilation. In the cats of this series, maximal respiratory alkalosis of 0.35 pH above control was associated with an average lowering of plasma $[Ca^{2+}]$ by 0.11 m*M*, in the extreme case by 0.3 m*M*. This had to be compared with the results of the following series of experiments.

Hypocalcaemia and neuromuscular function

In these experiments the plasma $[Ca^{2+}]$ of anaesthetized cats was lowered by the i.v. infusion either of citrate or of EGTA, while the contractions of EDL muscle and EMG were recorded (see Methods and Allen et al. 1985).

Peripheral tetany was detected in these cats by the increase of twitch tension, elevated base-line tension, slowed relaxation, and EMG afterdischarges evoked by motor nerve stimulation (Fig. 3). Spontaneous twitching and EMG discharges occurred in the (unstimulated) chest and face muscles of some, but not all cats, at lower $[Ca^{2+}]$ levels than did evoked afterdischarges. The very first signs of peripheral tetany occurred when plasma $[Ca^{2+}]$ decreased from its control level of greater than 1.0 m*M* to below 0.6 or 0.5 m*M*: corresponding to a change of at least 0.5, more usually 0.6 m*M*.

Compound muscle action potentials evoked by stimulating the motor nerve began to be depressed at about the same level of plasma $[Ca^{2+}]$ at which the first signs of tetany appeared. Contractile force of the muscle was, however, at first actually increased, because of the summation of contractions triggered by the multiple muscle action potentials fired by those muscle fibers in which neuromuscular transmission was preserved. When plasma $[Ca^{2+}]$ fell below 0.4 or 0.3 m*M*, twitch tension rapidly decreased. During recovery, neuromuscular block showed considerable hysteresis: plasma $[Ca^{2+}]$ had to recover to a higher level for transmission to be restored than the level at which blockade first occurred (see also Allen et al. 1985).

The minimum decrease in $[Ca^{2+}]$ that caused peripheral tetany was thus clearly greater than that induced by respiratory alkalosis. We concluded that the tetany of hyperventilation is due either to the alkaline pH itself or, more probably, the synergism of low $[H^+]$ and low $[Ca^{2+}]$.

We also tried to induce tetany by hyperventilating cats that were prepared as for the hypocalcaemia experiments. Clear signs of tetany were seen in three out of eight animals, in the form of increased twitch tension, 110–230%; the others showed borderline or no increase. Plasma $[Ca^{2+}]$ never decreased below 1.1 m*M* in these experiments, which is much above the threshold for tetany in pure hypocalcaemia. The pH increased to between 7.53 and 7.70 in this series.

The dependence of cerebral $[Ca^{2+}]_o$ and pH_o on plasma $[Ca^{2+}]$ and pH

It is generally believed that ion concentrations in CSF are regulated independently from those in blood. This is based in part on measurements of CSF ion concentrations during i.v. infusions of solutions of elevated ion concentrations (Davson 1967; Kemény et al. 1961; Ames et al. 1964) and in part on estimates of fluxes in and out of CSF of radiolabelled ions (Graziani et al. 1967). In all these studies there was, however, indication that CSF ion levels do change, albeit less than those in plasma. Because of this, and because CSF concentration need not accurately reflect interstitial fluid concentration, we measured $[Ca^{2+}]_o$ in cerebral cortex with ion-selective electrodes during experimentally induced acute hypo- and hypercalcaemia.

Plasma $[Ca^{2+}]$ was lowered by infusing either citrate or EGTA and raised by infusing $CaCl_2$ in anaesthetized cats. Cerebral $[Ca^{2+}]_o$ changed in the same direction as plasma $[Ca^{2+}]$ but only slightly and slowly (Fig. 4).

When respiratory acidosis or alkalosis was induced as already described, cerebral pH changed as expected (e.g., Eldridge et al. 1984), slowly approaching, but not quite reaching, the level attained in plasma. Brain $[Ca^{2+}]_o$ decreased

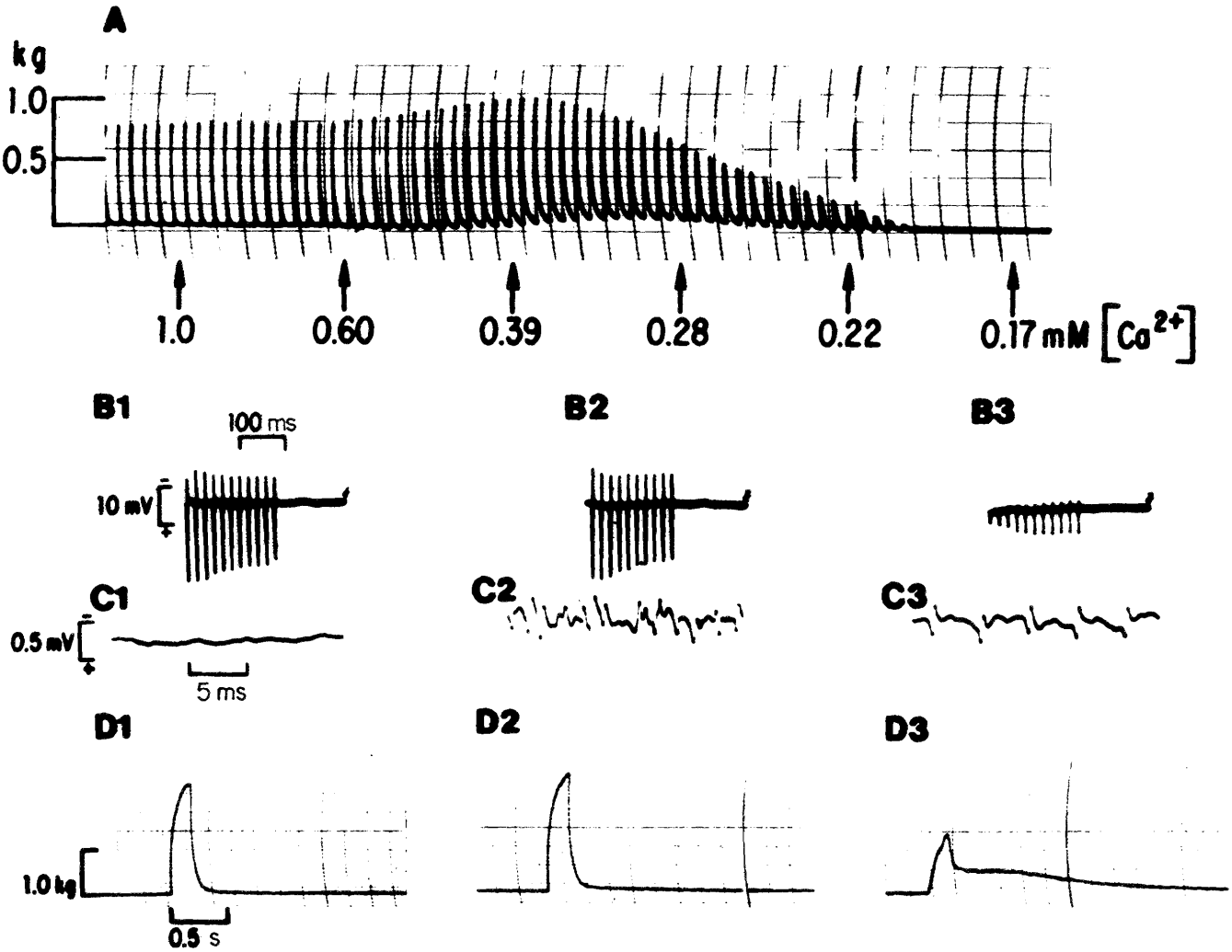

FIG. 3. The effect of hypocalcaemia on neuromuscular transmission. (A) Twitch tension evoked by single pulses to motor nerve. Numbers below tracing give plasma $[Ca^{2+}]$ in mmol/L. Note slowed relaxation, increased twitch tension, elevation of base-line tension, and eventual failure of contractions. (B–D) From another experiment. (B) Compound muscle action potentials (CMAP) evoked by a train of stimuli applied to the motor nerve. (C) EMG discharges recorded with a microelectrode in the same muscle immediately after the last CMAP: note high gain and sweep speed. (D) Contractile tension. (B1–D1) Control: $[Ca^{2+}]$ of 1.22 mmol/L; (B2–D2) 0.46 mmol/L; (B3–D3) 0.21 mmol/L.

during alkalosis and increased during acidosis. Changes in cerebral $[Ca^{2+}]_o$ induced by respiratory pH changes were modest, never amounting to more than 0.15 m*M*. Our next task was to determine how such changes affect brain function.

The effect of moderate changes of $[Ca^{2+}]_o$ on neuronal functions

It is well known that deficiency of $[Ca^{2+}]_o$ not only increases the excitability of nerve and muscle, but also lowers the output of transmitter at chemical synapses. Which of these two effects is dominant at a given level of $[Ca^{2+}]_o$ in a given system cannot be predicted from the results of experiments conducted on different tissues and at different calcium concentrations.

We decided to use isolated hippocampal tissue slices to test the effect of moderate changes of $[Ca^{2+}]_o$ in mammalian brains, because of the ready control of the milieu of this preparation. We either lowered the (total) calcium concentration in the bathing fluid from the control level of 1.2 to 0.8 m*M*, or we raised it to 1.8 m*M*.

Lowering $[Ca^{2+}]_o$ depressed focally recorded synaptic potentials (fEPSP) evoked by a given magnitude of afferent input (presynaptic volley) by about 38%. Raising $[Ca^{2+}]_o$ depressed the fEPSP by about the same amount. This figure is somewhat less than, but similar to that found earlier using a slightly different experimental protocol (Dingledine and Somjen 1981). Changes of neuronal excitability were estimated from the relation between orthodromic population spike amplitude and fEPSP. This changed surprisingly little. As a result, overall synaptic transmission (that is, the relation of population spike to presynaptic volley) changed in the same sense as the fEPSP: in hypocalcia the curve shifted to the right, in hypercalcia to the left (Fig. 5). This corresponded to an increase of the population spike amplitude evoked by a given presynaptic volley by an average of 36% in high and to a decrease by 30% in low $[Ca^{2+}]_o$ (see also Balestrino et al. 1986).

We saw no increased excitability of hippocampal neurons at this degree of hypocalcia. In these experiments the decrease of $[Ca^{2+}]_o$ was almost double the decrease of cerebral $[Ca^{2+}]_o$ measured during respiratory alkalosis in cats. It appears therefore that hypocalcia in itself does not explain the lowered seizure threshold of hyperventilating subjects. Thus our attention turned to changes of pH and of CO_2 itself.

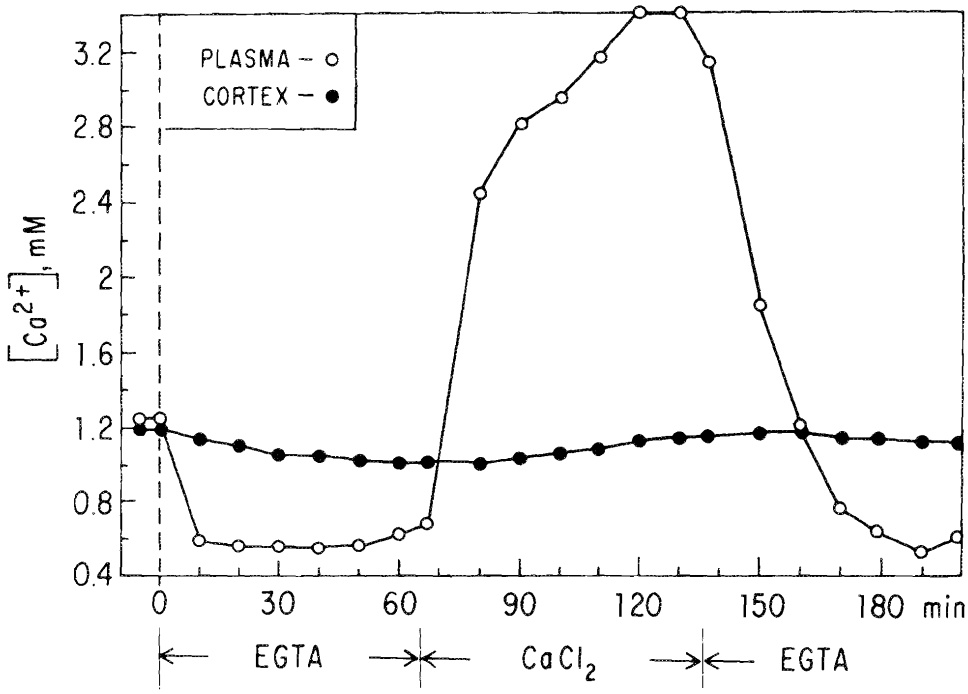

FIG. 4. Ionized calcium concentration in plasma and in cortical interstitial fluid during acutely induced hypo- and hyper-calcaemia of an anaesthetized cat.

Effects of pH and CO_2 concentration on neuronal excitability

In one series of experiments evoked potentials were recorded together with the pH of the interstitial fluid from hippocampal formation of anaesthetized rats, using ion-selective microelectrodes. Administering elevated CO_2 in inspired gas depressed the population spike of dentate granule cells evoked by perforant path stimulation; hyperventilating them with oxygen enhanced the response (Fig. 6). The amplitude of the population spike varied in direct proportion with pH. The effect was variable but usually quite strong. In 10 rats (15 "runs") the population spike changed on the average by more than 40% for each 0.1 pH_o change. Not surprisingly, paroxysmal afterdischarges (cf. Somjen et al. 1985) were more readily provoked in alkalotic than in acidotic brains.

In some rats $[Ca^{2+}]_o$, $[K^+]_o$, or Po_2 was measured in the same layer of fascia dentata with separate microelectrodes. Changes of $[Ca^{2+}]_o$ were even smaller than those found in cat cortex with similar changes of pH_o. $[K^+]_o$ increased slightly during respiratory acidosis and decreased during alkalosis. Since elevated $[K^+]_o$ increases neuronal excitability (Balestrino et al. 1986), it could not explain the depression actually observed during acidosis. Po_2 decreased during hyperventilation and increased during CO_2 administration. But neuronal excitability increased about the same degree during hyperventilation with room air as with oxygen, even though cerebral Po_2 was much higher in the latter case. In any event, Po_2 was kept constant in the next series of experiments, conducted on isolated hippocampal slices.

In hippocampal tissue slices the pH of the bathing fluid was changed either by varying the Pco_2 in both gas and aqueous phase in the slice chamber, or by adding HCl or NaOH to the ACSF. Acidification depressed and alkalinization enhanced the population spike evoked in CA1 pyramidal cells by a given input volley through the Schaffer collateral bundle. Unlike the effect of $[Ca^{2+}]_o$, the change in this case was mainly in the excitability of the postsynaptic neurons. This became apparent from the right shift of the curve relating population spike to fEPSP in

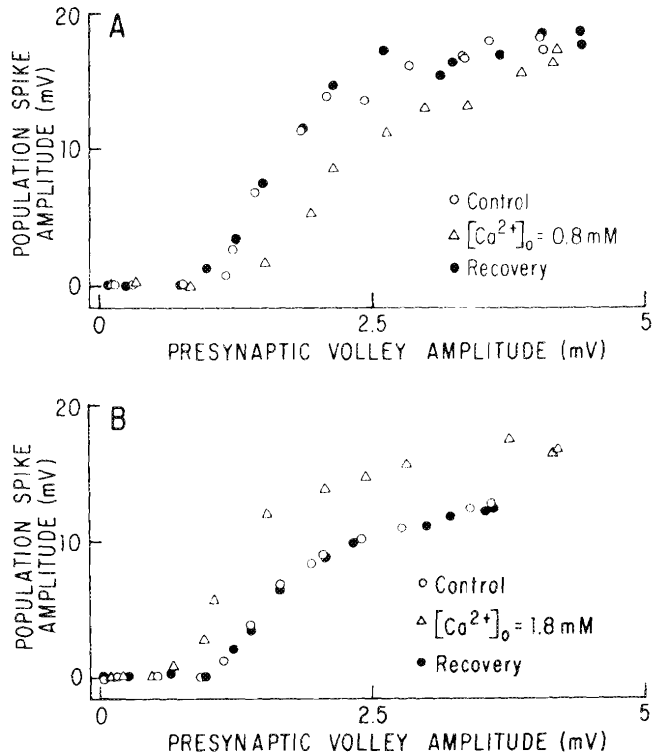

FIG. 5. Input–output curves from recordings in CA1 sector of two hippocampal tissue slices. In A the calcium concentration in the bathing solution was lowered, in B it was raised from its control level of 1.2 m*M*.

acid, and its left shift in alkaline solutions. There was little or no change in the relation of fEPSP to presynaptic volley.

The effect of changing CO_2 concentration was about twice that of adding strong acid or base: respectively, 32% (SD ± 19%, $N = 24$) and 15% (SD ± 19%, $N = 10$) average change of population spike amplitude for 0.1 pH change, determined by

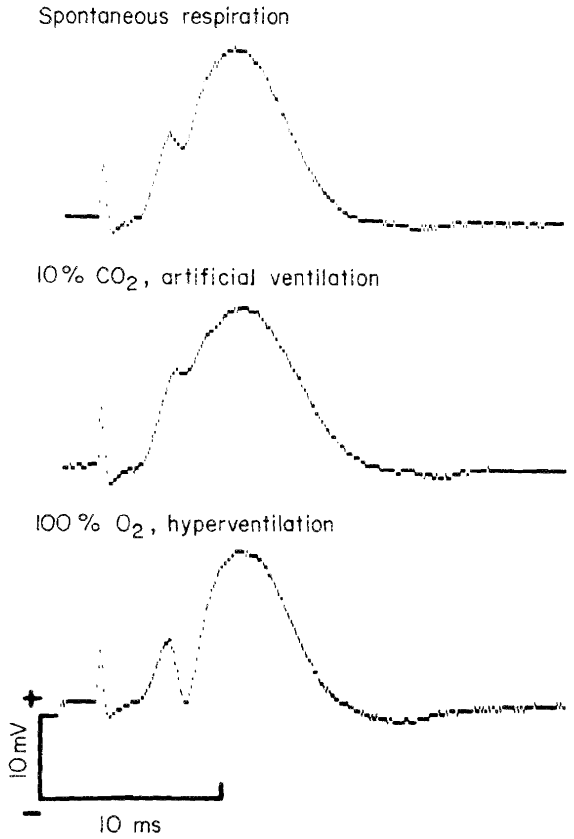

FIG. 6. The effects of hyper- and hypo-capnia on the potentials evoked in fascia dentata of an anaesthetized rat by stimulation of the perforant path (angular bundle). During control recording the rat breathed room air. Note marked changes of the population spike, (which is the negative notch on the rising phase of the fEPSP wave), and almost no change of the (positive) fEPSP.

regression analysis. The slope of the two regressions differed significantly ($p < 0.002$). We concluded that hydrogen ions in the extracellular fluid weakly depress neuronal excitability and that CO_2 enhances this effect. CO_2 could act by acidifying the neuronal cytoplasm or directly on the neuronal membrane (see Discussion).

Discussion and conclusions

From these observations certain facts stand out.

(1) Changes of ionized calcium concentration in the plasma of circulating blood induced by "metabolic" acidosis and alkalosis are greater than those induced by respiratory acidosis and alkalosis.

(2) The decrease of $[Ca^{2+}]$ caused by hyperventilation-induced hypocapnia is too small to account by itself for the neuromuscular tetany typical of this condition.

(3) Acute hypo- and hyper-calcaemia influence slightly cerebral $[Ca^{2+}]_o$.

(4) Moderate decrease of $[Ca^{2+}]_o$ depresses synaptic transmission in hippocampal formation. Increased irritability of central neurones was not seen at $[Ca^{2+}]_o$ levels that could be expected in hyperventilating subjects. Moderately increased $[Ca^{2+}]_o$ enhanced synaptic transmission.

(5) Hydrogen ions in the extracellular fluid have a weak depressant effect on neuronal excitability.

(6) CO_2 is a strong depressant of the excitability of neurones in the hippocampal formation.

One important limitation of our work is that it speaks only to acute changes of pH, P_{CO_2}, and of $[Ca^{2+}]$. From it we could not predict the effects of hypo- or hyper-calcaemia lasting more than an hour or so, nor that of prolonged acidosis and alkalosis.

From our data it does seem clear that the hyperirritability of the central and the peripheral nervous system of acutely hyperventilating subjects is caused by the hypocapnia and not the concomitant decrease of cerebral blood flow. Since CO_2 had comparable effects on neuronal excitability *in vitro* and *in situ*, changes in blood flow could not have been a major factor. This does not mean that all signs and symptoms of respiratory alkalosis are the direct consequence of low CO_2 concentration: the feeling of faintness and dizziness often reported by hyperventilating subjects may well be related to cerebral vasoconstriction.

It furthermore seems that lowered calcium contributes little or nothing to either the central or the peripheral hyperirritability associated with short-term hyperventilation. Not only are the changes of ionized calcium concentration too small, they are of the wrong kind: when $[Ca^{2+}]_o$ is just slightly lowered, synaptic transmission is depressed instead of enhanced and the threshold of firing is not significantly affected (Balestrino et al. 1986). Hypocalcaemic tetany occurs only at calcium levels that are substantially lower than those reached with hyperventilation.

The situation may be different with "metabolic" alkalosis. Our data are insuffficent to permit definitive conclusions, but the following must be borne in mind. In alkalosis induced by infusion of NaOH the reduction of plasma $[Ca^{2+}]$ was more pronounced than during equivalent respiratory alkalosis. Moreover, alkalosis induced with strong base (while $[Ca^{2+}]$ in the perfusion fluid remained constant) caused less hyperexcitability of neurones in hippocampal tissue slices than did the lowering of CO_2. Furthermore, it is well known that CO_2 levels tend to be increased rather than decreased in metabolic alkalosis of extended duration. Thus, *if* tetany is seen in metabolic alkalosis, then low Ca^{2+} may be at least a contributing factor.

The next question to be examined is, why, for a given level of acidification, is CO_2 more strongly depressant than is HCl? It has long been held that raising CO_2 would acidify the cytoplasm of cells, while adding strong acid to the extracellular fluid would not (e.g., Waddell and Bates 1969). However, more recent direct measurements with intracellular H^+-selective microelectrodes revealed that, at least in cold-blooded animals, if P_{CO_2} is raised intracellular pH becomes transiently acid but then it recovers partly or wholly in the face of sustained hypercapnia (Thomas 1976; DeWeer 1978; Moody 1984). The ability to regulate $[H]_i$ when exposed to excess CO_2 depends on the availability of HCO_3^- (e.g., Schlue and Deitmer 1987). Balestrino, LaManna, and Sick (unpublished) are reexamining the problem, using the intracellular indicator dye neutral red to measure pH of the cells in hippocampal tissue slices. Preliminary results showed that, in the bicarbonate-buffered perfusion fluid and within the range of pH used in the preceding series of experiments, neither changing CO_2 nor the addition of HCl and NaOH could significantly influence intracellular pH. If further experiments now in progress confirm this observation, then the depressant effect of CO_2 cannot be attributed to intracellular acidification.

If so, then CO_2 itself is a depressant gas. This direct effect of CO_2 appears to be additive to that of extracellular H^+ ions. When the concentration of CO_2 rises, synergism of the two effects results in a strikingly powerful depression of neuronal excitability. Therefore, by regulating breathing, and through breathing P_{CO_2}, the brain is in control of its own excitability.

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