THE EFFECTS OF RESPIRATORY ACIDOSIS ON A SENSORY RELAY SYSTEM*

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MORE THAN 100 YEARS of investigation and speculation have not yet explained the mechanism of action of carbon dioxide on the excitability of the nervous system. Reviews of the literature include many descriptions of differential sensitivity and phasic responses of increases and decreases in excitability.[1,2] The studies presented in this paper were initiated in an attempt to elucidate further the action of CO_2 on transmission at a central synapse.

One of the afferent pathways for the transmission of sensory information from peripheral receptors of the forelimb is via the dorsal column tracts of the spinal cord to the cuneate nucleus in the dorsal part of the lower medulla. Here these fibres terminate, making synaptic contact with relay cells whose axons project upwards as the medial lemniscal fibres to the thalamus, where they then relay to the somato-sensory cortex. The anatomical location of the cuneate nucleus makes it conveniently accessible for the observation and control of its input-output relationships. In recent years there have been numerous studies of the transmission of afferent activity through the cuneate nucleus[3–5] and of its anatomical and functional organization.[6,7] The synaptic contacts of its cells are relatively large,[8] and synaptic mechanisms have been demonstrated which include both excitatory and inhibitory influences from the periphery,[4] descending cortical projections[9,10] and the reticular formation.[11] These are some of the reasons which determined the choice of the cuneate nucleus synaptic relay for the present studies.

The effects of respiratory acidosis on the dorsal column cuneothalamic relay path were studied in decerebrate cats. Changes in orthodromic transmission were observed in medial lemniscal responses evoked by stimulation of afferent fibres in the forelimb and near their terminations in the cuneate nucleus. The inhibition of transmission was produced by the interaction of afferent volleys in different nerves. Stimulation in the cuneate nucleus evoked responses which were recorded as antidromic potentials in a peripheral nerve (reflecting the excitability of the presynaptic terminals[12]), and simultaneous orthodromic medial lemniscal discharges (representing both post-synaptic and trans-synaptic responses of the cuneate neurones[13]). Figure 1 illustrates the recording and stimulating sites of the experiments, and shows the level of transection in the mid-brain and some typical recorded potentials.

METHODS

The experiments were performed in 14 unanaesthetized cats, after mid-collicular decerebration under halothane anaesthesia. The animals were paralysed by the

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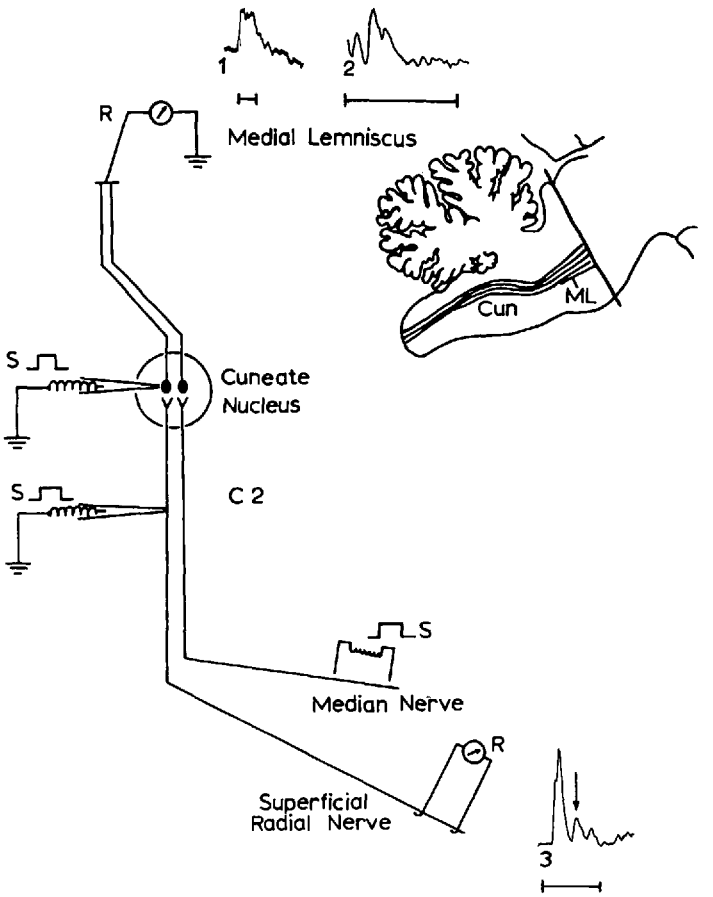

FIGURE 1. Diagram of stimulus (S) and recording (R) arrangements. Sagittal brain section shows level of decerebration. Typical orthodromic responses recorded at transected medial lemniscus are (1) evoked from stimulation of a peripheral nerve, and (2) evoked from stimulation in the cuneate (the α potential—the first smaller component; the β potential—the second component). The antidromic potentials (3) are evoked by stimulation of dorsal column fibres or terminals (arrow marks dorsal column reflex). Time scale 10 msec.

continuous intravenous administration of 0.1 per cent succinylcholine chloride and ventilation was controlled at a rate of 30/min by a Palmer pump with a non-rebreathing circuit. The rectal temperature was maintained at 36–37° C, and the mean arterial pressure was continuously monitored.

The dorsal surface of the medulla and upper spinal cord was exposed by removal of the posterior arches of the atlas and upper cervical vertebrae and a part of the occiput covering the cerebellum. The superficial radial and median nerves in the forepaw were dissected, cut, and mounted on bipolar platinum electrodes for recording and stimulation. Medial lemniscal potentials were recorded with a single platinum electrode from the surface of the transected brain-stem. The dorsal column fibres were stimulated at or near their pre-synaptic terminals

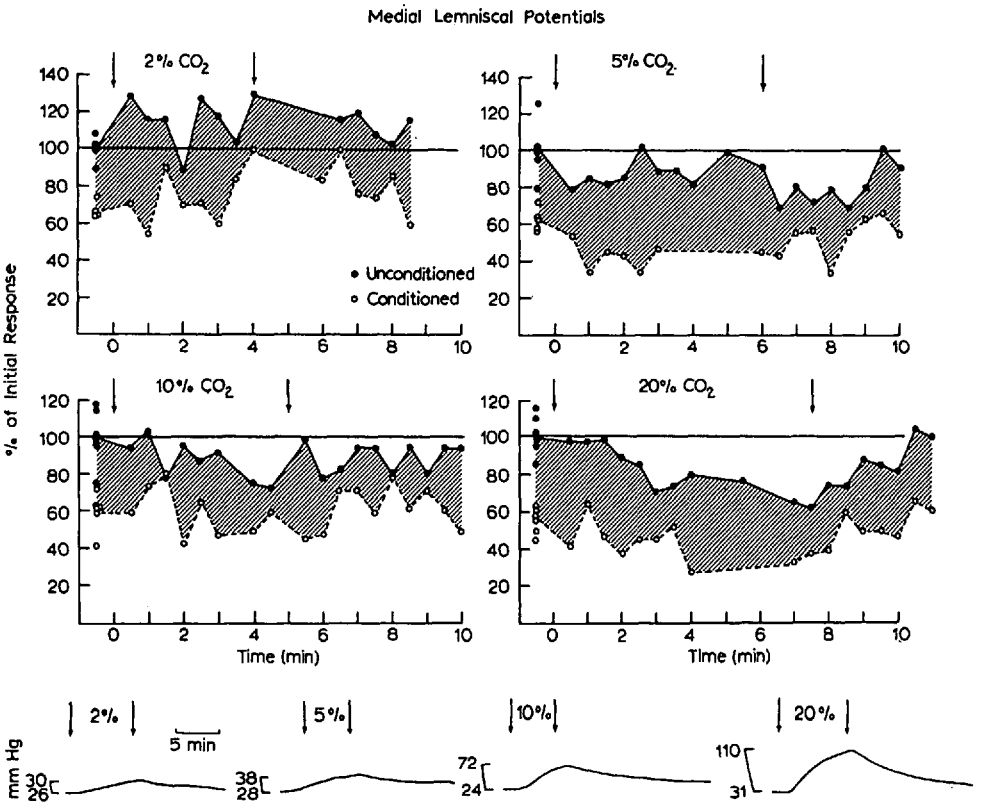

FIGURE 2. Variation of the amplitude of potentials (second peak, measured from baseline) recorded in the medial lemniscus and evoked by stimulation of the superficial radial nerve which was supramaximal for the medial lemniscal response, during the administration of different concentrations of CO_2. Filled circles (●) are responses to an unconditioned volley. Open circles (○) are responses which have been conditioned at an interval of 30 msec by a preceding volley in the median nerve. The shaded area represents the degree of inhibition caused by conditioning. The lower traces show the tissue P_{CO_2} levels recorded continuously during this experiment from the surface of the cuneate nucleus.

(2–4 mm caudal to the obex) and at the level of the second cervical segment (15–20 mm caudal to the obex), using glass microelectrodes filled with 1 M NaCl, with 30–60 μ tip diameters and resistances < 1 MΩ, which were introduced to depths of < 1 mm by a micromanipulator. Rectangular constant current pulses of $\leqq 0.2$ msec duration, at a frequency of 1 Hz, were employed for stimulation.

Carbon dioxide was introduced to the respirator inlet in concentrations varying from 2 to 20 per cent in oxygen, from a standard Boyle's anaesthetic machine. The average time of administration was five minutes; observations of recovery were made over a subsequent 20- to 30-minute period. Variations in P_{CO_2} were continuously monitored, whenever possible, on the exposed surface of the cuneate nucleus using a tissue P_{CO_2} electrode (supplied by Eschweiler & Co., Kiel, West Germany) as described by Siesjö[14], with a Beckman 160 Physiological Gas Analyzer and pen recorder. This electrode had a contact diameter of 2 mm, and, when prepared with an electrolyte solution of 0.001 M $NaHCO_3$ and 0.025 M KCl and

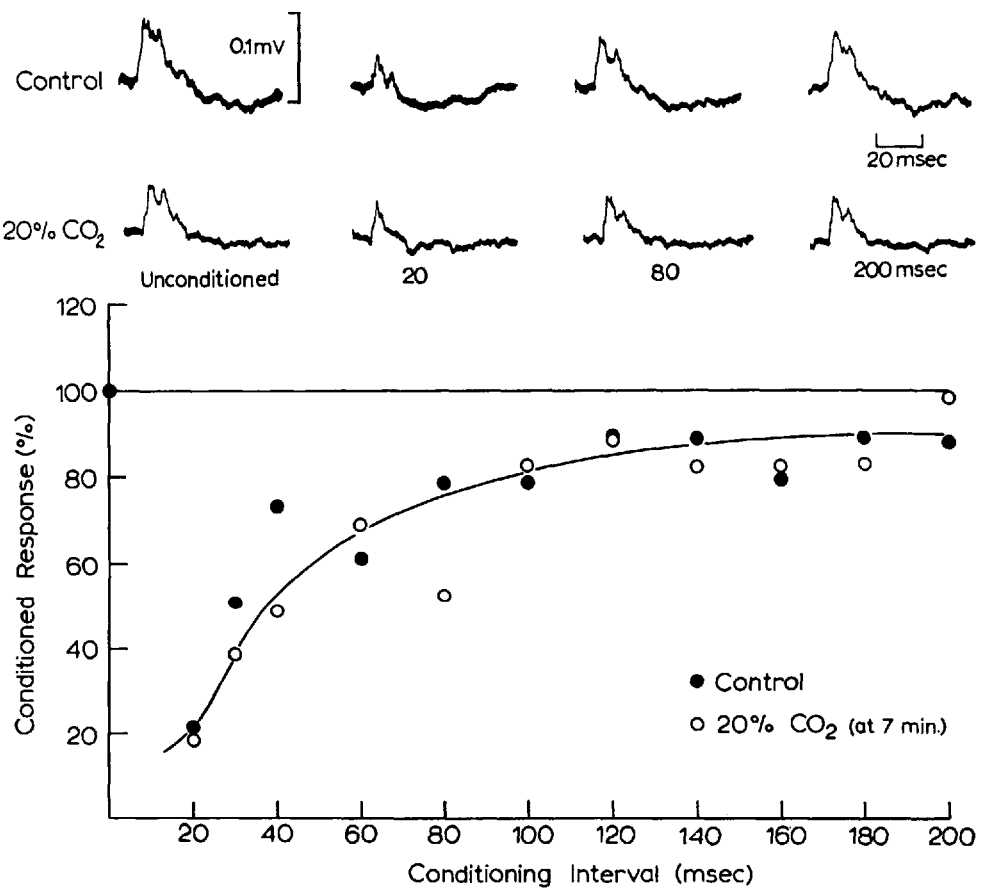

FIGURE 3. Effect of 20 per cent CO_2 on conditioning of orthodromic transmission from the superficial radial nerve by a preceding afferent volley in the median nerve. (Intervals between volleys were varied from 20 to 200 msec; stimulus intensities for both nerves were supramaximal for the medial lemniscal response.) In the upper part of the diagram are examples of traces of the responses evoked from the superficial radial nerve before and during Co_2 (at 7 min): an unconditioned response and responses conditioned at 20, 80, and 200 msec respectively.

I-Lastic membrane, had a response time of approximately 45 seconds (time required to reach 90 per cent equilibrium when the Pco_2 was increased by 20 mm Hg). It was calibrated at a temperature of 36° C in a system approximating the recording conditions on the brain. Baseline tissue Pco_2 levels were in the range of 25–30 mm Hg.

RESULTS

Orthodromic transmission evoked from peripheral nerve

Figure 2 demonstrates the effects in one experiment of different concentrations of inspired CO_2 on transmission through the cuneate nucleus, monitored by the medial lemniscal responses to peripheral nerve stimulation (at supra-maximal intensities). The time course and extent of the tissue Pco_2 changes at the surface

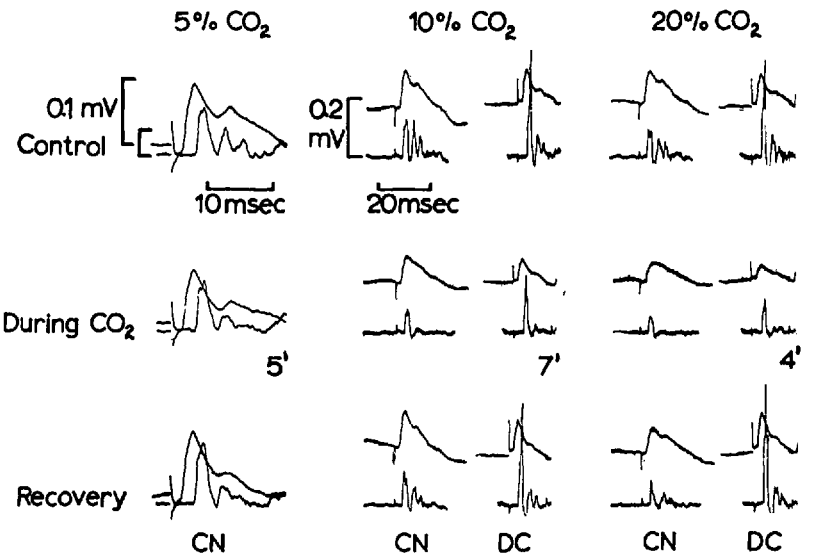

FIGURE 4. Effects of 5, 10, and 20 per cent CO_2 on orthodromic potentials recorded at times indicated in the medial lemniscus (upper traces), and antidromic potentials recorded in the superficial radial nerve (lower traces). Responses were evoked by microelectrode stimulation in the cuneate nucleus (CN) 2–4 mm below the obex, and in the dorsal column (DC) at a point 16 mm more caudal.

of the medulla are also shown in the lower part of Figure 2. Transmission was increased with 2 per cent CO_2 and depressed with higher concentrations. Similar effects were observed in three other experiments. During these studies the current required to evoke a threshold response remained approximately constant, and changes in conduction time were variable.

Transmission through the cuneate nucleus is inhibited by the interaction produced by a preceding volley in a second nerve.[13] It can be seen in Figure 2 that these inhibited responses (conditioned at an interval of 30 msec) were affected by changes in CO_2 in the same way as the unconditioned responses. Figure 3 shows the absence of effect of 20 per cent CO_2 (giving a tissue P_{CO_2} level of 110 mm Hg) on the time course of this inhibition, when intervals between conditioning and test volleys were varied between 20 and 200 msec.

Excitability changes in afferent fibres

1. Presynaptic terminals. Inspiratory CO_2 concentrations of 5 per cent or less caused varying changes in the excitability of the afferent fibre terminals; Figures 4 and 5 (from one of eight experiments) show increases of the antidromic action potentials with 5 per cent CO_2 and decreases with higher concentrations. The overall trend was one of increasing depression in response to graded increases of CO_2 above 5 per cent, the average maximal reduction in excitability with 20 per cent CO_2 being by 38 per cent of the initial control level. The changes varied in their rate of onset, and usually did not progress after the administration of CO_2 was

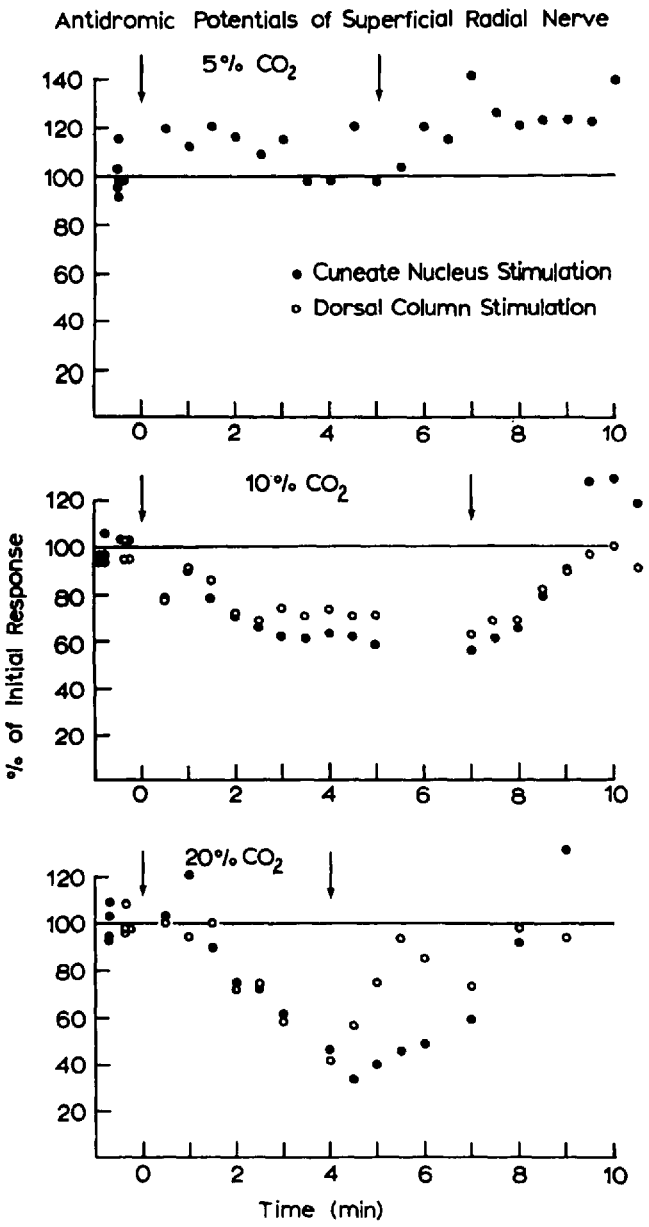

FIGURE 5. Effects of 5, 10, and 20 per cent CO_2 on excitability of terminals (filled circles, ●) and dorsal column fibres (open circles, O). (Measurements of potential amplitudes of the second component are from the experiment illustrated in Fig. 4.) Initial points show variability of responses during control period, before the start of CO_2 administration.

stopped. The recovery of excitability was often delayed and showed more fluctuation than was present before and during the administration of CO_2.

2. *Dorsal columns.* In one experiment stimulation through separate micro-

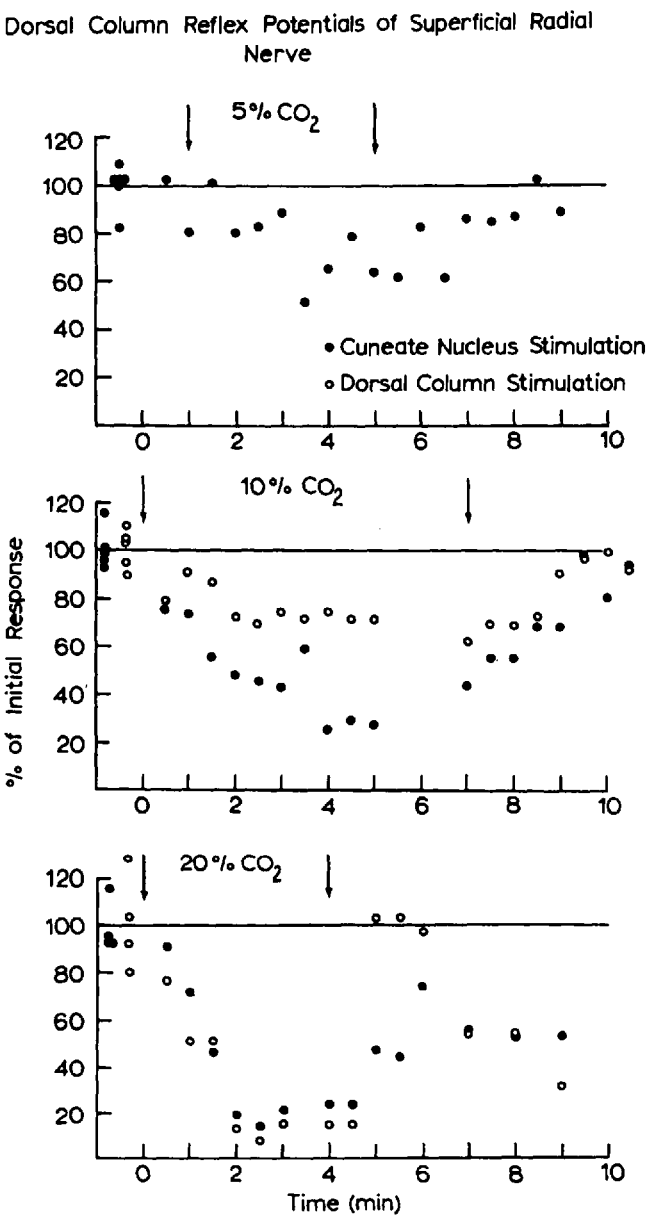

FIGURE 6. Effects of 5, 10, and 20 per cent CO_2 on antidromically conducted dorsal column reflexes evoked by stimulation in the cuneate nucleus (filled circles, ●) and the dorsal column (open circles, O). (Measurements of potential amplitudes of the initial dorsal column reflex peak are from the experiment of Fig. 4.)

electrodes placed in the cuneate nucleus and dorsal column (as described in the Methods section) allowed a comparison of the excitability of afferent fibres and their terminals. Increases in P_{CO_2} with 10 and 20 per cent CO_2 depressed the

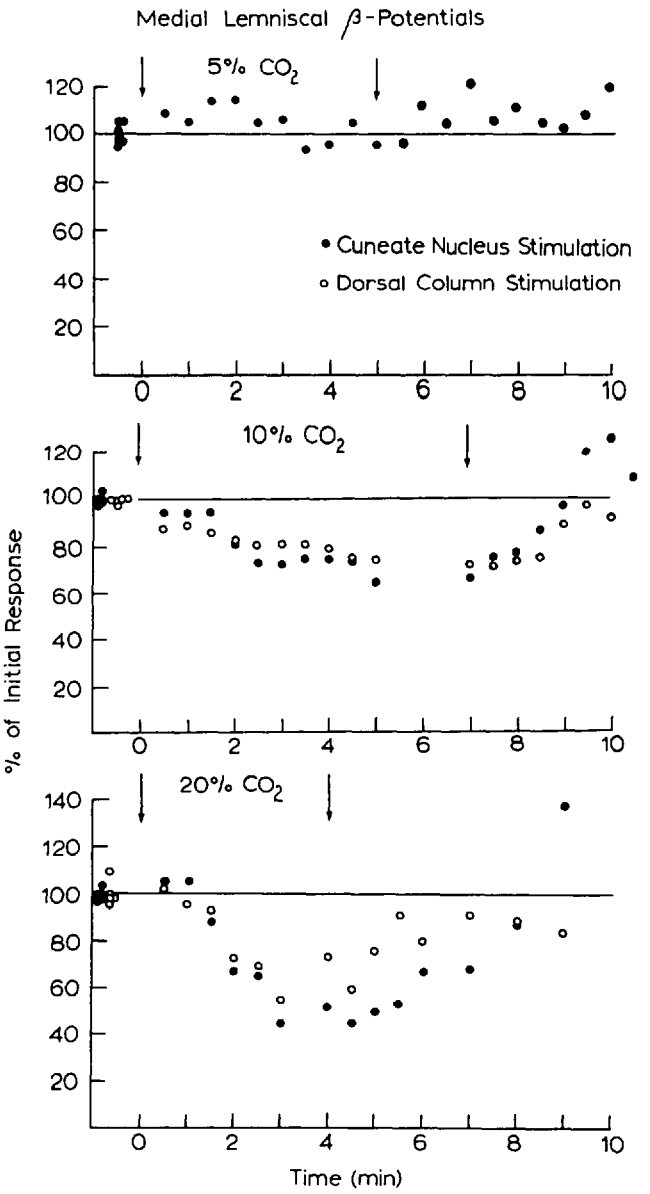

FIGURE 7. Effects of 5, 10, and 20 per cent CO_2 on medial lemniscal β potentials evoked by stimulation in the cuneate nucleus (filled circles, ●) and the dorsal column (open circles, O). (Measurements of potential amplitudes of the initial β peak are from the experiment of Fig. 4.)

excitability of the terminals more than that of the fibres lower down in the dorsal column (Figs. 5, 6, and 7).

3. *Dorsal column reflex.* The later component of the antidromically conducted action potentials (see Fig. 1), which may be identified as the dorsal column reflex by

its latency and its failure at stimulation frequencies > 20/sec,[15] was consistently depressed by all increases of P_{CO_2} (observations from six experiments). These changes were always more rapid and greater than the depression of excitability of the earlier components of the antidromic potentials (cf. Figs. 5 and 6), and there was only partial or slower recovery.

Orthodromic transmission evoked from cuneate nucleus stimulation

Medial lemniscal potentials evoked by stimulating the presynaptic terminals in the cuneate nucleus (the β or secondary potentials with latencies long enough to include a synaptic delay[13]) showed changes in response to hypercarbia which paralleled the time course and extent of the excitability changes of these terminals, as shown by the antidromic responses simultaneously recorded in the peripheral nerve (cf. Figs. 5 and 7). The average maximal depression in response to 20 per cent CO_2 was by approximately 35 per cent. These changes resemble those in orthodromic transmission evoked from the periphery, when comparisons are made within individual experiments.

Correlation of the orthodromic β potentials (transmission through the synapse) and the antidromic action potentials (presynaptic excitability), evoked by different intensities of stimulation in the cuneate, is shown in Figure 8. The input-output relation is not changed by 10 per cent CO_2 and the depression of transmission through the synapse can be accounted for by the decrease in fibre excitability (i.e. the number of fibres stimulated). Similar effects were seen in several different experiments, with different concentrations of CO_2, although 20 per cent CO_2 caused more variation in this relationship.

Excitability changes in post-synaptic responses

Careful placement of the stimulating electrode in the cuneate near the relay cells evokes an early component in the medial lemniscal response, the α potential (see Figs. 1 and 9). This potential, because its latency is too brief to include a synaptic mechanism, is considered to represent post-synaptic responses of the cuneo-thalamic relay cells or their axons.[13] Hypercarbia caused a depression of the α potentials, which was considerably less than that of synaptic transmission and terminal excitability. Figure 9 demonstrates the changes in alpha response caused by 20 per cent CO_2 in one experiment.

Discussion

The main effect of hypercarbia on transmission from a peripheral nerve through the first synaptic relay of the dorsal column somato-sensory pathway was a depressant one, similar to that which has been described for the monosynaptic reflex in the spinal cord.[16] Possible mechanisms of this effect are (*a*) block of invasion of the presynaptic terminals, (*b*) reduced synaptic efficiency, due either to reduced release of transmitter or to reduced sensitivity of the post-synaptic cells to transmitter, and (*c*) reduced electrical excitability of the post-synaptic cell. Stimulation near the synaptic contacts of the relay cells provides a test of the various possible factors involved in these changes in transmission. The β potential

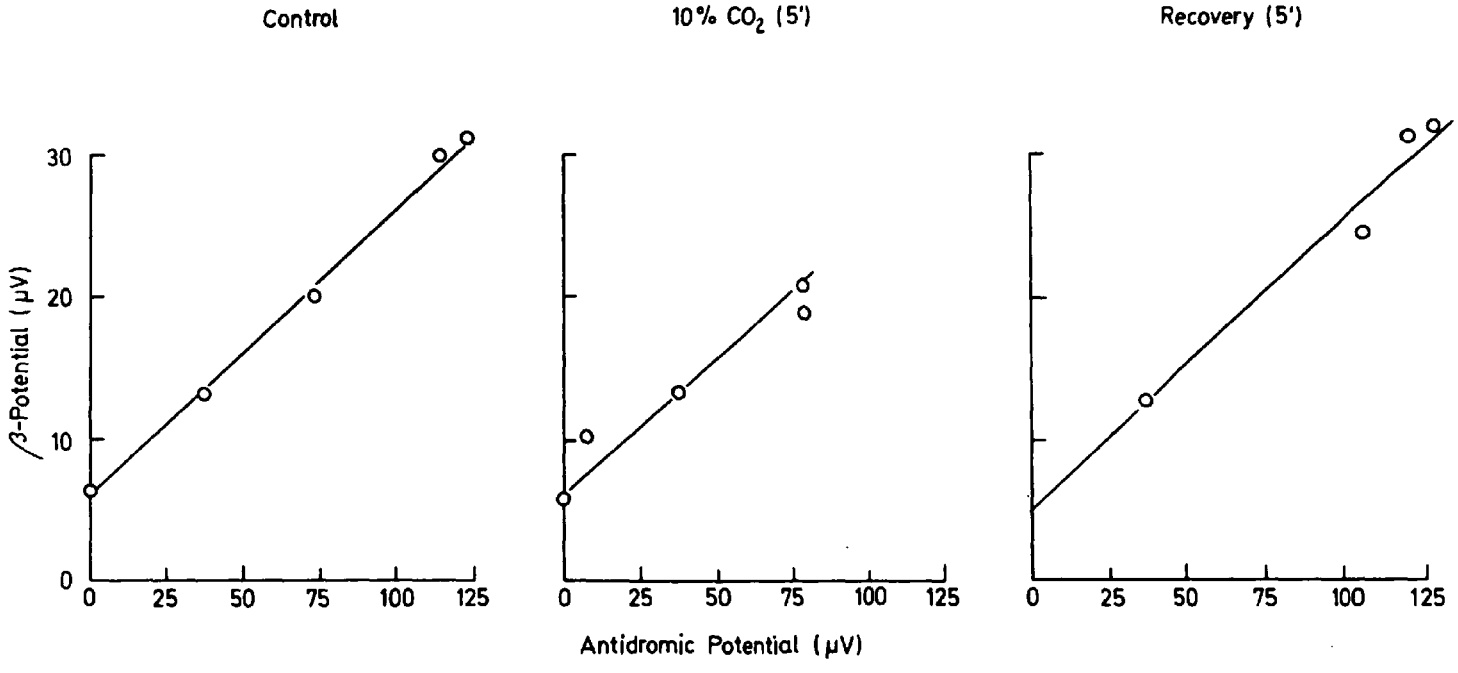

FIGURE 8. Input-output correlation of synaptic transmission (β potential of medial lemniscal response) and excitability of pre-synaptic terminals (antidromic potential at peripheral nerve) evoked by different intensities of stimulation in the cuneate, before, during, and after 10 per cent CO_2. (Measurements are of first peak amplitudes from one experiment; lines were drawn by eye.)

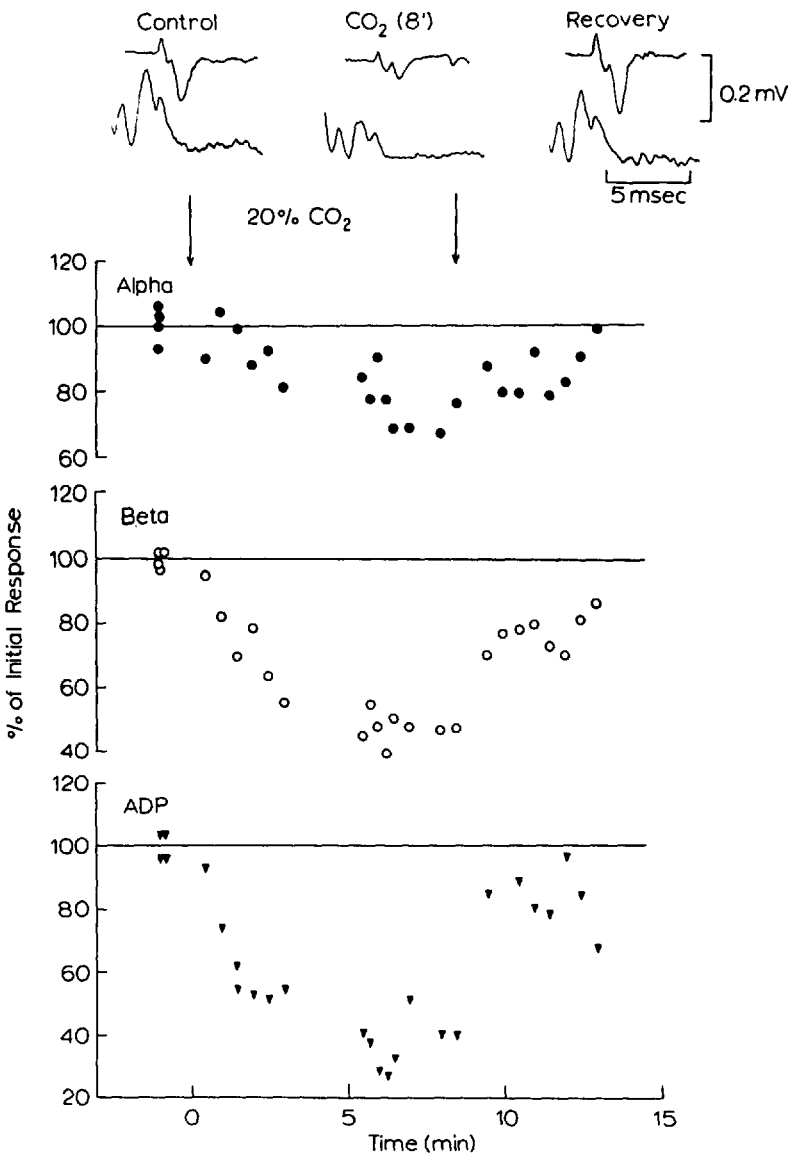

FIGURE 9. Effect of 20 per cent CO_2 on potentials evoked by microelectrode stimulation in the cuneate. Upper trace shows antidromic potential (corresponding to ADP (▼) in graph). Lower trace is medial lemniscal response (first smaller α potential, ●; second larger β component, O).

of the lemniscal response represents the orthodromic conduction through the synapse from the excited presynaptic terminals; at the same time the potentials conducted antidromically from the terminals give an estimate of the number of fibres initiating synaptic transmission. Wall *et al.*[17] have shown that the safety factor for transmission of information from the dorsal column to the peripheral nerve is equivalent to that in peripheral nerve, and much greater than for intraspinal con-

duction from peripheral nerve along the dorsal column. Therefore, it can be assumed that changes in these antidromic potentials give a reasonably accurate estimate of changes in terminal excitability.

Correlation of the input and output (antidromic fibre potentials and orthodromic β potentials) at the cuneate gives an index of the efficiency of the synaptic process. The marked changes in transmission through the synapse which were caused by progressive increases in tissue P_{CO_2} can be accounted for almost entirely by the similar changes in the afferent fibre excitability, and therefore the synaptic efficiency of individual active terminals was not depressed. Decreases in post-synaptic excitability were small, and made minimal contribution to the changes in transmission. However, maintenance of synaptic efficiency in the presence of post-synaptic depression raises the possibility of either increased release of transmitter, or increased chemical sensitivity of the post-synaptic membrane. Membrane hyperpolarization, which has been demonstrated by intracellular recording in cells of the cortex[18] and toad spinal motoneurones[19] during CO_2 administration, could increase spike amplitude, and so effect an increase in transmitter release.[20] This might explain the facilitation of transmission produced by low concentrations of CO_2, and increased synaptic efficiency at a time when pre- and post-synaptic excitability is depressed by higher concentrations of CO_2.

The depression of overall transmission by hypercarbia therefore can only be explained by presynaptic events. The mechanism of this depression of presynaptic excitability may well be due to a failure of invasion of terminals at their points of branching where the safety factor for conduction is low,[21] or at earlier intraspinal sites of branching.[17] The particular sensitivity to CO_2 shown by the dorsal column reflex, which may be generated from the regions of the terminals[15,22] and more distal intra-axonal junctions,[23] supports this. Temporal dispersal of the afferent volley by changes in conduction velocity[24] could also reduce its effectiveness in initiating transmission.

The apparent lack of effect of hypercarbia on the inhibition of transmission by a preceding volley suggests that excitatory and depressant effects of CO_2 are probably not mediated by changes in the efficacy of inhibitory mechanisms in the cuneate.

Summary

The effects of hypercarbia on afferent inputs and synaptic transmission in the cuneate nucleus were studied in decerebrate cats. Stimulation with microelectrodes placed in the cuneate nucleus and/or dorsal column evoked antidromic potentials in the superficial radial nerve; stimulation of afferent fibres in the forelimb, dorsal column, or cuneate nucleus evoked orthodromic responses in the medial lemniscus. Small increases of inspired CO_2 ($\leqslant 5\%$) often increased synaptic transmission and the excitability of afferent fibres. Further increases of P_{CO_2} depressed transmission and terminal excitability, while producing considerably smaller changes in post-synaptic excitability. CO_2 had no obvious effect on the inhibition of orthodromic transmission produced by afferent nerve interaction, but markedly depressed the dorsal column reflexes. The efficiency of synaptic transmission, as estimated from

the input-output relation of the cuneate, was maintained at individual active synapses. It is suggested that CO_2 does not depress release of transmitter at this synapse, and that the changes in transmission are effected mainly by presynaptic events, possibly by block of conduction in the intraspinal afferent fibres and/or their terminals.

Résumé

Nous avons étudié sur des chats décérébrés les effets de l'hypercarbie sur les influx afférents et sur la transmission synaptique dans le noyau cunéiforme. Une stimulation avec des micro-électrodes placées dans le noyau cunéiforme et/ou dans la colonne dorsale provoquent des potentiels antidromiques dans le nerf radial superficiel; une stimulation des fibres afférentes du membre antérieur, colonne dorsale, ou noyau cunéiforme provoque des réponses orthodromiques dans le lemniscus médian. De légères augmentations du CO_2 ($\lessapprox 5$ pour cent) augmentent souvent la transmission synaptique et l'excitabilité des fibres afférentes. De plus fortes augmentations de la Pco_2 déprime la transmission et l'excitabilité terminale, alors qu'elles produisent des changements beaucoup plus faibles de l'excitabilité post-synaptique. Le CO_2 n'a pas eu d'effet évident sur l'inhibition de la transmission orthodromique par l'interaction nerveuse afférente mais il a déprimé de façon marquée les réflexes de la colonne dorsale. L'efficacité de la transmission synaptique, appréciée d'après la relation influx afférents et efférents dans le cunéiforme a été maintenue aux synapses actives individuelles. Cela suggère que le CO_2 ne déprime pas la libération de l'agent transmetteur à cette synapse et que les changements dans la transmission sont produits surtout par les éventualités présynaptiques, peut-être par bloc de conduction dans les fibres afférentes intraspinales et/ou leurs terminaisons.

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