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Intermittent Hypoxia Induction Alters Onset of Anesthesia Time and Limb Withdrawal Reflex Time in Rats

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Abstract

Sodium thiopental is rapid-acting and intravenous anesthetic as non-opioid agents. Hypoxia is the subnormal concentration of oxygen in cells. Recent years, researchers discuss on hypoxia: Friend or Foe? The present study was conducted to examine the effect of intermittent hypoxia on thiopental anesthesia.

Keywords: Intermittent Hypoxia, Thiopental, Wistar Albino Rat

INTRODUCTION

Sodium thiopental is a rapid-acting and short-acting barbiturate derivative general anesthetic. Thiopental is an intravenous anesthetic and a non-opioid [1]. Thiopental sodium triggers off a hypnotic effect and loss of consciousness in clinical use, but the mechanism has not been completely enlightened [2]. The prefrontal cortex (PFC) of mammals brain has been reported to play an important role in the modulation and regulation of consciousness, recognition, and memory [3].

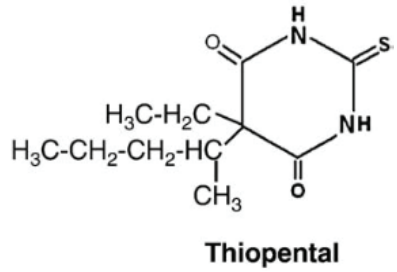

Figure 1. The structure of Thiopental [1].

Thiopental sodium inhibits glutamate releasing from cultured neurons or cerebrocortical slices [4]. Thiopental sodium shows decrease in the extracellular level of glutamate in the PFC [2]. Previous researches have reported that thiopental-triggered off electroencephalographic (EEG) alterations can be correlated with anesthetic depth [5].

The blood–brain barrier (BBB) constitutes a component of the neurovascular part as endothelial cells (ECs) and surrounded by astrocytes, pericytes and neurons [6]. Tight junction of ECs and efflux transporters contribute to regulating central nervous system homeostasis.

Hypoxia is the subnormal concentration of oxygen in cells. Acute hypoxia can increase blood-brain barrier permeability [7] and can breakdown the BBB by the reason of involvement of oxidative stress [8]. Decreased oxygenation or hypoxia is a characteristic of brain disorders that increases barrier permeability [9].

As regards to intermittent hypoxia (IH), this condition is either a friend or a foe. According to recent research, intermittent hypoxia protects the heart and brain from ischemic injury [10] [11]. IH can be conducted safe and easily therapeutic modality for triggering beneficial neuroplasticity [12].

Hypoxia may induce drugs altering drugs' metabolism and increase or decrease the causative toxicity of dependent on drug use, therefore change the effective therapeutic dose [13].

The Aim of this study is to investigate the effect of intermittent hypoxia on thiopental anesthesia.

MATERIALS and METHODS

Animals

16 male Wistar Albino rats (10-12 weeks old) were used in the present study. We provided them from Ankara University School of Medicine Experimental Animals and Research Laboratory. Rats were left for adaptation for 1 week. The subjects were maintained under 12 h light/12 h dark cycle at a temperature of 23-25 °C and damp (50-70%) controlled room. They were hosted in standard polycarbonate cages (3-4 rats per cage) and fed with *ad libitum*. Sterilized sawdust was used as a bedding material. The animals were randomly divided into two groups (*n=8 per group*). The first group is control group (Control) and rodents in the control group were given atmospheric air (20.9% O_2). Rodents in the second group (Hypoxia) were exposed to intermittent hypoxia. All experiments were carried out in a quiet room between 9:00AM and 3:00PM in order not to break the circadian rhythm.

Hypoxia Induction, Anesthesia and Sacrification

After the adaptation period, the hypoxia group was placed into a hypobaric hypoxic chamber which stimulates 69.3 kPA, 3000 meter altitude (520 mm-Hg, about 14% O_2), 6 hours/day for 21 days. On the 22nd day after weighing, sodium thiopental (50 mg/kg) was injected intraperitoneally into the subjects. Onset of anesthesia time and limb withdrawal reflex time were recorded. Blood glucose levels were measured in subjects. Under deep anesthesia, subjects were sacrificed and brain and cerebellum's volume and weight were measured. Brain and cerebellum weight/body weight ratio was calculated. The ethical approval was received from Institutional Animal Care and Use Committee for Ankara University.

Figure 2. Hypoxia Chamber

Figure 3. Brain and cerebellum of a rat.

Statistical Analysis

IBM SPSS Statistics 23.0 was conducted for statistical analysis. Normality and homogeneity of the groups was evaluated by Shapiro-Wilk Test. Whether the parameters showed above significantly alter between the groups was assessed using non-parametric Mann-Whitney U Test. Variables were pointed out as median used for central tendency, the interquartile range of upper 75% and lower 25% percentiles and presented in box-plot graph. Results were considered significant when $p<0.05$.

RESULTS

The comparisons of the parameters between control and hypoxia group were shown in figure 4, 5, 6, 7, 8, 9, 10 and table 1, 2, 3, 4, 5, 6. It was possible to compare all parameters between control and hypoxia group. There were no significant changes in body weight, brain weight, cerebellum weight, brain and cerebellum weight/body weight ratio and blood glucose level in hypoxia group when compared to control group. However body weight, brain weight and were higher but cerebellum weight and blood glucose level were lower in hypoxia group than control group. Interestingly it can be obviously seen that onset of anesthesia time and disappearance of limb withdrawal reflex time increased significantly in hypoxia group when compared to control group ($p<0.05$). After intermittent hypoxia (69.3 kPA, 3000 meter altitude, 520 mm-Hg, about 14% O_2, 6 hours/day for 21 days), onset of anesthesia time and disappearance of limb withdrawal reflex time increased significantly.

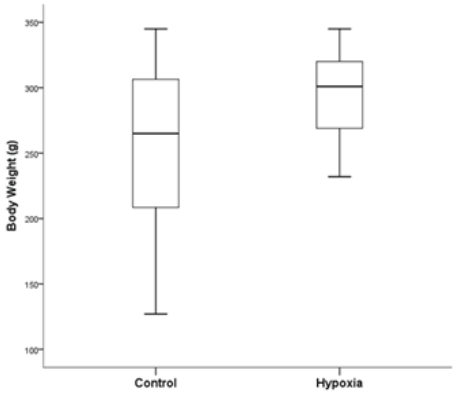

Figure 4. There were no significant changes between control and hypoxia group in body weight when Mann-Whitney U Test was conducted for the statistical analysis. $p>0.05$. For control group; median: 265.00, interquartile range: 108. For hypoxia group; median: 301.00, interquartile range: 57.

Table 1. How many rats are in range of body weight were shown in the table.

Range of Body Weight (g)			
Control		Hypoxia	
100-150	1	220-250	1
150-200	1	250-270	2
200-250	1	270-300	1
250-300	2	300-330	2
300-350	3	330-350	2

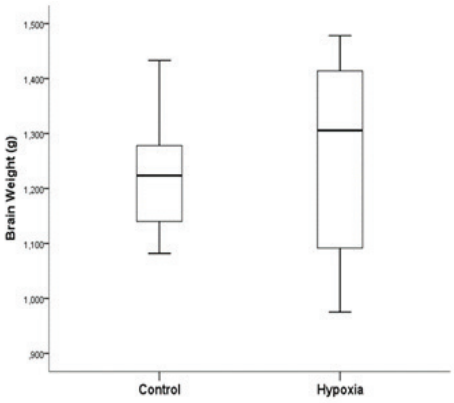

Figure 5. There were no significant changes between control and hypoxia group in brain weight when Mann-Whitney U Test was conducted for the statistical analysis. p>0.05. For control group; median: 1.224, interquartile range: 0.177. For hypoxia group; median: 1.306, interquartile range: 0.375.

Table 2. How many rats are in range of brain weight were shown in the table.

Range of Brain Weight (g)			
Control		Hypoxia	
1.000-1.100	2	0.900-1.000	1
1.100-1.200	1	1.000-1.100	1
1.200-1.300	3	1.200-1.300	1
1.300-1.400	1	1.300-1.400	1
1.400-1.500	1	1.400-1.500	2
		1.500-1.600	2

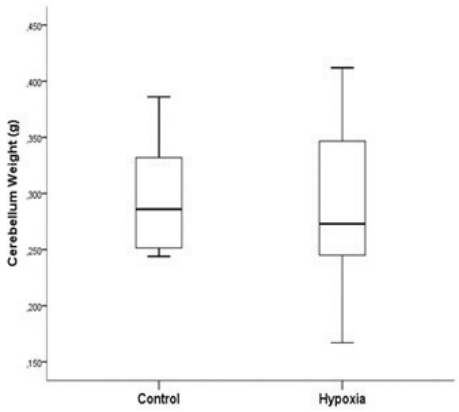

Figure 6. There were no significant changes between control and hypoxia group in cerebellum weight when Mann-Whitney U Test was conducted for the statistical analysis. p>0.05. For control group; median: 0.286, interquartile range: 0.097. For hypoxia group; median: 0.273, interquartile range: 0.107.

Table 3. How many rats are in range of cerebellum weight were shown in the table.

Range of Cerebellum Weight (g)			
Control		Hypoxia	
0.200-0.250	2	0.150-0.200	1
0.250-0.300	3	0.200-0.250	1
0.300-0.350	1	0.250-0.300	3
0.350-0.400	2	0.300-0.350	2
		0.350-0.400	0
		0.400-0.450	1

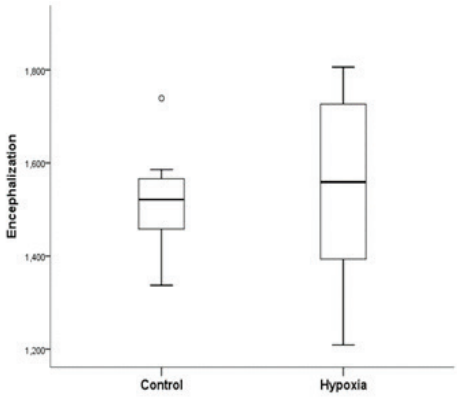

Figure 7. There were no significant changes between control and hypoxia group in encephalization when Mann-Whitney U Test was conducted for the statistical analysis. p>0.05. For control group; median: 1.522, interquartile range: 0.123. For hypoxia group; median: 1.559, interquartile range: 0.368.

Table 4. How many rats are in range of encephalization were shown in the table.

Range of Encephalization			
Control		Hypoxia	
1.300-1.400	1	1.200-1.300	1
1.400-1.500	2	1.300-1.400	1
1.500-1.600	4	1.400-1.500	1
1.600-1.700	0	1.500-1.600	1
1.700-1.800	1	1.600-1.700	2
		1.700-1.800	1
		1.800-1.900	1

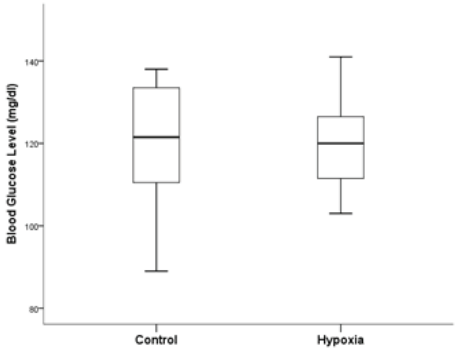

Figure 8. There were no significant changes between control and hypoxia group in blood glucose level when Mann-Whitney U Test was conducted for the statistical analysis. p>0.05. For control group; median: 121.50, interquartile range: 24. For hypoxia group; median: 120.00, interquartile range: 19.

Table 5. How many rats are in range of blood glucose level were shown in the table.

Range of Blood Glucose Level (mg/dl)			
Control		Hypoxia	
80-90	1	100-110	2
90-100	0	110-120	2
100-110	1	120-130	2
110-120	1	130-140	1
120-130	2	140-150	1
130-140	3		

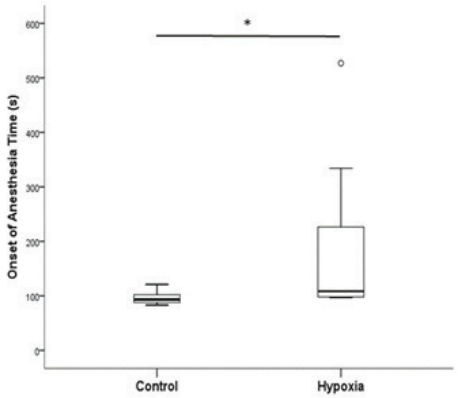

Figure 9. Onset of anesthesia time significantly increased in hypoxia group when compared to control group. Mann-Whitney U Test was conducted for statistical analysis. *p<0.05. For control group; median: 93.50, interquartile range: 17. For hypoxia group; median: 108.50, interquartile range: 182.

Table 6. How many rats are in range of onset of anesthesia time were shown in the table.

Range of Onset of Anesthesia Time (s)			
Control		Hypoxia	
80-90	3	0-100	3
90-100	3	100-200	3
100-110	1	200-300	0
110-120	0	300-400	1
120-130	1	400-500	0
		500-600	1

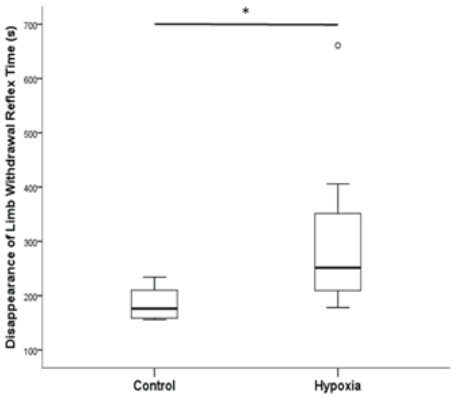

Figure 10. Disappearance of limb withdrawal reflex time significantly increased in hypoxia group when compared to control group. Mann-Whitney U Test was conducted for statistical analysis. *p<0.05. For control group; median: 176.00, interquartile range: 60. For hypoxia group; median: 251.50, interquartile range: 174.

Table 7. How many rats are in range of disappearance of limb withdrawal reflex time were shown in the table.

Range of Disappearance of Limb Withdrawal Reflex Time (s)			
Control		Hypoxia	
140-160	3	100-200	1
160-180	2	200-300	5
180-200	1	300-400	0
200-220	0	400-500	1
220-240	2	500-600	0
		600-700	1

DISCUSSION

Intermittent hypoxia (IH) can effect physiological and pharmacological mechanism. For instance, IH may alter the rate of drug metabolism and so the effective therapeutic dose [13]. This condition is life-sustaining for organisms. The presented study is related to an important agent of human and animal operation. The goals of preoperative medical assessment are to reduce the morbidity or mortality. Our results demonstrated that onset of anesthesia time and disappearance of limb withdrawal reflex time increased significantly. Disappearance of withdrawal reflex time (deep sleeping of mark for rodent) was prolonged and the effect of thiopental reduced.

It can cause some proteins such as tight junction and nuclear factor kappa B (NF-κB) protein increasing against blood brain barrier permeability. According to Brown et al, hypoxia trigger off an increase in the expression of tight junction proteins claudin-1 and actin [14]. Our IH protocol may cause similar increase in tight junction of BBB.

Because of the change of onset time of anesthesia, hypoxia must be considered as a preoperative risk determination. General important point for anesthesia: 1. Assessment of the patient's overall health status. 2. Uncovering of hidden conditions that could cause problems both during and after surgery. 3. Perioperative risk determination. 4. Optimization of the patient's medical condition in order to reduce the patient's surgical and anesthetic perioperative morbidity or mortality [15]. Further, other studies reported that hypoxia induced drug metabolism and therapeutic dose [16]. Some physiologically different conditions such as hypoxia and hypothermia are risk for surgical operation of human and animal.

Interestingly, other parameter of glucose level and body weight did not change, but previous studies reported that hypoxia can effect glucose metabolism and regulation adversely [17] [18]. Glucose metabolism can depend on some conditions such as species, hypoxia protocol (acute or chronic, treatment of day).

As regards to brain weight, cerebellum weight and whole brain weight; they did not change significantly during the hypoxia. Rat body weight, brain mass, whole brain weight and cerebellum conform to other study [19]. Whole brain mass and body weight were reported respectively 1.802±0.313 and 315.1±102.9 g [19]. Our results showed body weight (294.63±12.985 g), whole brain weight (1.259±0.0676 g) and cerebellum weight (0.289±0.0273 g).

There are several limitations in the current study. Tight junction proteins of the BBB, such as claudin-1 and actin would be analyzed by molecular methods. Drug metabolites and hormones (glucagon and insulin for glucose metabolism) can be measured.

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