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Cerebral Energy State and Glycolytic Metabolism during Enflurane Anesthesia in the Rat

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Abstract The effects of enflurane anesthesia on the cerebral cortical energy state and glycolytic metabolism were studied in rats. Twenty four rats were devided into four groups with increasing concentrations of enflurane in the arterial blood, i.e., control (1.9±0.3 mg/dl, means±SEM), level I (16.1±1.1 mg/dl), level Ⅱ (26.0±1.6 mg/dl), and level Ⅲ (32.9±0.9 mg/dl). At level I, high voltage 1-3 Hz slow waves superimposed with low voltage 10-12 Hz waves were predominant, and at levels Ⅱ and Ⅲ, spiking activity and burst suppression were recorded in the EEG. The duration of suppression at level Ⅲ was significantly longer than that at level Ⅱ. During enflurane anesthesia, there were no significant differences compared with the control group in the cerebral energy state or energy charge. Glycolytic metabolism remained unchanged except for an increase in glucose at levels Ⅱ and Ⅲ. Effects of hypocapnia and hypercapnia were examined in additional 12 rats with enflurane concentration in the blood similar to that at level Ⅱ. Irrespective of $Paco_2$ levels, there were no significant changes in cerebral energy charge and glycolytic metabolites except for a decrease in glucose and an increase in lactate at hypocapnia. It was concluded that there was neither evidence of derangement of energy state nor increased anaerobic metabolism in the cerebral cortex during enflurane anesthesia.

Key Words: Anesthetics; enflurane, Brain; metabolism, glycolysis, high energy phosphates

Introduction

Enflurane anesthesia is associated with the EEG seizure and twitching of the muscles at deep levels, particularly during hypocapnia[1-3], and much attention has been raised concerning cerebral circulatory and metabolic responses during seizure[4,5]. Although the cerebral metabolic rate for oxygen ($CMRo_2$) at 2.2% enflurane (end-tidal) significantly decreased by 34% from that of the control in dogs, $CMRo_2$ during seizure produced by the combined stimuli of hypocapnia and repetitive hand clapping at 3.4% enflurane increased by 48% from the preseizure value[4]. It is well known that the common convulsants strikingly increase $CMRo_2$ and alter the normal intracellular state of metabolism[6]. Thus, it is reasonable to suspect a possible cerebral metabolic derangement

during enflurane-induced seizures. To clarify this problem, the concentrations of high energy phosphates and glycolytic intermediates and endproducts during enflurane anesthesia need to be determined. The present study was designed to evaluate the effects of enflurane on the cerebral energy state and glycolytic metabolism during enflurane anesthesia. It was found that during deep enflurane anesthesia, even with spiking activity in the EEG, there was no evidence of increased anaerobic metabolism in the cerebral cortex.

Materials and Methods

Thirty-six unstarved male rats, weighing 260–395 g, were randomly divided into six groups of six rats each, i.e., control, level I, level Ⅱ at hypocapnia, at normocapnia and at hypercapnia, and level Ⅲ. The levels I, Ⅱ and Ⅲ are defined according to the concentration of enflurane in the arterial blood. All rats were anesthetized with 3.0% enflurane (after tracheotomy, reduced to 1.5%), and 70% nitrous oxide in oxygen. The rats were ventilated via a tracheotomy with an animal ventilator (Rodent respiration pump 681®, Harvard Apparatus Co., U.S.A.) and were paralyzed with d-tubocurarine, 0.5 mg/kg initially followed by 0.25 mg/kg every 30 min. The right femoral artery and vein were catheterized for monitoring arterial blood pressure, blood sampling, and the injection of fluid, drugs, and blood. After the rats were turned to a prone position, the skull was exposed and the EEG was recorded from bipolar frontoparietal leads, using screw electrodes. After completion of the operation, enflurane was discontinued in the control group and the rats were ventilated with 70% nitrous oxide in oxygen. In the level I group, anesthesia was maintained with 1.5% enflurane (inspired), and 70% nitrous oxide in oxygen. In the level Ⅱ and Ⅲ groups, inspired concentration of enflurane was increased to 2.0%. High voltage spikes usually appeared at 2.0% enflurane. Then, the concentration of enflurane was increased to 2.5% (level Ⅱ), and further to 3.5% (level Ⅲ). Phenylephrine (10 μg/ml) was required in order to maintain the blood pressure in the level Ⅲ group (maximum dose was 60 μg). In the level Ⅱ group, desired $Paco_2$ (hypocapnia, normocapnia, and hypercapnia) was obtained by changing the concentration of inspired carbon dioxide, while the ventilation was kept constant. In all groups, the brain was frozen in situ by pouring liquid nitrogen into a funnel over the intact skull bone following Pontén's technique[7]. During the freezing of the brain, the ventilation was maintained, and blood samples were taken for the determination of enflurane concentrations by gas chromatography (Gas chromatograph GC-4A PTF, Shimadzu, Japan). Blood samples for gas analysis (ABL 2 Radiometer, Denmark) were taken at frequent intervals, including a sample immediately before freezing the brain. Blood loss due to sampling was replaced by fresh heparinized blood. Body temperature was kept at 37.1±0.1°C by a warming blanket, hematocrit was maintained at 43±1.5% and blood glucose was measured by enzymatic analysis. Cerebral cortical tissue samples were stored and dissected in liquid nitrogen. After weighing, the cerebral tissue was extracted with methanol-perchloric acid below 0°C. The techniques of Lowry and Passonneau[8] were used for the determination of phosphocreatine (PCr), ATP, ADP, AMP, glucose, glucose-6-phosphate (G-6-P), lactate (L), and pyruvate (P) concentrations in the cerebral cortical tissue. The energy charge (EC) was calculated as proposed by Atkinson[9]. All enzymatic analyses were done with a spectrophotometer (124 Hitachi, Japan) with an attached linear-log recorder. Enzymes and coenzymes for the assay were purchased from Boehringer Mannheim GmbH, West Germany.

Statistical differences were tested by the one-way analysis of variance with critical-difference testing. The significance of results in the EEG analysis was tested by Wilcoxon's rank sum test. $P<0.05$ was considered to be significant.

Results

The representative EEG changes with increasing concentrations of enflurane in the arterial blood are shown in figure 1. Control EEG during 70% nitrous oxide in oxygen was characterized by 4–6 Hz waves. During inhalation of 1.5% enflurane, high voltage slow waves (1–3 Hz) superimposed with low voltage 10–12 Hz waves were predominant (level I). With increasing inspired concentrations of enflurane, slow waves were accom-

panied by random high voltage spikes; and irregular spikes and waves developed with burst suppression (level Ⅱ and Ⅲ). The isoelectric period became longer with deepning anesthesia. In order to quantify the EEG change, the frequency of spikes (greater than 100 μV) and the percentage of time occupied by the periods of suppression (electrical silence 1 sec in duration or longer) were determined in the EEG for the 30 sec immediately before freezing brain (Table 1). Frequencies of spikes were higher at levels Ⅱ and Ⅲ, and at level Ⅱ they were lower during hypercapnia than those during normocapnia or hypocapnia. The isoelectric period became significantly longer at level Ⅲ than that at level Ⅱ.

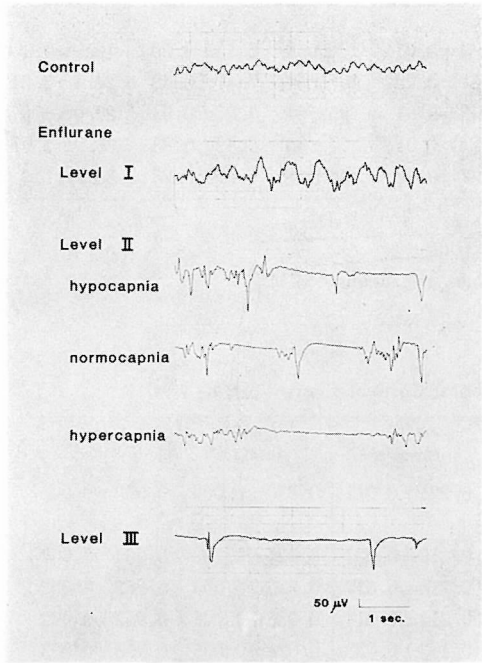

Fig. 1 The representative EEG of 6 groups, i.e., control, level I, level Ⅱ-hypocapnia, normocapnia, and hypercapnia, and level Ⅲ. Three different levels were defined according to concentrations of enflurane in the arterial blood.

Table 2 summarizes the physiological parameters and blood enflurane concentrations in the rats. Tables 3 and 4 summarize the cerebral cortical high energy phosphates, EC, glycolytic metabolites, the lactate/pyruvate ratio (L/P) and brain to blood glucose concentration ratio. During enflurane anesthesia, there were no significant differences compared with the control in the cerebral energy state or energy charge. Glycolytic metabolism remained unchanged except for an increase in glucose at levels Ⅱ and Ⅲ. Irrespective of $Paco_2$ levels, there were no significant changes in the cerebral energy charge and glycolytic metabolites except for a decrease in glucose and an increase in lactate during hypocapnia. There was significant increase in the brain to blood glucose concentration ratio at level Ⅱ-normocapnia.

Table 1 The Frequencies of Spikes and the Percentages of the EEG Occupied by Burst Suppression

Group	Spikes frequencies/min	Burst suppression %
Control	—	—
Enflurane		
Level Ⅰ	2±1	—
Level Ⅱ-hypocapnia	34±4	29±10
Level Ⅱ-normocapnia	29±4	27±2
Level Ⅱ-hypercapnia	9±2*	39±8
Level Ⅲ	17±4	79±6*

*Significantly different from level Ⅱ-normocapnia ($P<0.05$).
The values are means±SEM.

Table 2 The Physiological Parameters of Control and Enflurane Groups and Enflurane Concentrations in the Arterial Blood

Group	n	Pao_2 torr	$Paco_2$ torr	pH	MAP torr	Blood glucose μmol/ml	Enflurane mg/dl
Control	6	118±7	39.5±1.2	7.43±0.02	150±9	6.20±0.86	1.9±0.3
Enflurane							
Level Ⅰ	6	124±4	40.7±0.6	7.44±0.01	108±6#	6.73±0.50	16.1±1.1
Level Ⅱ-hypocapnia	6	131±6	23.0±0.8#*	7.60±0.02#*	107±6#	7.47±0.48	24.4±3.5†
Level Ⅱ-normocapnia	6	132±7	37.2±0.8	7.43±0.01	96±6#	5.87±0.64	26.0±1.6
Level Ⅱ-hypercapnia	6	122±3	64.7±2.0#*	7.24±0.01#*	97±8#	6.89±0.83	26.9±1.5†
Level Ⅲ	6	124±6	41.0±1.4	7.37±0.02	110±2#	7.07±0.28	32.9±0.9

Significantly different from control ($P<0.05$).
* Significantly different from level Ⅱ-normocapnia ($P<0.05$).
† Not significantly different from normocapnia. The values are means±SEM.
MAP: mean arterial pressure.

Table 3 Effects of Enflurane Anesthesia on the Cerebral Cortical Energy State

Group	PCr μmol/g	ATP μmol/g	ADP μmol/g	AMP μmol/g	EC
Control	5.67±0.23	3.57±0.07	0.221±0.011	0.036±0.006	0.962±0.003
Enflurane					
Level Ⅰ	5.52±0.20	3.40±0.07	0.210±0.021	0.024±0.003	0.964±0.003
Level Ⅱ-hypocapnia	5.66±0.21	3.34±0.08	0.218±0.017	0.030±0.005	0.962±0.002
Level Ⅱ-normocapnia	5.93±0.21	3.48±0.08	0.214±0.014	0.036±0.006	0.962±0.002
Level Ⅱ-hypercapnia	5.54±0.19	3.52±0.10	0.183±0.023	0.028±0.005	0.968±0.003
Level Ⅲ	5.72±0.22	3.39±0.06	0.234±0.020	0.031±0.004	0.960±0.003

The values are means±SEM. PCr: phosphocreatine, ATP: adenosine triphosphate, ADP: adenosine diphosphate, AMP: adenosine monophosphate, EC: energy charge.

Table 4 Effects of Enflurane Anesthesia on the Cerebral Cortical Glycolytic Metabolism

Group	Glucose μmol/g	G-6-P μmol/g	Lactate μmol/g	Pyruvate μmol/g	L/P	Glucose brain/blood
Control	3.26±0.36	0.243±0.040	1.92±0.18	0.117±0.020	18.1±2.4	0.56±0.08
Enflurane						
Level Ⅰ	3.38±0.19	0.158±0.027	1.49±0.20	0.104±0.010	15.4±2.9	0.52±0.06
Level Ⅱ-hypocapnia	3.91±0.16*	0.193±0.063	1.76±0.18*	0.138±0.015	13.4±1.8	0.53±0.02*
Level Ⅱ-normocapnia	4.59±0.21#	0.188±0.024	1.08±0.04	0.105±0.018	12.6±2.9	0.84±0.12#
Level Ⅱ-hypercapnia	5.07±0.19#	0.197±0.025	0.76±0.07	0.084±0.011	9.9±1.6	0.81±0.13
Level Ⅲ	5.16±0.35#	0.222±0.058	1.73±0.33	0.108±0.015	16.8±3.1	0.74±0.08

Significantly different from control ($P<0.05$).
* Significantly different from normocapnia ($P<0.05$). The values are means±SEM.
G-6-P: glucose-6-phosphate, L/P: lactate/pyruvate ratio.

Discussion

The present study clearly demonstrated that during enflurane anesthesia, there were no significant differences compared with the control group in the cerebral energy state. The spiking activity observed at levels Ⅱ and Ⅲ suggested increased cerebral irritability, which has been well recognized during deep enflurane anesthesia, particularly with hypocapnia[1-3]. In the present study, even at level Ⅱ-hypocapnia, enflurane did not cause any reduction in the cerebral energy charge.

Seizures induced by common convulsants, i.e., bicuculline, pentylenetetrazol, homocysteine, or electrical stimulation are associated with increases in both cerebral blood flow (CBF) and $CMRo_2$. The increase in CBF usually exceeds that of $CMRo_2$[6]. However, such seizures were accompanied by decreases in PCr and ATP, and increases in AMP and ADP, indicating metabolic derangement [10]. It has been reported that 2.2% enflurane, which is insufficient to produce typical EEG seizures, decreased $CMRo_2$ by 34%[4]. However, reported changes in $CMRo_2$ during enflurane-induced seizures have been variable. Michenfelder and Cucchiara observed EEG seizures and spontaneous skeletal muscle activity induced by combined stimuli of hypocapnia and repetitive hand clapping at 3.4% enflurane, and found that $CMRo_2$ increased by 48% from the pre-seizure value, reaching near the control values, and this was accompanied by a similar magnitude of increase in CBF[4]. On the other hand, Sakabe reported that $CMRo_2$ decreased further during deep enflurane anesthesia (enflurane concentration in blood: 27.0 ± 1.3 mg/dl) with typical EEG seizure[5]. This discrepancy may be related to the differences of anesthetic depth, the method used to induce seizure, and the presence or absence of spontaneous skeletal muscle activity. In both studies, however, the mean $CMRo_2$ was lower than that of the control, while CBF was maintained at near control values. Thus, the enflurane-induced seizure is considered to be different from seizures induced by common convulsants. Furthermore, the present results indicate that increased cerebral irritability does not accompany any significant changes in the cerebral energy state. It has generally been accepted that anesthesia in clinical concentrations is not accompanied by signs of energy failure. Nitrous oxide, halothane, pentobarbital were reported not to change the tissue concentration of ATP, ADP or AMP[11]. The recent study by Michenfelder and Theye, however, revealed that more than 2.3% halothane anesthesia particularly at the level with isoelectric EEG activity in the dog, caused a progressive decrease in $CMRo_2$ accompanied by decreases in PCr and ATP[12]. Although direct comparison is difficult due to the difference of species, no evidence of energy failure during deep enflurane anesthesia with predominant burst suppression in the EEG suggests that enflurane, unlike halothane, has not detrimental effects on the cerebral cortex, at least from the biochemical view point. However, it must be added that there is a possibility of metabolic perturbation occuring in parts of the brain other than the cerebral cortex. Recently, Myers and Shapiro suggested that the epileptogenic foci for the seizures induced with enflurane in rats are located in the hippocampus and related structures[13].

Glucose increased significantly during spiking activity (levels Ⅱ and Ⅲ) at normocapnia in this study. Increase in brain to blood glucose concentration ratio similar to that at level Ⅱ - normocapnia has been reported with barbiturate and halothane[11], and this might be interpreted by two possible mechanisms; reduced consumption of glucose or an increased transport of glucose from the blood to the brain. However, Siesjö suggested that the increase in tissue glucose can be explained only by the lowered metabolic rate, showing that the brain to blood glucose

concentration ratio increases with continued reduction in $CMRo_2$[6]. Evidence of the activation of phosphofructokinase by hypocapnia has been recognized, and lower glucose level and higher lactate level during hypocapnia compared with the level at normocapnia in the present study can be explained by the stimulation of glycolysis as reported by Norberg[14]. One may suspect that increased lactate levels during hypocapnia are a manifestation of increased anaerobic metabolism. This is, however, unlikely since biochemica derangement due to hypocapnia by itself occurs only at 10 torr of $Paco_2$[15].

In summary, during enflurane anesthesia even at deep levels with seizure activity in the EEG, there was no evidence of increased anaerobic metabolism in the cerebral cortex.

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