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CORTICAL CO_2 TENSION AND NEURONAL EXCITABILITY

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It has been known for a long time that hypercapnia has rather striking effects on the human central nervous system (Brown, 1930; Sieker & Hickam, 1956). Although a variable amount of excitation may be seen, the predominant effect is loss of consciousness. According to Brown (1930), most subjects cannot tolerate more than 10–12 % of CO_2 for longer than 2 min.

The mechanism and site of action of CO_2 have never been very clear. From early studies on the electrocorticogram of the 'cerveau isolé' (Bremer & Thomas, 1936), it seemed that the cortex was affected directly by CO_2, low doses exciting and large doses depressing the neurones. Although later publications in Russia (cf. Ivanov, 1962) have agreed with this interpretation, some other authors (Gellhorn & French, 1953; Gellhorn, 1954; Dell & Bonvallet, 1954) have claimed that the brain stem is particularly sensitive to CO_2 and that cortical arousal is only secondary to activation of the mid-brain and hypothalamus. The sole direct action observed was a strong depression of the isolated cortex by large amounts of CO_2 (Gellhorn & French, 1953).

A recently developed method for the continuous recording of the cortical CO_2 tension (P_{CO_2}) (Siesjö, 1961) has been combined in the present experiments with a technique for testing directly changes in the excitability of single neurones. It will be shown that even in the isolated cortex, many cells are affected by a small increase in P_{CO_2}, and that the resulting changes in excitability are rather complex, with evidence of both excitation and depression.

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METHODS

Thirteen cats and one monkey have been studied. They were anaesthetized mostly with a mixture of allobarbitone and urethane (Dial compound, Ciba Ltd), supplemented whenever necessary with pentobarbitone (Nembutal, Abbots Ltd). In one experiment, chloralose (Hopkins & Williams Ltd, 80 mg/kg) was used, and in another the brain stem was cut through the superior corpora quadrigemina while the cat was under ether anaesthesia, the ether being then withdrawn to allow recording from the unanaesthetized 'cerveau isolé'.

Exposure of brain. The skull was usually opened widely over one hemisphere but after resecting the dura, the cortical surface was protected from drying and cooling by covering with pieces of thin polythene sheet, except for the small areas through which neuronal potentials and P_{CO_2} were to be recorded. In addition, a slow drip of warm physiological solution was kept flowing over the region of electrical recording; the latter was otherwise covered by a pressor, consisting of a Perspex disk with a central hole through which micropipettes were inserted. Enough pressure was applied to reduce local pulsations without impeding the pial blood supply, clearly visible through the transparent pressor.

Undercutting the grey matter. In several experiments indirect actions of CO_2 were reduced or eliminated by undercutting a given portion of cortex. A narrow knife was inserted at 2 points in an adjacent area and a wire, bent at a right-angle, was passed under the cortex through each hole and rotated several times to sever all deep connexions. In some cases, the procedure was limited to cut only the most direct connexions while in others the tip of the undercutting wire was worked carefully all around a certain area right up to the surface, but taking care to avoid damaging the superficial blood supply. The resulting lesion therefore varied from substantial undercutting to practically complete isolation, as checked by subsequent examination.

The undercut area was usually in the anterior half of the suprasylvian gyrus, where it occupied the full width of the gyrus for a length not less than 1 cm. But for intracellular recording the post-cruciate region was more convenient, and the lateral portion of the posterior sigmoid gyrus was undercut instead.

Electrical recording and tests of neuronal excitability. Unit spikes were recorded extracellularly with multibarrelled micropipettes, as described by Krnjević & Phillis (1963*a*). One barrel was filled with 2·7 M-NaCl and acted as a recording channel; another barrel contained L-glutamate or ACh, which could be released from the common tip by iontophoresis. Nearly all cortical units are readily excited by L-glutamate (Krnjević & Phillis, 1963*a*) and many of the deeper cortical units are also sensitive to ACh (Krnjević & Phillis, 1963*b*). These substances (L-glutamate in particular) were therefore used to test neuronal excitability, by initiating a more or less steady level of discharge with a slow iontophoretic release maintained for several minutes (e.g. Fig. 2) or by short applications of a fixed amount of excitant, giving standard responses at regular intervals (Fig. 4). The short applications lasted 5–10 sec; if conditions were particularly favourable, very short pulses of current could be used, having a duration of only some 10 msec.

After suitable amplification the electrical responses were displayed on an oscilloscope and either photographed (Fig. 8) or led into an EKCO ratemeter, giving a D.C. output proportional to pulse frequency for recording on paper (Fig. 1).

Intracellular recording. Cortical cellular resting potentials were measured in the usual manner with fine glass micropipettes containing 3 M-KCl. To ease the insertion, the pial layer was removed with fine forceps. Tissue movements were reduced as much as possible by various methods: cisternal drainage (after enlargement of the foramen magnum); bilateral pneumothorax with artificial respiration; suspension of the body by clamps on the spine, to relieve pressure on the viscera; and the application of a pressor on the region of cortical recording, as described above. Strong respiratory movements during hypercapnia caused much instability; in a few experiments this was prevented by administering tubocurarine (Roche Ltd).

Spinal cord studies. The lumbo-sacral spinal cord was exposed in two cats by dorsal laminectomy and then covered by a pool of warm liquid paraffin. Dorsal and ventral spinal roots were cut and placed on platinum electrodes; antidromic and orthodromic potentials were recorded from motoneurones and other cells with micropipettes.

Administration of CO_2. CO_2 was obtained from The British Oxygen Company, Ltd, either as a pure gas or in various mixtures with air (2–20 % of CO_2). Pure CO_2 was mixed with known volumes of air in a continuous flow by using an anaesthetics machine. The CO_2 concentrations of several mixtures were checked with a Lloyd–Haldane apparatus. The gases were moistened by passing over water in a 2 l. flask and then led into the tracheal cannula.

Measurement of the mean cortical P_{CO_2}. P_{CO_2} on the surface of the exposed cerebral cortex was measured with a 'tissue P_{CO_2}' electrode (Siesjö, 1961). It has been shown (Gleichmann, Ingvar, Lübbers, Siesjö & Thews, 1962) that such an electrode measures a tension which is very close to the mean tissue P_{CO_2}, as estimated by integration along the tissue capillary and over the cross-section of the tissue cylinder in Krogh's model (1919). A 'flat' membrane glass electrode and a calomel reference electrode were incorporated in this instrument, and it had a water-jacket for temperature control (Siesjö, 1964). Since the present work was mainly concerned with changes in P_{CO_2} rather than absolute values, water at 37° C was circulated through the water jacket of the electrode in all experiments, taking no account of any variations in cortical temperature. The electrode was calibrated at 37° C with gas mixtures of known CO_2 content, which were preheated and saturated with water vapour in the water-bath.

The electrode was supplied by Eschweiler and Co., Kiel, West Germany; it was assembled with a solution containing 0·001 M-$NaHCO_3$ and 0·025 M-KCl and with a 6 μ Teflon membrane. The response time to 100 % equilibrium was about 1 min for a 20 mm Hg change in P_{CO_2} and 60 % equilibrium was reached in about 15 sec. Its stability was such that any drift was usually less than 3 mV in 10 hr. Thus there was no necessity to recalibrate the electrode at intervals shorter than 4–5 hr. Previous studies have shown that similar values of P_{CO_2} are recorded over different areas of the cortex (Siesjö, 1961). Therefore, our values of P_{CO_2}, though measured some distance away, probably give an adequate indication of the tissue P_{CO_2} at the point where micropipettes were inserted.

Since the cortical surface was exposed through a fairly wide craniotomy, the electrode could be rigidly clamped in a position where it touched the surface of the brain without any risk of exerting undue pressure. The construction of the electrode (Siesjö, 1964) made it easy to apply in such a way that CO_2 was not lost by diffusion from the surface. The electrode was connected to an electrometer ('Vibron' E.I.L.) whose output was led to a pen recorder.

RESULTS

Changes in cortical P_{CO_2}

When a given concentration of CO_2 was administered, there was always a certain delay before the onset of any recorded changes in cortical P_{CO_2}, due partly to the respiratory dead space and the circulation time, and partly to the properties of the P_{CO_2} electrode.

An abrupt change of the CO_2 concentration in the inspired air leads to a gradual increase in the mean tissue P_{CO_2} until it reaches a new plateau. Such an equilibrium is usually obtained in 5–10 min but it reaches its half-value within about 1 min (Siesjö, 1964). The new level is then usually upheld for prolonged periods (30–40 min) with only very small variations. At the end of the administration, the marked increase in ventilation

caused by CO_2 leads to a rapid return of the P_{CO_2} to its original value, which is often reached in 1 min. In some of the present preparations, notably in the 'cerveau isolé', the ventilatory response seemed to be out of phase with the P_{CO_2} increase, sometimes leading to a small further increase (Fig. 1), sometimes to a secondary decrease (Fig. 2) in P_{CO_2}, after the initial peak. The 'cerveau isolé' preparation also showed delayed oscillations in P_{CO_2} when the P_{CO_2} returned to the base line (Figs. 1, 2). These oscillations, which are not infrequently seen in animals with intact brain stems, were less marked when giving higher CO_2 concentrations (Fig. 3).

In order to allow a quicker repetition of the experiment, CO_2 was usually administered for only a short period, so there was no time for the P_{CO_2} to reach full equilibrium. In some cases, as in the experiments illustrated in Figs. 1–3, the CO_2 was given long enough to observe most of the full time course. In other cases, and in order to study the excitability changes during a fall in P_{CO_2}, CO_2 was administered for longer periods before it was suddenly discontinued.

When the animals were both anaesthetized and given curare to prevent excessive movements, and were therefore ventilated artificially, the P_{CO_2} changed more slowly. The difference is shown specially clearly by the rate of fall after stopping the administration of CO_2. Thus in Fig. 11, where 4% CO_2 was given for nearly 2 min (and the increase in P_{CO_2} was comparable to that seen in Figs. 1 and 2) the P_{CO_2} did not return to the initial level for about 5 min after the end of the administration. Changes in P_{CO_2} during CO_2 administration and hyperventilation were often speeded up by increasing the artificial respiration. The P_{CO_2} level in the paralysed animals was, of course, dependent upon the setting of the respirator. This was adjusted to give the same cortical P_{CO_2} as in the previous period of spontaneous ventilation.

Changes in neuronal excitability

The responses illustrated in Figs. 1–3 were observed in an undercut portion of the cortex of a 'cerveau isolé'. The upper traces (as in all other similar figures) indicate the P_{CO_2} level; since the voltage output of the P_{CO_2} electrode is recorded, the ordinate is not linear, being proportional to log P_{CO_2}. The lower traces in Figs. 1–3 show the frequency of firing of a particular neurone, whose spikes have been amplified and led into a ratemeter.

In Fig. 1 the unit was responding fairly regularly to a constant iontophoretic release of L-glutamate from the micropipette (by an inward current of 6 nA). Between the two arrows, 3% CO_2 was administered causing the observed change in P_{CO_2}. There was a transient phase of sharp

excitation while P_{CO_2} was rising rapidly, followed by a short-lasting depression. When P_{CO_2} fell, there was some indication of a small transient increase in excitability.

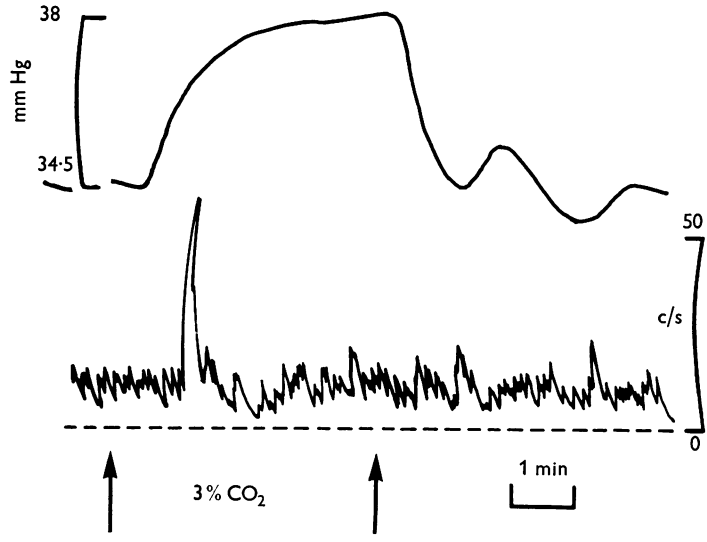

Fig. 1. Upper trace: cortical P_{CO_2}. Lower trace: rate of discharge of neurone in anterior suprasylvian cortex isolated by undercutting; neurone was excited throughout by steady release of L-glutamate (6 nA) from micropipette. Between arrows, cat inspired 3% CO_2 in air. Unanaesthetized 'cerveau isolé'.

In a second experimental run with the same unit (Fig. 2), the same concentration of inspired CO_2 (3%) was used but there was a faster initial rate of discharge owing to a greater release of glutamate (16 nA instead of 6 nA). The effects produced by CO_2 were comparable to those in Fig. 1, but the initial increase in excitability was relatively small, since the firing rate at the peak was about the same as in Fig. 1; moreover, the response occurred somewhat earlier (the apparent lag in the P_{CO_2} trace was due partly to the curvilinear properties of the penwriter system and partly to the response time of the P_{CO_2} electrode). As might be expected the excitatory effect was less evident against a background of strong excitation.

When the cat was given a higher concentration of CO_2 (10%), the main effect was a strong depression of the glutamate-induced firing (Fig. 3). There may have been a transient small phase of excitation at the start, and there was some rebound of excitation after the return of the P_{CO_2} to its initial level, but neither effect was comparable in magnitude to the main depression.

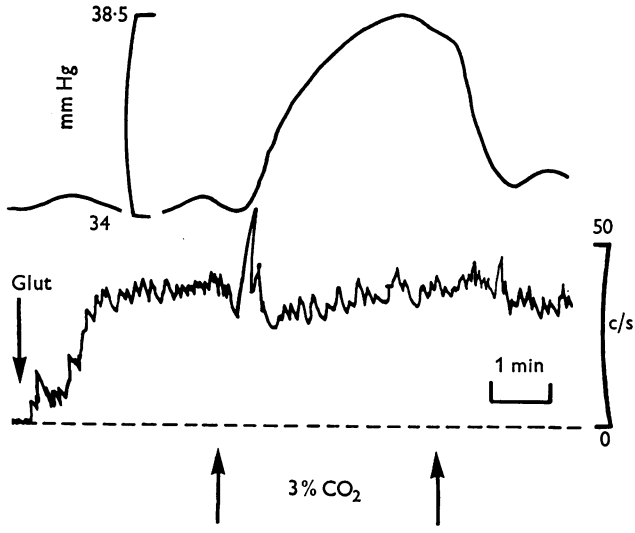

Fig. 2. Upper trace: cortical P_{CO_2}; Lower trace: frequency of discharge of neurone already illustrated in Fig. 1. First arrow indicates start of L-glutamate release, at faster rate (16 nA) than in Fig. 1. Between two other arrows, cat inspired 3 % CO_2.

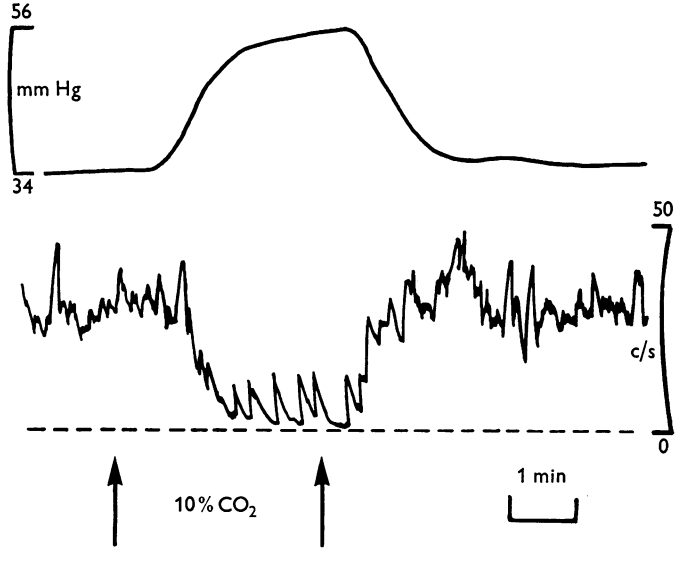

Fig. 3. Upper trace: cortical P_{CO_2}. Lower trace: frequency of discharge of neurone already illustrated in Figs. 1 and 2, during steady release of L-glutamate (16 nA). Between two arrows, cat inspired 10 % CO_2.

The changes in neuronal excitability were also tested with short applications of glutamate. This is shown in Fig. 4. Here we have a cortical unit whose responses to identical 5 sec applications of glutamate, repeated every 30 sec, were recorded with the rate-meter (lower trace). The cat had received two injections of a carbonic anhydrase inhibitor, acetazolamide (Diamox sodium, Cyanamid Ltd, 40 mg/hr I.V.), ½ and 1 hr previously. As a result there was a progressive increase in cortical P_{CO_2} to the relatively high basal level seen in Fig. 4. Carbon dioxide (7 %) caused the cortical P_{CO_2} to rise further, as shown by the upper trace. This led to a temporary increase in the evoked firing during the initial period of rapid increase in P_{CO_2}, followed by a prolonged depression, with a short phase of hyperexcitability again seen during the fall of P_{CO_2}. There was no evidence that acetazolamide alters radically the effects of CO_2 on neurones.

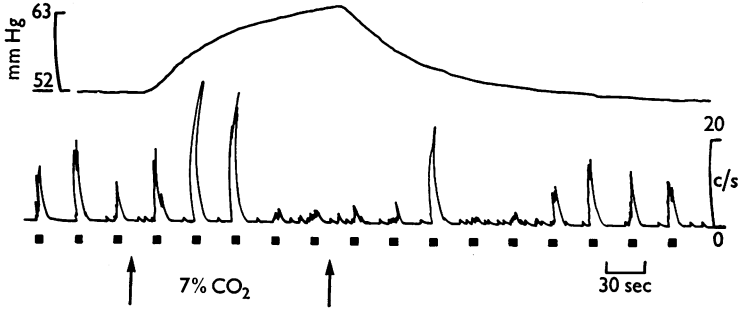

Fig. 4. Upper trace: cortical P_{CO_2}. Lower trace: neuronal discharge elicited by 5 sec applications of L-glutamate (60 nA), indicated by black squares; neurone was in the undercut mid-suprasylvian cortex of a cat under Dial. Between arrows, 7 % CO_2 was administered. The animal had received earlier two injections of acetazolamide (40 mg/kg I.V.).

In some cases the late excitation was much more evident than early excitation. This is shown rather clearly by the unit illustrated in Figs. 5–7. In Fig. 5, 2 % CO_2 caused a slight increase in the firing evoked by a steady application of glutamate, and there was a further transient acceleration as soon as P_{CO_2} began to fall. With 7 % CO_2 (Fig. 6) firing was depressed but there was again a pronounced excitation after the end of CO_2, though somewhat later than in Fig. 5. Figure 7 shows that 15 % CO_2 had a particularly strong depressant action on the same unit and this persisted for several more minutes after the period shown in Fig. 7; there was a corresponding potentiation of the late phase of excitation which now occurred even later during the phase of falling P_{CO_2}.

The traces in Figs. 1–7 are representative of the variety of effects observed, showing how the emphasis varied between depression and early and late excitation, depending partly upon the amount of change in P_{CO_2}.

Although L-glutamate was used in most cases to test excitability, similar observations were also made on cortical cholinoceptive cells excited with iontophoretic applications of ACh (Krnjević & Phillis, 1963*b*).

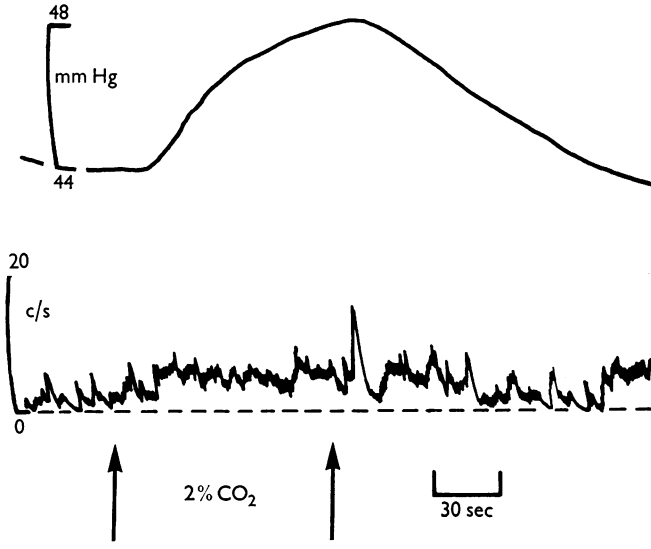

Fig. 5. Upper trace: cortical P_{CO_2}. Lower trace: frequency of firing of neurone in mid-suprasylvian gyrus of cat under Dial, during steady release of L-glutamate (24 nA); 2% CO_2 was given between arrows.

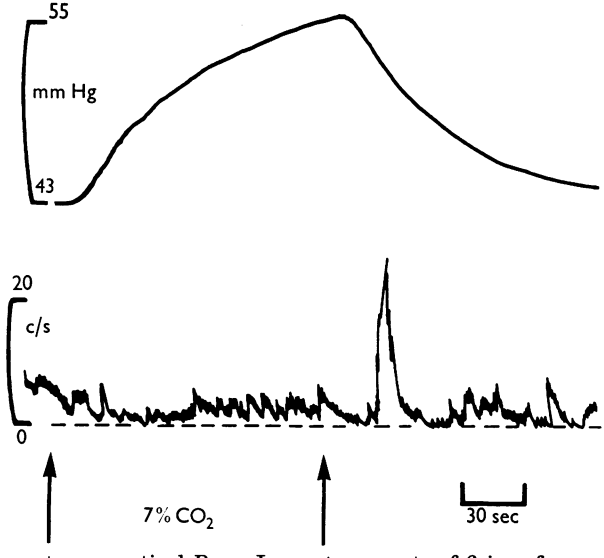

Fig. 6. Upper trace: cortical P_{CO_2}. Lower trace: rate of firing of neurone already illustrated in Fig. 5 during continued steady release of L-glutamate (24 nA). 7% CO_2 was given between arrows.

In most experiments the range of concentrations of inspired CO_2 was 2–20 %; but clear effects were seen even with 1 % CO_2 on the few occasions when this concentration was tried. For instance, in the monkey, where depressant effects were particularly marked, it was possible to stop a brisk discharge with as little as 1 % CO_2, which caused the cortical P_{CO_2} to change by only 1 mm, from 36 to 37 mm Hg.

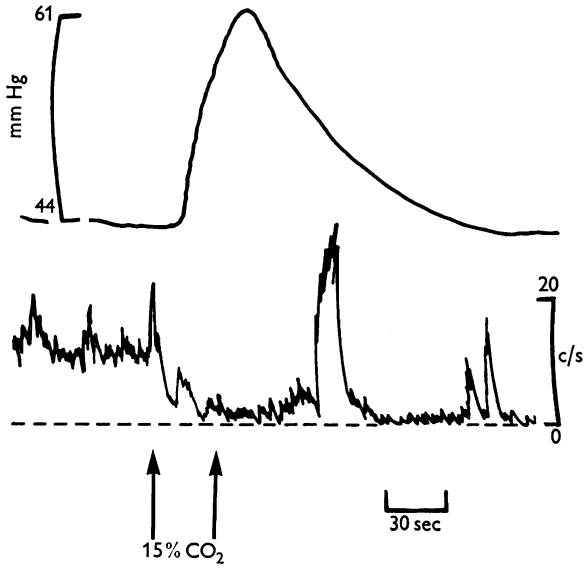

Fig. 7. Traces as in Figs. 5 and 6, showing effect of 15 % CO_2 on same neurone.

In three experiments the effect produced by a fall in P_{CO_2} was also examined, either by artificial hyperventilation, or by stopping a prolonged administration of CO_2, after the cortical P_{CO_2} had remained stable at a raised level for some 10 min. Most commonly, there was a sharp temporary reduction in excitability, which later returned towards its initial level either spontaneously, or when the P_{CO_2} was again raised by inspired CO_2 or by changing the rate of ventilation (Fig. 8).

The changes in excitability produced by hypercapnia were often associated with a marked alteration in the size of unit spikes. This might have been partly due to small movements of tissue at the tip of the micropipette, resulting from changes in blood flow or respiratory movements, but as the spikes in most cases became smaller, hypercapnia may have had a direct depressant action on the spike mechanism comparable with that described by Arvanitaki & Chalazonitis (1954).

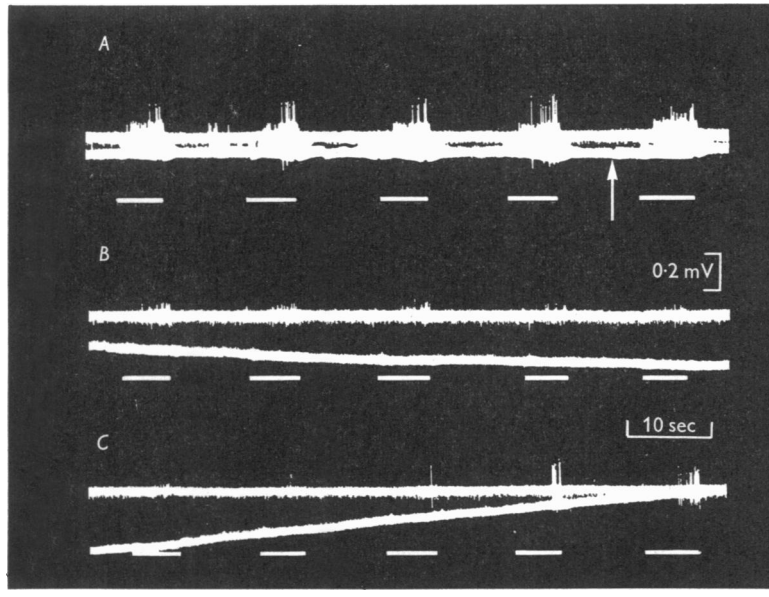

Fig. 8. Change in excitability produced by hyperventilation. In each pair of traces, upper one shows discharge of 2 units caused by 5 sec application of L-glutamate (20 nA) as indicated by short horizontal lines; lower trace records cortical P_{CO_2}. At arrow in *A*, rate of artificial ventilation was doubled and P_{CO_2} fell (*B*), from initial level of 33 mm Hg to a minimum of 25 mm Hg (there was a gap of 45 sec between end of *A* and start of *B*). Four minutes after end of *B*, animal was given 6% CO_2 causing P_{CO_2} to rise again as shown in *C*. Units were in undercut post-central cortex of Rhesus monkey under Dial.

Changes in spontaneous activity and in responses evoked from the periphery

Most of the preparations in which the cortex was undercut had no spontaneous activity; but many cells were discharging spontaneously in areas which had not been undercut. They were affected in a similar way by changes in P_{CO_2}, showing a mixture of excitatory and depressant effects.

Responses evoked by peripheral stimulation were also studied in a cat anaesthetized with chloralose. Electrical pulses were applied to the paws through needle electrodes, and the responses recorded in the primary somatosensory area. As usual, two kinds of evoked potentials were seen: sharply localized unit spikes, and slow waves distributed much more diffusely. Changes in P_{CO_2} had a different action on these two types of responses. Low concentrations of P_{CO_2} (2–7%) tended to reduce the slow waves and to abolish the spikes; but high concentrations (> 10%), which also blocked the spikes, potentiated the slow waves, sometimes to an enormous extent, as in teh example shown in Fig. 9. With intermediate

concentrations of CO_2 a biphasic effect was also observed in some cases; an initial depression, followed by an increase in amplitude.

Such a striking dissociation between spike and slow-wave responses, giving a large increase in slow waves at a time when spikes are completely abolished, was no longer seen when the chloralosed animal was given some pentobarbitone. As Fig. 10 shows, increasing concentrations of CO_2 now had the usual blocking action on the spike response, but there was no tendency towards an increase in the slow wave.

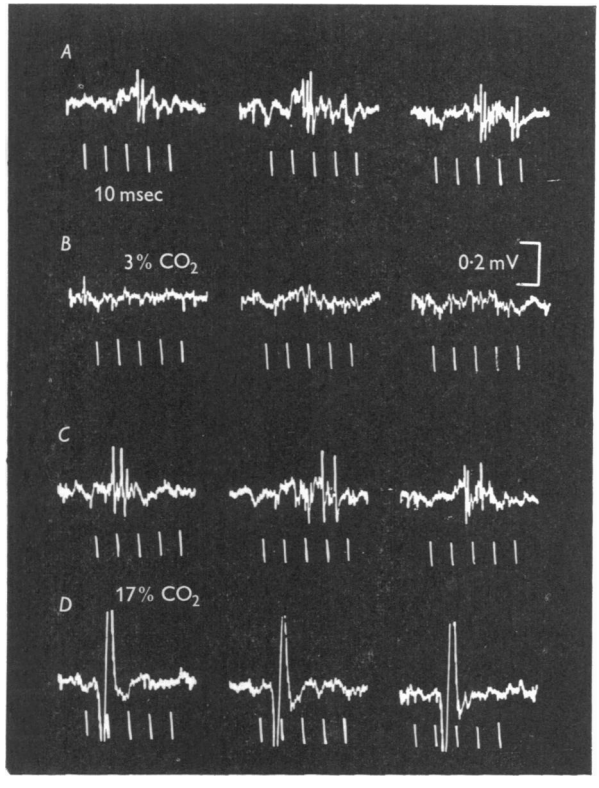

Fig. 9. Spike and slow-wave responses in somatosensory cortex of cat under chloralose during single shock electrical stimulation of ipsilateral forepaw. *A*, 3 controls; *B*, during administration of 3% CO_2; *C*, after end of 3% CO_2; *D*, during administration of 17% CO_2. Time course of slow waves was much altered by short time constant of recording.

Changes in membrane resting potentials

In four experiments, resting potentials were recorded inside cells in the post-cruciate area. Many potentials were detected, but very few remained stable enough for prolonged observation. Six cells showed consistent effects similar or comparable with those in Fig. 11. Hypercapnia was

associated with an increase in resting potential and the period of falling P_{CO_2} with depolarization. One can see from Fig. 11 that the resting potential did not bear a simple relation to the P_{CO_2}: as soon as P_{CO_2} began to drop the potential fell very rapidly, reaching a minimum long before the P_{CO_2} approached its initial level. There was a gradual repolarization, and sometimes even an overshoot of potential, while the P_{CO_2} was still falling.

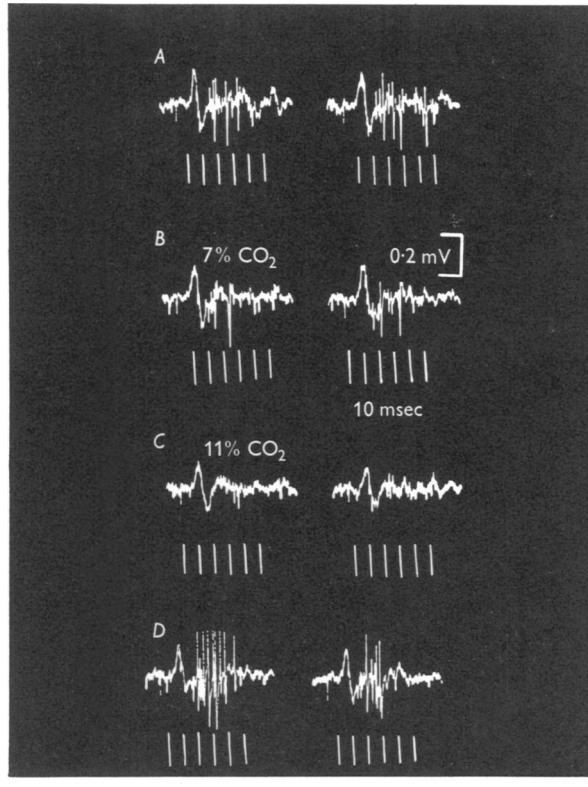

Fig. 10. Spike and slow-wave responses evoked by stimulating contralateral forepaw, in same cat, but after an intravenous dose of pentobarbitone. *A*, 2 controls; *B*, while giving 7 % CO_2; *C*, while giving 11 % CO_2; *D*, recording after end of CO_2 administration.

Although the changes in resting potential were small, varying between 1 and 5 mV, the effect was quite consistent and could be repeated several times. The amount of change bore some relation to the increase in P_{CO_2}; thus, in Fig. 11, 20 % CO_2 had a substantially greater effect than 4 % CO_2.

The effect of a maintained increase in P_{CO_2} was also tested on the same cell, as shown in Fig. 12*A*. The resting potential remained at a higher level not only for the same period but also after the P_{CO_2} had been allowed to

fall to the basal level. It returned to its initial value only after a further administration of concentrated CO_2 (30 %).

Spinal neurones

Hypercapnia appeared to have relatively little effect in the spinal cord. For instance, 10 % CO_2 given for 10 min failed to alter significantly the submaximal response of a Renshaw cell to ventral root stimulation, and

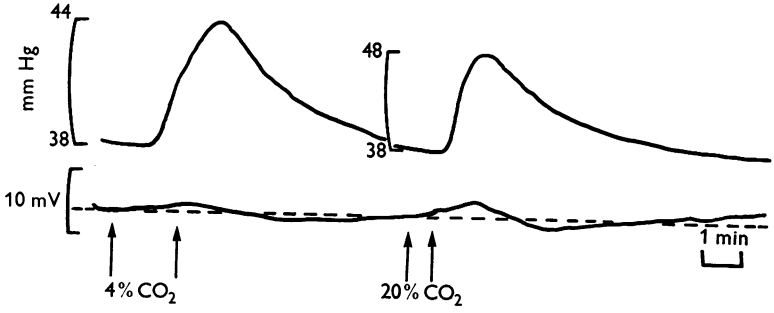

Fig. 11. Upper trace: cortical P_{CO_2}. Lower trace: resting potential of cell in posterior sigmoid gyrus of cat under Dial. Interrupted line indicates initial stable level at −50 mV, hyperpolarization being shown by an upward displacement. Cat was given 4 % CO_2 between first pair of arrows and 20 % CO_2 between second pair.

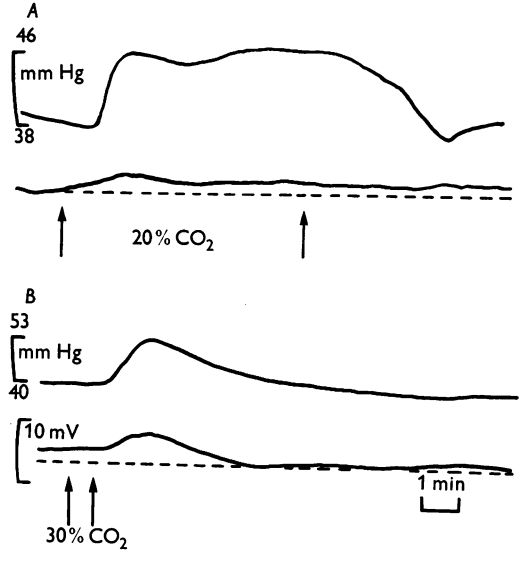

Fig. 12. Traces as in Fig. 11. *A*, between 2 arrows CO_2 in inspired air was first raised and then adjusted to maintain an approximately constant plateau of P_{CO_2}. In *B*, immediately following *A*, a high concentration of CO_2 (30 %) was given for a short period.

other synaptic responses recorded inside cells showed only inconclusive changes. The resting potentials of motoneurones (Fig. 13) and other neurones were not altered consistently even by large variations in P_{CO_2}.

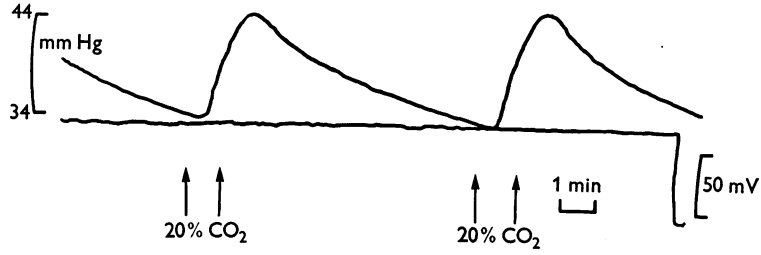

Fig. 13. Lack of any detectable changes in resting potential of lumbar motoneurone (lower trace) during large variations in spinal P_{CO_2} (upper trace). Each pair of arrows indicates administration of 20 % CO_2. Electrode was moved out of the cell at extreme right.

DISCUSSION

Cortical cells are remarkably sensitive to variations in P_{CO_2}; even changes of the order of 1–2 mm Hg may be sufficient to produce a clear alteration in their excitability. On the whole, the main effect of hypercapnia is depressant, since the excitatory changes tend to be transient, at the beginning and the end of the administration of CO_2, except when giving a very low concentration.

As these effects are observed equally well in the isolated cortex, they must be direct, and cannot be mediated by an ascending activating pathway, such as the hypothalamo-cortical system of Gellhorn (1953, 1954).

Mechanism of depressant action

The simplest explanation for the depressant effect of hypercapnia is a general hyperpolarization of cortical neurones, like that seen in a number of cells while recording the resting potential. The rapid depolarization recorded when P_{CO_2} began to fall could explain the rebound of excitation often seen at that time. But we have insufficient evidence to decide whether these changes in membrane potential are large enough to account for the observed variations in excitability, nor indeed is it certain that they occur in all, or even most cells. Nonetheless, they are in the right direction, and there is a reasonable possibility that they may provide a sufficient explanation.

Mechanism of membrane hyperpolarization

There is general agreement that an increase in P_{CO_2} tends to reduce the excitability of vertebrate nerve fibres (Davis, Pascual & Rice, 1928; Necheles & Gerard, 1930; Lorente de Nó, 1947) probably by raising the membrane potential (Lorente de Nó, 1947; Shanes, 1948). Why this

occurs is not known; a metabolic mechanism has been suggested (Lorente de Nó, 1947) but without much evidence in support. The only well-known effect of a change in P_{CO_2} is a corresponding alteration in 'intracellular pH' (Arvanitaki & Chalazonitis, 1954; Caldwell, 1958; Kostyuk & Sorokina, 1961). One possible explanation is that a fall in internal pH may increase the membrane potential by reducing the membrane's permeability to Na^+; but a direct action of CO_2 on the membrane cannot be excluded.

The rebound of excitation, which was paralleled by a rapid depolarization, suggests that high P_{CO_2} initiates a relatively slow reaction, possibly an active removal of H^+ from the cell, which may lead to an overshoot of pH when the P_{CO_2} is rapidly lowered.

Initial excitation during hypercapnia

Although membrane potential changes were observed which could account for the main depression and rebound of excitation, no initial depolarization was seen corresponding to the common early excitation. A depolarizing action, such as that of CO_2 on giant neurones of invertebrates (Chalazonitis, 1963) therefore cannot be involved. The membrane properties may be affected by an alteration in free Ca^{2+}, or there may be more complex effects, possibly involving the hydration of the cells.

Changes in blood flow

One of the clearest effects of a rise in blood P_{CO_2} is a sharp increase in cerebral blood flow (Gibbs, Gibbs & Lennox, 1935; Kety & Schmidt, 1948; Sokoloff, 1959). If the resting potential and excitability are immediately dependent upon the general state of the neurone they might be affected by an alteration in the balance between the supply and utilization of O_2 and various metabolites. However, this is not likely to be an important factor in the present experiments. According to Söderberg (1964) CO_2 does not produce any marked circulatory changes in the isolated cortical slab of the 'cerveau isolé', which nevertheless clearly showed the usual effects of CO_2 (Figs. 1–3).

Evoked responses

The dissociation between the strong depression of unit responses by hypercapnia and the relatively slight or even opposite changes in evoked slow waves shows how misleading slow waves can be as an index of cortical excitability. If slow waves are generated either presynaptically or as local post-synaptic responses, they could be enhanced by the removal of background excitation and by a general hyperpolarizing tendency. The gross potentiation seen under chloralose is likely to be related to its convulsant properties (Adrian & Moruzzi, 1939; Albe-Fessard, 1961).

In conclusion, it is clear that the effects of CO_2 on cortical neurones are not less pronounced than those observed previously in the brain stem (von Euler & Söderberg, 1952; Bonvallet, Hugelin & Dell, 1956), where neurones are usually thought to react particularly strongly to changes in P_{CO_2}. Spinal neurones, on the other hand, seem much less sensitive, in agreement with previous evidence (King, Garrey & Bryan, 1932) that the depressant effect of CO_2 (cf. Brooks & Eccles, 1947) is mediated by inhibition from higher centres.

SUMMARY

1. Changes in the excitability of cortical cells (mainly in cats under Dial) were tested by micro-iontophoresis of L-glutamate during hypercapnia, recording simultaneously the cortical P_{CO_2}.

2. Many cells clearly reacted to even small changes in P_{CO_2} (e.g. 4 mm Hg). Moderate doses of CO_2 led to both excitation and depression; typically there was an initial phase of excitation during the rise in P_{CO_2}, a subsequent longer period of depression, and some sharp excitation during the fall of P_{CO_2}.

3. With relatively low concentrations of inspired CO_2 ($< 10\%$) the relative importance of excitant and depressant effects varied between cells and between different animals; with strong doses of CO_2 ($> 10\%$) the depressant action was always predominant.

4. Similar changes in excitability were observed in cortical slabs isolated from the rest of the brain by undercutting; CO_2 therefore probably acts directly on the cortex.

5. Cortical responses evoked by peripheral stimulation were also examined; moderate hypercapnia depressed the evoked firing of units, but had relatively little effect on the slow-wave responses. Under chloralose anaesthesia, slow waves were very much potentiated by strong hypercapnia.

6. Membrane potentials of cortical cells showed a consistent tendency towards hyperpolarization during hypercapnia and a sharp depolarization when P_{CO_2} fell again. These changes in resting potential may account for some of the observed variations in excitability.

7. Spinal neurones appear to be less sensitive than cortical neurones to variations in P_{CO_2}.

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