Cortical activity and respiratory responses to intermittent hypoxia were studied in anesthetized, paralyzed, vagotomized, and artificially ventilated rats. Respiratory responses to hypoxic exposure consisted of stimulation of phrenic and hypoglossal activity and a subsequent decline of the activity up to apnea. The respiratory response to hypoxia was accompanied by a gradual decrement of the total power of EEG. Relative EEG power increased in the delta frequency range and decreased in the remaining frequency ranges. During hypoxic bradypnea or apnea, the total power of EEG strongly diminished or ceased. Each episode of hypoxia caused similar respiratory and cortical effects. However, in comparison with the baseline level, the total power of EEG decreased gradually while the power of the delta frequency range increased in subsequent hypoxic episodes. EEG activity after the last hypoxic exposure recovered within 40-60 min. We conclude that hypoxia initially induces modest changes in the cortical activity that grow with the severity of hypoxia. The persistence of changes in EEG activity following intermittent hypoxia may contribute to disorders present in the sleep apnea syndrome.

Key words: EEG, intermittent hypoxia, respiratory activity

INTRODUCTION

Hypoxia induces cardio-respiratory reflex responses. Behavioral effects suggesting the involvement of cortical activity in the broad response to hypoxia are also observed in both conscious and sleeping subjects subjected to low oxygen environment or suffering from the sleep apnea syndrome. The relation between cortical activity and respiratory responses to episodic hypoxia in
conscious man is unclear. Enhancement (1), depression (2, 3) or no effect (4) on EEG alpha activity has been described during hypoxic respiratory stimulation. A feeling of anxiety associated with hypoxic respiratory stimulation can be a source of some changes in EEG activity (4). In anesthetized animals, no behavioural or emotional effects of hypoxia can be expected. However, the state of cortical activity during anesthesia influences respiratory activity and its response to hypoxia (5, 6). Intermittent hypoxic episodes model the type of hypoxia noticed in the sleep apnoea syndrome. The respiratory response to hypoxia consists of an initial increase in tidal volume and frequency of breathing, followed by hypoxic ventilatory decline that may last for some time after the end of hypoxic exposure. The present study addresses the question of whether, and to what extent, the breathing pattern would be correlated with cortical activity in the anesthetized animals subjected to acute intermittent hypoxic episodes.

MATERIAL AND METHODS

The study protocol was approved by a local Ethics Committee.

General preparation

Seven adult male Wistar rats weighing 290-310 g were used in the study. All experimental procedures were carried out under anesthesia with a combination of urethane (Sigma-Aldrich, Poznañ, Poland) and α-chloralose (Fluka Chemie, Germany), 700 mg/kg and 120 mg/kg, respectively, injected intraperitoneally. Catheters were placed in a femoral artery and vein. Following tracheostomy, the animals were paralyzed with 0.08 mg/kg pipecuronium bromide (Arduan; Gedeon, Hungary) and mechanically ventilated. Both vagus nerves were cut in the mid-cervical region to avoid respiratory reflexes from the lungs induced by mechanical ventilation. Arterial blood pressure was continuously recorded (MCK 4011S, Femed, Zabrze, Poland). Arterial blood gases and pH were measured with an AVL Compact 2 Blood Gas Assembly (Roche Diagnostics Graz, Austria). Rectal temperature was maintained at 37-38°C with an external heating pad. Drugs and fluids were administered via femoral vein.

Neurophysiological recordings

The electroencephalogram (EEG) was recorded from the frontal cortex (A 2.0, L 2.0) via stainless steel screws implanted into the skull. A reference electrode was placed in the frontal skull bone. A C5 phrenic nerve rootlet (PH) and hypoglossal nerve (HG) were cut distally and placed on bipolar silver electrodes.

EEG potentials were filtered at 0.5-50 Hz band pass, phrenic and hypoglossal nerve activities were filtered at 5-2500 Hz band pass and amplified using a NeuroLog System (Digitimer, Welwyn Garden, UK). Nerve activities were integrated with a time constant of 70 ms. Cortical and neural signals were digitized at a rate of 500 Hz and 5000 Hz, respectively, with a CED1401 interface and Spike 2.v5 data acquisition system (Cambridge Electronic Design, Cambridge, UK). EEG, raw, and integrated nerve signals and arterial blood pressure were displayed on-line and stored for further analysis using Spike 2.v5 software.
Experimental design

Acute intermittent hypoxia consisted of five episodes of breathing with a hypoxic mixture containing 14% O$_2$ in N$_2$ and 1.7% CO$_2$. Hypoxic episodes took 1.5 min each and were introduced every 3 min.

Following the surgical procedure, the animals were ventilated with air enriched with oxygen to keep the oxygen pressure in the arterial blood above 110 mmHg to minimize stimulation of respiration by peripheral chemoreceptors during both control and recovery after each hypoxic episode. To avoid hypocapnia due to hypoxic hyperventilation, a small amount of CO$_2$ was added. Arterial blood gases and pH were corrected, whenever required, by adjustment of the respirator or injection of 8.4% NaHCO$_3$. The animals’ condition was allowed to stabilize for 30 min before the application of intermittent hypoxia and the start of recordings. The variables were continuously recorded throughout the intermittent hypoxic procedure and for an additional hour after its termination. Arterial blood samples were taken before and during the first, third, and fifth hypoxic episode.

Data analysis

An analysis of EEG was performed with Spike 2 v.5 software. EEG power spectra of consecutive 5 s epochs were calculated using Fast Fourier Transform function (FFT) with Hamming window and 512 points. Four EEG frequency bandwidths were distinguished as follows: delta (1.9-3.9 Hz), theta (3.9-7.8 Hz), alpha (7.8-13.7 Hz), and beta (13.7-35.2 Hz), and a relative power for each frequency band was calculated as a percent of the total EEG power within the frequency range of 1.9-35.2 Hz. Total and relative EEG power in each band range was averaged over the periods of time corresponding to the baseline and to the respiratory response to hypoxic episodes. PH and HG activity amplitudes, frequency of inspiratory bursts per min, and their products f x PH and f x HG were calculated and presented as a percent of control. The respiratory response to hypoxia was divided into following parts: stimulation of breathing, decline of the response, hypoxic apnea, and recovery. Respiratory and EEG activities were averaged in these compartments. Values were presented as means ±SE. Statistical analysis was performed with Student’s t test or two-way ANOVA followed by a post hoc LSD test. P<0.05 was taken as indicative of significant differences in all comparisons.

RESULTS

Respiratory parameters and arterial blood pressure

Each hypoxic episode of intermittent hypoxia caused a repeatable sequence of temporal changes in the phrenic and hypoglossal activity. The respiratory response to hypoxia was typically biphasic. It consisted of initial stimulation (about 30 s) of both PH and HG peak amplitudes and an increase in frequency (f) of bursts, followed by a decline of the response toward the control values. The amplitude of hypoglossal activity amounted to higher levels than that of phrenic activity. The decline of the response consisted of a decrease in frequency of respiratory bursts down even to apnea of different duration, while inspiratory bursts remained high when present. After removal of hypoxia, inspiratory amplitude attenuated in comparison with that present before termination of hypoxia, frequency of bursts...
increased, and minute respiratory activity slowly recovered within 1-2 min. During subsequent hypoxic episodes (*Table 1*), increments in minute activity of PH and HG gradually diminished. Changes in HG activity were more dynamic than those in PH activity during intermittent hypoxia.

*Table 2* presents the values of arterial blood gases and pH, arterial oxygen saturation, and the mean blood pressure in the control condition and during hypoxic episodes at the time when the respiratory response declined but before it ended up in apnea. The respiratory response was accompanied by a gradual fall in the mean arterial pressure that reached the nadir of 32.8 ±3.2 mmHg in apnea.

**EEG during intermittent hypoxia**

Intermittent hypoxia caused a depression of EEG activity. During the initial hypoxic respiratory stimulation, EEG activity slightly decreased. The activity was then gradually declining along with the respiratory decline (*Table 3*). The most
prominent effect of hypoxia on EEG was observed during bradypnea or apnea accompanied by severe hypotension, when the EEG activity was markedly suppressed or at times even became isoelectric.

Spectral power analysis of EEG revealed that the dominant baseline frequency was in a range of theta activity. During the course of hypoxia, a reduction of EEG total power was due to decreases in the spectral power of theta, alpha, and beta frequencies, while the power of delta slightly increased (Fig. 1). The relative power of EEG frequencies did not reach the baseline values immediately after hypoxic stimulus withdrawal. The lowered power of theta, alpha, and beta frequencies were sustained during at least 30 min after cessation of intermittent hypoxia. Delta EEG activity continued to increase over the same time span. The EEG activity returned to the baseline level within 40-60 min.

Fig. 2 shows that the EEG delta activity increased during intermittent hypoxia, while the alpha activity gradually decreased. The theta and beta EEG did not show such a tendency. Regression analysis of changes in the total EEG power and MAP during intermittent hypoxia showed a high positive correlation (r=0.90) between the two, while the correlation between the respiratory rate, on the one side, and the total EEG power and MAP, on the other side, was weaker; r=0.64 and r=0.68, respectively.

DISCUSSION

The present study shows that a single episode of acute hypoxia and repeated intermittent hypoxic episodes lasting less than 30 min elicit a gradual decrease in

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![Fig. 1. Percent changes in spectral power density of the total EEG and the delta, theta, alpha, and beta frequency bands during respiratory responses to intermittent hypoxia, immediate recovery, and 30 min after intermittent hypoxia. Values are calculated as the mean of five hypoxic episodes, normalized to preceding baseline level of each. Abbreviations: C-control, HS-hypoxic stimulation, HD- hypoxic decline, HA- hypoxic apnea, R-recovery.](image-url)
the total EEG power during both the stimulatory and inhibitory phases of the respiratory response. The stimulatory phase is thought to originate from carotid body chemoreceptors and the inhibitory one may be of central origin (7). One could expect that stimulation of breathing by hypoxia might be associated with EEG arousal, having in mind that an augmentation of ventilation often results from increased arousal (5). Since the EEG activity actually declines during both stimulatory and depressant phases of the respiratory response to hypoxia, it is probable that the hypoxic drive from chemoreceptors to respiratory neurones initially overcomes the central depressant effect of hypoxia on cortical and respiratory neurons. At any rate, until the appearance of apnea, the net EEG activity during hypoxia cannot be used as an indicator of the specific phase of the hypoxic respiratory response.

The hypoxic respiratory depression, associated with a progressive suppression of EEG activity, might have a component of an additive interaction between EEG and respiratory activity. In the present study when hypoxic exposure continued, respiratory depression changed into apnea and EEG activity could be completely silenced as long as apnea lasted. Also, cortical activity during the time of low arterial blood pressure and strong bradypnea started to recover during reoxygenation in parallel with an increase in respiratory activity. During recovery from hypoxia, changes in EEG and in respiratory activity were apparently correlated.

While lowering of oxygenation evokes a biphasic respiratory response, the associated alternations in arterial blood pressure are unidirectional. A fall in the arterial blood pressure even during normocapnic normoxia elicits a decrease in the amplitude of EEG (8). Since hypoxic systemic hypotension elicits cerebral
hypoxia and ischemia (9), progressive hypotension during episodes of hypoxia in the present experiments seems an important factor of the EEG suppression.

Similarly to cyclic changes in respiratory activity, intermittent hypoxia evokes a sequence of EEG changes being repeated with each hypoxic episode, although with consecutive hypoxic trials both background EEG activity and the amount of suppression of the total EEG power gradually decline. That means that severe hypoxia alternating with normoxia or mild hyperoxia elicits persisting changes in neuronal activity probably resulting from fluctuations of cerebral flow (10, 11). An analysis of spectral power of EEG frequency bands in relation to respiratory activity during hypoxia showed that both stimulation and decline in the respiratory response were associated with some increase in the power of delta EEG activity and a decrease in the theta and in higher frequency bands, while the total power of EEG activity decreases. Moreover, in the course of intermittent hypoxia, delta EEG activity gradually increased and alpha activity decreased. This effect manifested as a progressive depression of cortical neuronal activity. In this study performed on urethane-chloralose anesthetized animals, the dominant EEG frequency was in a lower range of theta frequency. These results are different from the EEG pattern described under urethane anesthesia, where an augmentation of delta activity occurs during deep anesthesia, while theta rhythm dominates when anesthesia becomes shallower (12). Chloralose causes an appearance of slow EEG activity, but the frequency range has not been estimated (13). The EEG changes we observed in the present study in response to intermittent hypoxia might be comparable to those taking place in response to anesthesia or increasing depth of sleep (14), but also may correspond to the effects of brain ischemia (8).

There are some discrepancies between the effects of intermittent hypoxia seen in the present study, performed in anesthetized animals, and those found in sleeping animals by other authors. Boon et al (5) have reported that in the urethane-anaesthetized rats, breathing air and hypoxic or hypercapnic gas mixture, ventilation increases in rapport with cortical activation in a manner that is similar to that observed in unanesthetized animals. Hypoxia itself influences the states of sleep and slow wave sleep is less frequently observed during hypoxia (5). During slow wave sleep, hypoxia induces more wake episodes and increases the faster frequencies in EEG (6), which implies that hypoxia may have some arousal-like action. In the present study, there was no sign of hypoxic arousal in EEG. One explanation for these differences between the present and previous results might be that some anesthetic agents attenuate or blunt EEG responses to noxious stimuli (15). Also, in contrast to the previous studies cited above, in this study acute intermittent hypoxia repeatedly provoked respiratory apnea, severe hypotension, and a deep attenuation of cortical activity. Such prominent oscillations in the respiratory, cardiovascular, and cortical neural outputs might result in complex changes in neurotransmitters levels and neuronal activities controlling the respiratory and vascular systems. These factors, taken together,
may cause a dominance of CNS depression that is maintained long after cessation of intermittent hypoxia.

In conclusion, in anesthetized animals intermittent hypoxia produces sequential changes in respiratory activity, arterial blood pressure, and in EEG. The excitatory and early depressant effects on respiratory activity of hypoxia are only slightly reflected in EEG activity, but become prominent during respiratory apnea. The EEG changes during intermittent hypoxia are strongly correlated with changes in the arterial blood pressure accompanying hypoxia. The persistence of changes in EEG activity following intermittent hypoxia may potentially contribute to disorders present in the sleep apnea syndrome.

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