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TRANSIENT NEOCORTICAL AND HIPPOCAMPAL EEG SILENCE INDUCED BY ONE MINUTE INHALATION OF HIGH CONCENTRATION CO_2 IN SWINE.

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SUMMARY

Swine were exposed twice to 80% CO_2 for 1 min during simultaneous recording of the EEGs from the neocortex and the hippocampus. In five of the animals myoclonic jerks started at 28±1 s of CO_2 exposure and lasted for 6±2 s. Neocortical slow-wave activity and increased amplitude of the hippocampal (5-7 Hz) waves had developed before the period of myoclonic jerks. After this period the EEG activity declined, resulting in neocortical EEG silence at the end of the exposure which lasted for on average 1 min. The return of the neocortical EEG activity exhibited a pattern reverse to its disappearance, but was much prolonged in comparison to its extinction. Pre-exposure neocortical EEG pattern was not regained until 3-5 min post-exposure. In eight out of 11 experiments the CO_2 inhalation also induced hippocampal EEG silence lasting for on average 30 s. The observed changes in the neocortical and hippocampal EEGs suggest that the present swine were unconscious already when they exhibited motor reactions.

INTRODUCTION

The justification for using high concentration CO_2 inhalation as pre-slaughter anaesthesia in swine has been debated because of the possibility of stress reactions developing before an adequate depth of narcosis is reached, and because of lacking information on the duration of the induced anaesthesia.

With the ultimate goal of obtaining an objective evaluation of the ethics and the narcotic efficiency of using CO_2 for this purpose, an experimental series involving electroencephalographic (EEG) recordings was started. As a first step, the effects of 1 min ventilation with 80% CO_2 upon the neocortical EEG and sensory evoked cortical potential were investigated in the rat (Forslid et al. 1986b). In that species the CO_2 exposure caused a marked reduction in the EEG activity concomitant with a temporary extinction of the evoked responses.

The second step of the series was the development of a technique for simultaneous recordings of neocortical, hippocampal, and amygdaloid EEGs in the awake, unrestrained swine, and the use of that technique for obtaining information on the normal EEG activity in these brain regions (Forslid et al. 1986a). That information formed the basis for a subsequent study of EEG changes induced by inhalation of high concentration CO_2 in swine (Forslid 1987). The latter study is reviewed here.

METHODS

Animals. The six Yorkshire swine (body wt 40-70 kg) used for the reported experiments were supplied with recording electrodes implanted into the frontal neocortex and the dorsal hippocampus. The animals (numbers I-VI) had previously, from the second to the fifth or sixth post-implantation day, been employed for EEG recording from these brain regions during the awake, unrestrained state (Forslid et al. 1986a). Concerning implantation/recording techniques, electrode localization, and registered normal EEGs in the present animals see Forslid et al. (1986a).

Exposure to CO_2. With a 48 h interval, 1 min exposure to approximately 80% CO_2 was performed twice in each animal between the sixth and ninth post-implantation day. About 10 min after the second exposure, the swine were killed with CO_2 exposure prolonged for 5 min and ended by exsanguination.

Exposure technique. The swine was placed in a wooden cage resting on a hydraulic table which was lifted until the head of the animal was about 1.5 m above the floor. The table and the lower part of the cage were enclosed in a roofless 1.5 m high rectangular (1.1 x 1.8 m) perspex chamber taped tightly onto the floor. At the bottom, the chamber was supplied with an inlet from a cylinder containing compressed 100% CO_2. A CO_2 monitor (Binos, Leybold-Heraeus, FRG) was taped onto the inner frontal wall of the chamber 0.8 m above the floor. The CO_2 (being heavier than air) was layered from the chamber bottom until the concentration reached 80% at the measuring point, where it was then maintained constant by intermittent inlet of small volumes of the gas. Repeated checks with the CO_2 monitor temporarily placed 1.2 m above the floor, revealed that the CO_2 concentration at the level remained <10% throughout the experiments.

During continuous EEG recording, the table was lowered 0.9 m (to the floor) whereby the snout of the swine reached the 0.8 level after 20 s. Thus, exposure to high concentration CO_2 apparently did not occur until about 15 s after the start of the descent. To delimit the exposure to roughly 1 min, the ascent of the table was commenced 70 s after the start of its descent.

EEG recordings from the various brain regions were started 4-5 min before the animal was lowered into the CO_2, and were continued until 5-10 min after the end of the ascent, that is, when the EEGs appeared 'normalized', the animals had become fully awake, and had taken up standing position again.

The EEGs were recorded by means fo a Mingograf EEG Junior (Elema Schönander, Sweden) using a high pass filter at 1 Hz and a low pass filter at 30 Hz.

Figures in the text preceded by ± represent standard error of means (SE).

RESULTS

Visible influences of the CO_2 inhalation

The present experiments were not aimed at studying systemic or behavioural effects of the CO_2. However, some general observations regarding the reactions of the swine were made.

Motor reactions. With the exception of animal III, all swine exhibited more or less pronounced myoclonic jerks commencing at 28±1 s after the estimated start of exposure to high concentration CO_2 and lasting for 6±2 s. At the end of this motor reaction, the swine had taken recumbent position and appeared entirely reactionless. The first sign of returning motor control (head movements) was generally seen 2-3 min after the end of the exposure. Taking up the standing position again was on no occasion observed until 4 min after the exposure.

Respiration. A gradual increase in respiratory frequency occurred during the initial 25 s of CO_2 inhalation. However, in connection with the brief period of myoclonic jerks, the respiration was con-

verted into low frequency gasping (about 10 per min) continuing for about 30 s after the exposure period. Then the respiratory frequency again increased, reaching about 60 per min within a minute, whereafter the tachypnoea gradually declined. However, the respiratory frequency had not yet returned to pre-exposure level (about 20 per min) at the end of the observation period some 10 min later.

EEG effects of the CO_2 exposure

EEG silence. The general influence of the CO_2 inhalation upon the different EEGs is demonstrated in Fig. 1. The figure shows strips of the EEG obtained by simultaneous recordings from the dorsal hippocampus (RH, LH) and the frontal neocortex (RC, LC) in both hemispheres. In that experiment (first exposure, animal I) seemingly complete EEG silence was registered simultaneously at all four brain sites within 10 s after the termination of the 1 min CO_2 inhalation.

The onset of apparent neocortical isoelectricity appeared at 52.5±2.5 s of CO_2 inhalation, whereas the corresponding onset as regards the dorsal hippocampus (experiment n=8) was delayed until 6±4 s post-exposure. The mean duration of the silent period was longer in the neocortex than in the dorsal hippocampus (58±9.5, range 19-113 s, n=10 vs 30±7 s, range 0-67 s, n=11). In three of the experiments, the dorsal hippocampal activity was never completely suppressed.

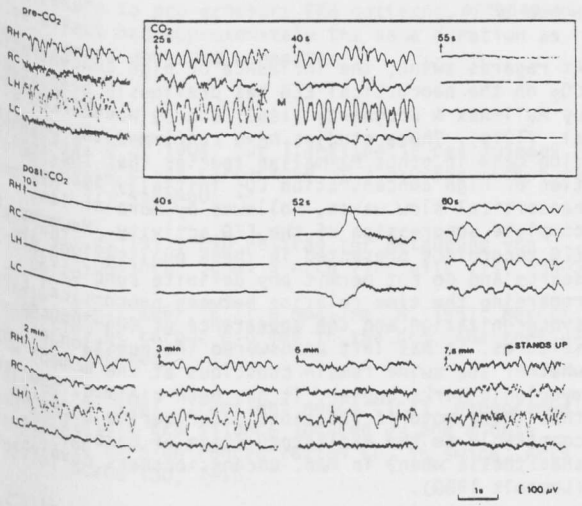

Fig. 1. Strips (3 s) of continuous EEG recordings from the right and left dorsal hippocampus (RH, LH) and the right and left frontal neocortex (RC, LC) in animal I before (pre-CO_2), during (CO_2), and after (post-CO_2) 1 min exposure to approximately 80% CO_2. During the interval marked M the swine exhibited myoclonic jerks for 4 s. Note the presence of apparent EEG silence at all leads 10 s post-CO_2. (From Forslid 1987.)

Neocortical EEG changes. A change in the EEG recorded from the frontal neocortex became visible at 13±0.4 s (range 11-15 s) after the estimated onset of high concentration CO_2 inhalation. Then waves in the frequency range 1-3 Hz (= low delta activity) started to appear more frequently, becoming the dominating activity 10-15 s later. Thus, delta activity had become fully developed before the brief period of myoclonic jerks (Fig. 1, M) occurring in all animals except in animal III. High frequency (about 30 Hz) activity with a gradually diminishing amplitude remained superimposed upon the delta waves and outlasted the latter for a few seconds before the development of EEG silence. The pattern is demonstrated in Fig. 2a, which shows a continuous record from the left frontal neocortex in animal III during the exposure period.

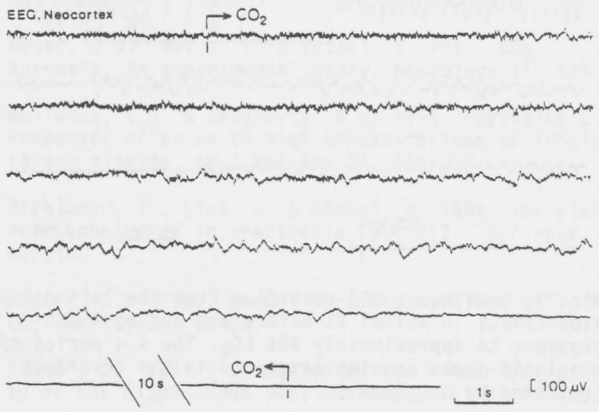

Fig. 2a. Continuous EEG recording from the right frontal neocortex in animal III immediately before and during 1 min exposure to approximately 80% CO_2. The animal did not exhibit myoclonic jerks during the exposure. Note the gradual increase in delta (1-5 Hz) activity followed by extinction of all EEG activity. An intersection of 10 s has been made during the EEG silence. (From Forslid 1987.)

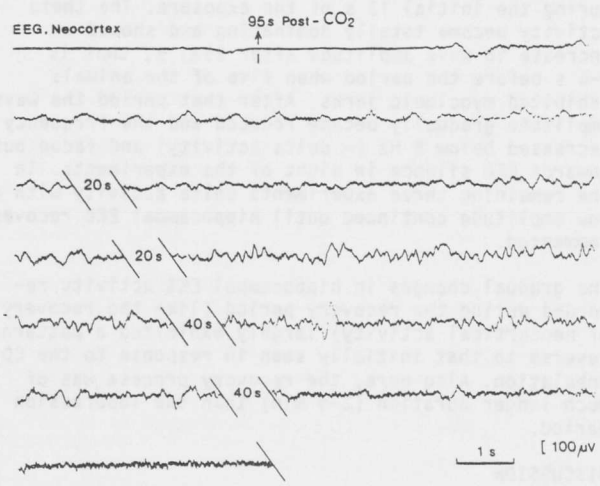

Fig. 2b. Continuous recording of the neocortical EEG activity in this animal starting about 100 s after the CO_2 inhalation. Intersections (20 and 40 s) have been made in the record to demonstrate the long duration of the recovery process. (From Forslid 1987.)

After the period of apparent isoelectricity, the gradual return of neocortical EEG activity exhibited a pattern reverse to its disappearance. However, in comparison to the EEG extinction, the recovery process was much prolonged (Fig. 1; Fig. 2a vs. b). Thus, a neocortical EEG showing obvious resemblance to that recorded immediately before the CO_2 inhalation was not observed until 3-5 min post-exposure.

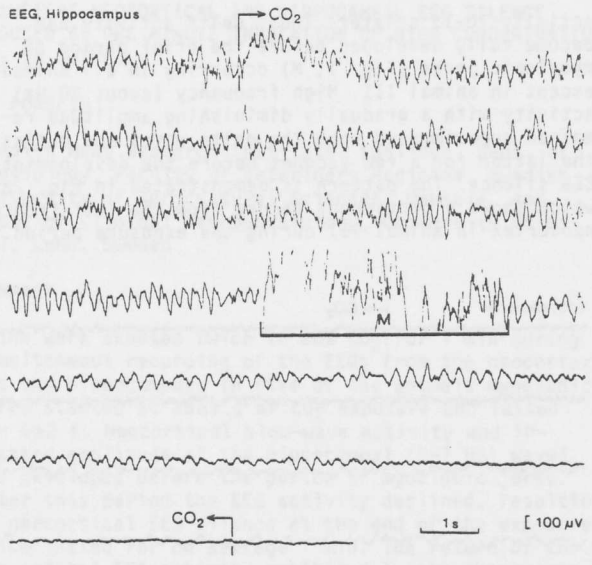

Fig. 3. Continuous EEG recording from the left dorsal hippocampus in animal VI before and during 1 min exposure to approximately 80% CO_2. The 4 s period of myoclonic jerks causing artefacts in the record is indicated by M. (From Forslid 1987.)

Hippocampal EEG changes. The obvious CO_2 influences on hippocampal EEG activity roughly coincided with those recorded from the neocortex. The pre-exposure irregular theta (5-7 Hz) activity, being intermingled with activity of higher frequency, changed to more continuous theta rhythm at 17±2 s after the start of CO_2 inhalation in five of the animals. Animal VI (Fig. 3) was an exception in as much as a corresponding accentuation of the theta activity was here seen during the initial 12 s of the exposure. The theta activity became totally dominating and showed an increase in wave amplitude after 25±1 s, that is 2-6 s before the period when five of the animals exhibited myoclonic jerks. After that period the wave amplitude gradually became reduced and the frequency decreased below 5 Hz (= delta activity) and faded out towards EEG silence in eight of the experiments. In the remaining three experiments delta activity with a low amplitude continued until hippocampal EEG recovery commenced.

The gradual changes in hippocampal EEG activity recorded during the recovery period (like the recovery of neocortical activity) largely exhibited a pattern reverse to that initially seen in response to the CO_2 inhalation. Also here, the recovery process was of much longer duration (2-5 min) than the suppression period.

DISCUSSION

The narcotic properties of CO_2 inhalation were already recognized at the beginning of the previous century (see Cantieni 1977). However, 'convulsions' are mentioned in practically all papers dealing with the effects of high concentration CO_2. This has precluded the utilization of CO_2 as an anaesthetic in man. As regards farm animals, the potential value of CO_2 inhalation as pre-slaughter anaesthesia was evaluated in the sheep more than 70 years ago, but the gas was found to have an insufficient narcotic effect in that species (Bendersky 1904). Carbon dioxide was introduced as pre-slaughter anaesthesia for swine in the beginning of the 1950s by Hormel Packing Co., USA (Slater 1952), and has subsequently become widely used also in other countries.

Experimental studies of the effects of hypercapnoea (involving neocortical EEG recordings) have been made in man (Woodbury & Karler 1960) and several other mammalian species (Meyer et al. 1961, Eisele et al. 1967), and have provided a rather uniform picture. Inhalation of CO_2 within the concentration range of 20-35% seems to increase neocortical activity via a stimulatory influence upon the brain-stem reticular activating system (Ingvar 1958). This could be the explanation why myoclonic jerks or convulsions generally appear during exposure to CO_2 of that moderately high concentration. Short-lasting motor reactions often occur also initially during exposure to CO_2 at and above 40%. At these higher concentrations the gas induces anaesthesia associated with progressing neocortical changes similar to those seen during the second and deeper stages of barbiturate anaesthesia (Pichlmayr et al. 1984), that is, initial appearance of slow, high amplitude waves, followed by a decline of all neocortical EEG activity. Evidence has been produced that this synchronization and gradual decline of the EEG activity is not cuased by the hypercapnoea as such but rather by the simultaneously developing cerebral acidosis (Meyer et al. 1961).

There does not seem to be any previous reports on the influence of high concentration CO_2 inhalation upon the hippocampal EEG. However, the changes occurring in hippocampal electrical activity at different depths of anaesthesia have been investigated (Stumpf 1965). During the second stage of barbiturate anaesthesia (characterized by cortical depression, unconsciousness and subcortical hypersensitivity; Pichlmayer et al. 1984) the amplitude of the hippocampal theta waves increases. This increase in amplitude gradually becomes converted into attenuation of the theta activity during the 'surgical' (third), and deeper stages of anaeshesia.

As regards swine, the influence of high concentration CO_2 on the neocortical EEG has previously been studied by Mullenax & Dougherty (1963) and by Hoenderken et al. (1979). These studies have confirmed the observation made in other mammalian species that the inhalation of high concentration CO_2 initially induces neocortical slow waves, followed by more or less complete suppression of the EEG activity. However, the EEG recordings presented in these publications are scarce and do not permit any definite conclusion regarding the time relation between neocortical EEG synchronization and the appearance of CO_2-induced seizures. It has left unanswered the question of whether the swine remain conscious at the moment when myoclonic jerks appear. It has been claimed, however, that these motoric reactions occur during a period comparable to the excitatory stage of barbiturate anaesthesia when, in man, unconsciousness develops (Lomholt 1980).

In five of the present animals, myoclonic jerks were seen about 30 s after the estimated onset of high concentration CO_2 inhalation, whereas no such reaction took place in animal III (Fig. 2). In every instance the jerks were preceded by the development of neocortical delta waves of the kind seen during the second stage of barbiturate anaesthesia (Fig. 1, RC, LC). It suggests that under the present experimental conditions, the swine were already anaesthetized when they exhibited the motor reactions. This does not exclude that CO_2-independent stress/arousal factors present in a slaughterhouse environment may facilitate the development of jerks, with the result that such reactions become manifest before the neocortical EEG exhibits and anaesthesia pattern. It is by no means self-evident, however, that motor activity appearing after some latency during slaughterhouse exposure to CO_2 should be regarded as a conscious adversive reaction.

Obviously, the present animals must have remained narcotized below the stage of surgical anaesthesia as long as neocortical EEG silence persisted, that is, for about 1 min after the CO_2 inhalation. From the ethical point of view, this implies that exsanguination might safely be performed within the first minute after the moment when the swine is removed from the high concentration CO_2 environment. However, the slow return to a pre-exposure neocortical EEG pattern suggests that the animals remained anaesthetized for at least 1 min longer. Thus, an EEG pattern corresponding to that seen during stage 2 of barbiturate anaesthesia (Pichlmayr et al. 1984) was generally recorded as late as 2.5-3 min post-exposure (Fig. 1, RC, LC; Fig. 2b).

Accentuation of the hippocampal theta rhythm occurs during arousal and attentive behaviour, and is regarded as an index of brain-stem reticular excitation (Kemp & Kaada 1975). Accentuation of that kind was here observed 17±2 s after the start of CO_2 exposure, suggesting that the swine then had entered into the excitatory (first) stage of anaesthesia (Pichlmayr et al. 1984). During the second stage of anaesthesia, the amplitude of the hippocampal theta waves increases (Stumpf 1965). This happened here a few seconds before the appearance of myoclonic jerks. Hence, like the presently observed neocortical EEG changes, also the alterations in hippocampal theta wave activity suggest that the animals had reached stage 2 of anaesthesia just before they exhibited the motoric reaction.

The fact that during eight of 11 exposures EEG-silence developed also in the hippocampus (Fig. 1, RH, LH) demonstrates that the pronounced neurodepressive effect of high concentration CO_2 inhalation also embraced subcortical brain ragions. To judge from the slow return to pre-exposure EEG patterns, this depressive effect had approxomately the same duration as that exerted upon the neocortex.

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