

The Effect of Premedication Dexamethasone on Exacerbation of Acute Hypoxic Brain Injury in Adult Mice

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Abstract

Premedication as an important component of pre-anesthetic drugs can be either very useful or dangerous and may disrupt the outcome of a surgery. For example, premedication dexamethasone may change normal body responses to stressors such as acute hypoxia through various mechanisms, such as inhibiting the pituitary-hypothalamic axis. Using nonselective nitric oxide synthase inhibitors (10mg/kg L-Name) and a selective neuronal blocker (40mg/kg NOS 7-NI), this study did not prevent the negative effects of dexamethasone as a premedication. Concomitant injection of dexamethasone 0.2 mg/kg as a pretreatment, a NO precursor blocker (L-arginine 60mg/kg) and an NMDA receptor blocker (0.5mg/kg Ketamine) 30 minutes before acute hypoxia also could not be a stimulus for adult mice normal responses to hypoxia. The results of this study showed that injection of premedication dexamethasone at a dose of 0.2mg/kg or higher three days before surgery decreased the response to acute hypoxia and disrupted Tail Test 60 minutes after hypoxia in the adult mouse completely and its effect remained for several hours.

Keywords: Premedication, Dexamethasone, acute hypoxia, Brain Injury, NMDA receptor, nitric oxide, L-arginine, 7-NI, Ketamine, Tail test, Anesthesia box chamber, behavioral changes

INTRODUCTION

Corticosteroids have been used for several years to treat metabolic and inflammatory diseases. This synthetic drug, unlike natural cortisol and corticosterone, has potent corticosteroid activity [1, 2]. The beneficial anti-inflammatory and immunosuppressive effects of dexamethasone have been shown in many inflammatory and autoimmune diseases. They have also shown beneficial effects in reducing edema in brain tumors [3-5]. Dexamethasone has varied and wide effects against hypoxia, either acute or chronic, and many studies have been conducted in this regard. Hypoxia studies have generally focused on the neonate of animals and few studies have been conducted on adult animals. Fletcher et al examined the effect of dexamethasone on reducing cerebral edema following acute hypoxia in neonates by activating or inhibiting Caspase and the mood of mice [6] to prevent Y neuropeptides in sheep neonates [7]. The aim of this study was to evaluate the effects of premedication corticosteroids on the response and resistance of mouse to acute hypoxia directly and its subsequent effects given the positive effects of dexamethasone on the brain of neonates of the animals during acute hypoxia. Due to different and sometimes conflicting studies in premedication administration [2, 4, 5, 8], necessary information can be provided on the appropriate use of these drugs to prevent surgery complications imposed on

physicians and patients. Corticosteroids affect the pituitary-hypothalamic axis and can influence creatures' responses to stress reactions [9, 10]. In this study, the response of adult animals to corticosteroid injection against acute hypoxia was evaluated and the rate of behavioral disorder and resistance of animals to acute hypoxia was recorded and evaluated. What is the resistance power against hypoxia if premedication corticosteroid is injected assuming that it has been dissociated from the oxygen source. Is resistance to this stress decreased or increased?

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METHODOLOGY

The study animals were NMRI mice, weighing 20-30 ±2 g, aged 8 -12 weeks, collected from the Laboratory Animal Center (Shohaday-e Tajrish, Tehran, Iran). They were randomly divided into 6 groups and at least 6 animals were studied in each group. All animals were kept at a temperature of 21-23 ±2 ° C with observing 12-h lightness and 12-h darkness cycle and with an adequate supply of water and food. All interventions and injections were performed between 11:00-17:00 by a skilled person working with laboratory animals. Standard conditions with maximum comfort facilities and minimum stress and annoyance were considered for animals equally and in accordance with guidelines of (National institutes of health publication National Animal Care Guidelines 80-23, revised 1978) and Laboratory Animal Protection Rules used in scientific projects (Directive 2010 / 63, EU) and approved by the Animal Work Guide issued by the Research Deputy of the Ministry of Health of Iran.

Drugs

The drugs used in this study included dexamethasone, N₂O, O₂, sterile distilled water, 5% sterile dextrose, CO₂ (all manufactured in Iran) and L-Name, L-Name 7.NI of sigma company and Ketamine (STEROP-Belgium Company). Dexamethasone was injected intraperitoneally (IP) at a volume of 5cc/kg and the dose of all drugs was selected based on previous studies [8, 11].

Treatment

Dexamethasone at a dose of 0.2mg/kg was used in animal groups at 6, 24, 48 and 72 h intervals before hypoxia. The rate of drug administration was based on pilot studies and valid results [9]. For further investigations, dexamethasone was injected intraperitoneally at doses of 0.2mg/kg, 0.4mg/kg, and 0.6mg/kg 36 hours before induction of acute hypoxia and the drug volume was set on 5cc/kg by saline or sterilized distilled water. In addition, nonselective NOS inhibitor drug such as 10mg/kg L-Name [12, 13] and a selective NOS neuron blocker such as 7-NI 40mg/kg, [14] and a NO precursor blocker such as 60 mg/kg L-arginine [15, 16], a NMDA receptor blocker, such as ketamine 0.5mg/kg, was selected 30 min before acute hypoxia and based on previous studies [17], was for precise assessment of the acute hypoxia-induced by separation from the anesthesia machine. Injection liquid volume 5cc/kg or 0.1 Tween 80 with distilled water or sterile saline is selected to control the mood effects of the control group before and during the intervention.

Behavioral Test (Tail test)

We use Tail Test to evaluate the mood status of the animal before and after specific interventions and the rate of stress and changes caused by acute hypoxia. Based on the study conducted by John Kerian et al, this test was proposed in 1985 [18-20]. In this test, the mouse is hung from the tail by a hook in a container with an opening diameter of 20cm and a height of 15cm. The animal mood status is calculated by an equipped

chronometer based on the animal's mobility and immobility in 360 seconds. The obtained results can be used to investigate the effects of a drug, depression, behavioral disorders, and sexual motivation. It should be noted that before any intervention in this study, this test is used for exclusion criteria, and we exclude the mice that are ill and sedentary and have mood disorders, as the inclusion criterion for the mice was 50% + 1 activity in 360 seconds. We also perform a test 60 minutes after acute hypoxia to record the mice movement and return to normal conditions.

Anesthesia machine, Anesthesia box chamber

The anesthesia machine is a device with vaporizer and flowmeters of oxygen and N₂O that connects to the Anesthesia Box Chamber through an interface. Anesthesia box chamber is a container of the length of 31cm and a width of 31cm and a height of 35cm located in the rectangular form, in front of a removable upward sliding wall to open and close the oxygen and other gases' inlet chamber door. This chamber is the site of induction of anesthesia with gas or intravenous anesthesia along with oxygen used in this study to induce anesthesia and acute hypoxia [21, 22].

Statistical Analysis

All data were analyzed as SEM ± mean using SPSS software and one-way ANOVA was used to compare simple group and two-way ANOVA was used to compare several different groups [23-25] and by Tukey's Post-test [26] was used for follow-up and evaluation. P values below 0.05 are usually acceptable.

The Effects of Acute Hypoxia on Animals

We placed the animals in an anesthetic chamber based on their group. First, the gases were adjusted to 2 liters of oxygen and 8 liters of N₂O (80% to 20% ratio), and 4 minutes later, the oxygen to N₂O ratio was changed from 8% to 92%. Since this time, respiratory, consciousness and sleepiness status were recorded. In case of respiratory arrest, the animal will be removed and resuscitated. In minute 8, we add 1% CO₂ to the anesthetic chamber, and we immediately remove the mouse with respiratory arrest to remove the last mouse in each group. For each group, we record immobility and sleepiness to respiratory arrest changes based on second and one-hundredth of a second and keep for analysis. Each animal is resuscitated with oxygen after removal from the anesthesia chamber and we record the time of waking, moving and returning to normal status (the criteria for removing any sample from the anesthetic box is respiratory arrest). After 60 minutes, we perform mood tests for each group. Our monitoring during acute hypoxia is based on observation and heart and lung hearing aid with a neonatal green stethoscope.

RESULTS

Effect of Dexamethasone on Acute Hypoxia

Figure A-1 illustrates the effects of dexamethasone (0.2mg/kg) injection based on time criterion on immobility and respiratory arrest induced by acute hypoxia. The main

group with complete hypoxia and finally with the addition of CO₂ immediately had a complete respiratory arrest, but the control group had respiratory arrest several seconds after CO₂ in addition to more movement and activity during acute hypoxia. Dexamethasone 0.2mg/kg did not change the status of immobility and respiratory arrest 6 hours before acute hypoxia. Moreover, injection 24 hours before acute hypoxia reduces action, but it does not cause a significant change ($P < 0.01$) but it causes significant effect 3 days before acute hypoxia ($P < 0.01$ and $F < 4.49 = 8.90$). Respiratory arrest with adding CO₂ individually in animals received dexamethasone is not very significant, because the animal has become immobile and sleepy before this time, and even some of them have had respiratory arrest, but with summing up of immobilization and respiratory arrest time, the reduction in time of injections 3 days before acute hypoxia is significant. The highest rate of negative effect was seen at 72 hours before acute hypoxia, seen in groups with 6 members. In Figure 1B, we experienced an injection of dexamethasone at different doses against acute hypoxia. One-way ANOVA evaluation after test showed the significant effect of dexamethasone on animals against acute hypoxia at all doses of 0.2mg/kg, 0.4mg/kg, 0.6mg/kg, 0.8mg/kg in the monitor and it was 0.2mg/kg ($P < 0.01$ and $F < 4.49 = 8.90$) and 0.8mg/kg, 0.6mg/kg ($p < 0.01$, $F < 4.43 = 8.90$) and 0.4mg/kg ($p < 0.01$, $F < 4.35 = 8.90$) and the maximum effect was seen at a dose of 0.4mg/kg. All three doses had a decreasing effect on acute hypoxia compared to the control group, but the dose of 0.2mg/kg had the minimum negative effects. It should be noted that each group had 6 members. (Table 1, 2)

Table 1: Comparison of Statistical Groups in Terms of P and F values

Statistical group	P	F
0.2mg/kg Dexa	$P < 0.01^{**}$	$F < 4.49 = 8/90$
0.4mg/kg Dexa	$P < 0.001^{***}$	$F < 4.35 = 8/90$
0.6mg/kg Dexa	$P < 0.01^{**}$	$F < 4.43 = 8/90$
0.8mg/kg Dexa	$P < 0.01^{**}$	$F < 4.43 = 8/90$

Table 2: Comparison of statistical groups in terms of P and F values

Statistical group	P	F
Control	---	$F \leq 8/90$
6h Dexa	$P > 0.05$	$F < 7.54 = 8/90$
24h Dexa	$P > 0.05$	$F < 6.31 = 8/90$
48h Dexa	$P < 0.05^*$	$F < 5.35 = 8/90$
72h Dexa	$P < 0.05^*$	$F < 4.49 = 8/90$

Note: In this study $P < 0.05$, $P^{**} < 0.01$, $P^{***} < 0.001$

Blocking Effect on NOS

- The Effect of L-Name against Acute Hypoxia

NO is an effective factor for acute responses in the body. In this study, we tested the inhibitory effect of L-Name a nonselective NO synthase with or without dexamethasone in acute hypoxia. Figure 2-A illustrates the effects of pure L-Name and in combination with dexamethasone. In this Chart it has been plotted, L-Name 10mg/kg as IP and dexamethasone 0.2mg/kg from 3 days before alone or in combination with dexamethasone against acute hypoxia. L-Name 10mg/kg used 30 minutes before hypoxia, dexamethasone 0.2 mg/kg for 72 hours before intervention in control and combination group. This chart illustrates that the control group and the L-Name group responded well to acute hypoxia-induced stress, but the group who took dexamethasone 3 days before acute hypoxia did not respond accurately to hypoxia-induced stress and rapidly affected by complications effects of hypoxia. The control group and L-Name alone were almost identical and no change was observed. However, L-Name 10mg/kg group and combined dexamethasone had a slight decrease in response, whereas response in the dexamethasone group alone decreased significantly compared to the control group. Two-way ANOVA analysis showed that dexamethasone 0.2mg/kg/72 h ($P < 0.001$ and $F = 4.49 = 8.90$) was completely significant and L-Name ($p > 0.005$ and $F < 8.65 = 8.90$) and L-Name and dexamethasone ($p > 0.005$ and $F < 7.53 = 8.90$) were not significant. (Table 3)

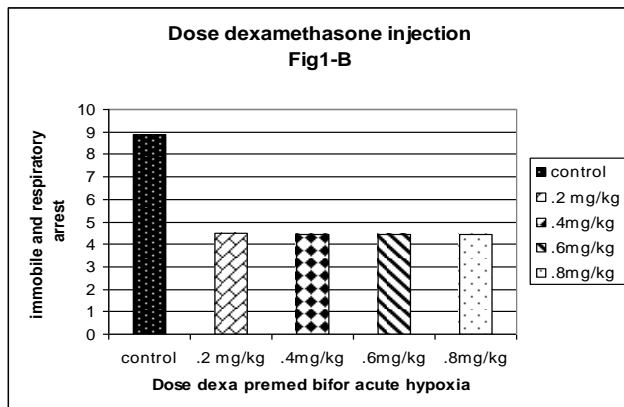
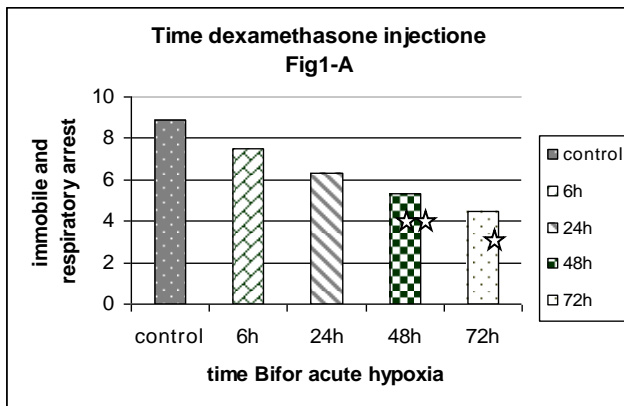


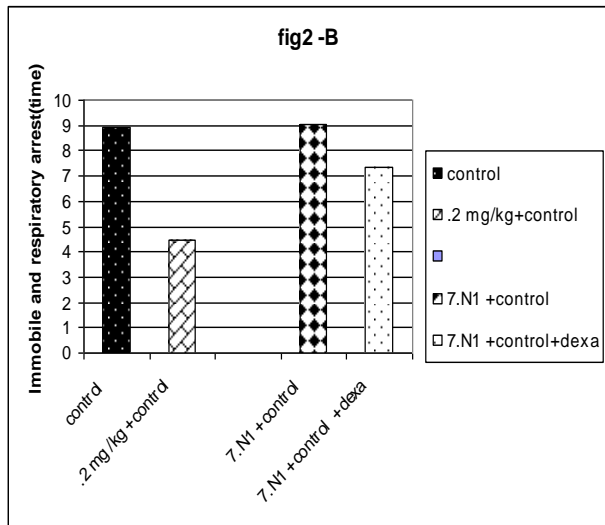
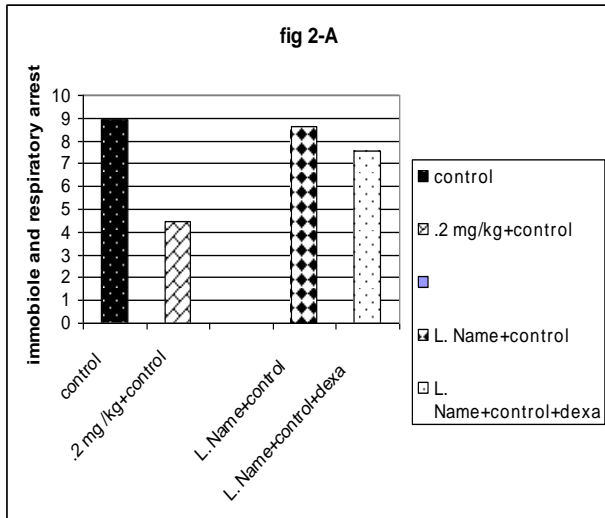
Table 3: Comparison of Statistical groups in Terms of P and F values

Statistical group	P	F
Dexamethasone	P<0.01**	F<8.90 = 4.49
Saline+ L-Name	P>0.05	F<8.90 = 9.65
Control+Dexamethason+ L-Name	p> 0.05*	F< 8.90= 7.53

significant and the dexamethasone group alone had a significant increase. Two-way analysis and evaluation of ANOVA showed that 0.2mg/kg/ 72 (p<0.001, F<4.49 = 8.901) and Dex. 7NI (P> 0.5, F<8.9=9.03) and Salin (P> 0.5, F=7.23=8.901). (Table 4)

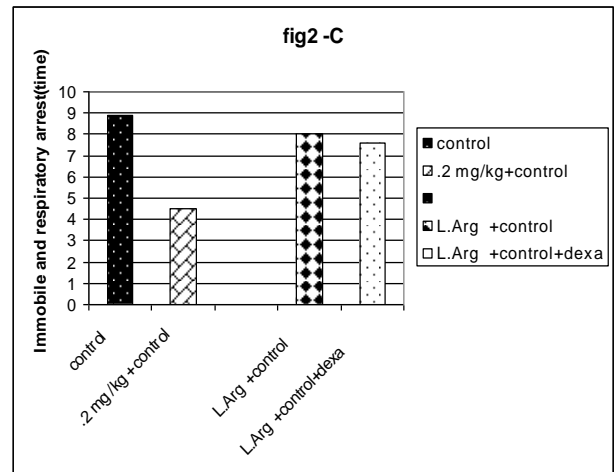
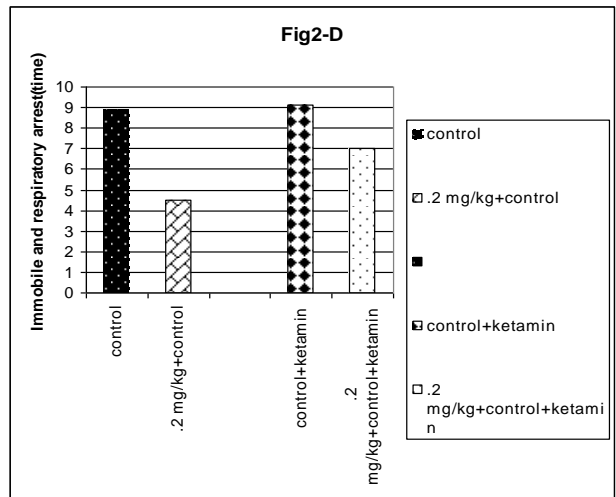
Table 4: Comparison of statistical groups in terms of P and F values

Statistical group	P	F
Dexamethasone	P<0.01**	F<8.90 = 4.49
Saline+ 7.NI	P>0.05	F<8.90 = 9.03
Control+Dexamethason+ 7.NI	p> 0.05	F< 8.90= 7.40



• **Effect of 7.NI against Acute Hypoxia**

7. NI, which is a specific NO nervous system inhibitor, is used to prevent the interference of the effects of dexamethasone and NO system inhibitors against the acute hypoxic on animals. Figure 2-B illustrates that the responses are different in the dexamethasone and control groups received saline, and in the dexamethasone and control groups received 7.NI. Reduction in the response of the control group received saline and group 7.NI was almost identical (with a slight increase in group 7.NI) and the reduction in the dexamethasone group with 7.NI has been slightly compensated but it was not



• **L-Arginine Effects against Acute Hypoxia**

In Figure 2C, the interactive effects of dexamethasone acute hypoxia, L-arg 60mg/kg as IP alone and in combination with dexamethasone with a dose of 0.2mg/kg/72h before surgery were compared with acute hypoxia. 30 minutes before acute hypoxia, we injected L.arg 60mg/kg as IP. The control group received saline, the second group received saline and dexamethasone 0.2mg/kg, the third group received L-

arginine, saline, and dexamethasone, and the fourth group received dexamethasone alone. In this study, resistance to acute hypoxia in the fourth group was significantly reduced ($P < 0.01$). Compared to the control group, by two-way ANOVA analysis showed that dexamethasone alone had a negative effect ($P < 0.01$) and L-arginine. F ($P < 0.001$, $F < 8.9 = 4.49$) alone did not show a significant effect on acute hypoxia compensation compared to the control group ($p > 0.05$ $F < 8.9 = 8.04$ or $P > 0.05$ $F < 8.9 = 7.59$). (Table 5)

Table 5: Comparison of Statistical Groups in Terms of P and F values

Statistical group	P	F
Dexamethasone	$P < 0.01^{**}$	$F < 8.90 = 4.49$
Saline+ L-arg	$P > 0.05$	$F < 8.90 = 9.03$
Control+Dexamethason+ L-arg	$p > 0.05^*$	$F < 8.90 = 7.40$

- **Blocking Effects of N.M.D.An against Acute Hypoxia**

We injected Ketamine intraperitoneally with a dose of 0.5mg/kg 30 min before hypoxia in the group received dexamethasone three days earlier. In Figure 2-D, the effects of NMDA receptor blocking effects against acute hypoxia in the four control groups of control, control + dexamethasone (2mg/kg), control + ketamine, and dexamethasone (2mg/kg) + control + ketamine were investigated. According to the analysis, the effect of dexamethasone (2mg/kg) + control on acute hypoxia was significant. However, the control group + ketamine and control group alone did not show any significant changes relative to each other. Based on ANOVA evaluation in the ketamine- dexamethasone group, the resistance status was improved but it was not significant, and in the ketamine-saline group, an effect is seen on hypoxia resistance, which was not statistically significant compared to the control group (Table 6).

Table 6: Comparison of statistical groups in terms of P and F values

Statistical group	P	F
Dexamethasone	$P < 0.01^{**}$	$F < 8.90 = 4.49$
Saline+ Ketamine	$P > 0.05$	$F < 8.90 = 9.11$
Control+Dexamethason+ketamin	$p > 0.05$	$F < 8.90 = 7.03$

DISCUSSION

Studies on dexamethasone against hypoxia have been conducted in pregnant animals and mothers. With regard to clinical response to acute hypoxia in adult male and female animals, there was no valid and accurate study of ischemia in their neonates. Dexamethasone is one of the drugs used often as an anti-inflammatory drug in trauma and edema caused by it [3-5, 27-29]. However, it has not been clarified yet whether this

drug has the same effects as the premedication. In our study, we found that the use of NOS inhibitors such as L-Name and a selective neuronal NOS inhibitor such as 7-NI could not compensate for or inhibit the inhibitory effects of the pituitary-hypothalamic dexamethasone. When a combination of NOS inhibitors is injected with dexamethasone, responses to acute hypoxia are enhanced, but its effect in the control and dexamethasone groups is not clear. The use of a precursor blocker such as L-arg also could not change the dexamethasone status and enhance the response. NMDA receptor blockers such as ketamine increase the response of animals receiving dexamethasone, but this increase is not statistically significant since their inhibitory effect site is different on the two parts and they did not replace each other, but the rate of response of the mice increased non-significantly. With the use of dexamethasone as a precursor, we achieved a very interesting and remarkable conclusion that administration of dexamethasone at any dose, even at doses above 0.2mg/kg and more than 48 hours before surgery, caused disorder in reactive response adult mice compared to acute hypoxia and the sleepiness and immobility continue after resuscitation and hypoxia. In a similar study in 2014, Lee et al with dexamethasone injections and with psychological tests such as T.S.T and F. S.T examined the behavioral changes of mice and observed a significant reduction in movements [30].

They also examined the associative effect of dexamethasone and NMDA receptors role and they achieved results similar to those of our study [31]. By evaluating the effect of dexamethasone as a premedication on laboratory mice undergoing general anesthesia and assuming that the oxygen tube has been isolated for a few moments for any reason, we concluded that the worst time for injection of dexamethasone on different mice with same injection dose was seen 72 hours before acute hypoxia and the animal will have minimum response in the anesthesia box and TST activity. In addition, it seems that with dose of 0.2mg/kg dexamethasone or higher doses, hypothalamic-pituitary axis would be inhibited 3 days before surgery and with increasing the dose, the axis inhibition would be longer and cannot be compensated by other stimulatory drugs [12-14, 17, 30, 32-34]. NMDA blockers such as ketamine with slightly different performance compensate for the hypothalamic-pituitary axis inhibition, but its status does not change significantly compared to the control group. However, a single-dose injection of ketamine improves the status slightly compared to the control group, which is not statistically significant but acceptable compared to the group receiving dexamethasone [30, 31].

In this study, all factors affecting the acute response induced by acute hypoxia such as NOs, NMDA receptors, selectively or non-selectively, were evaluated by inhibitory drugs. The results of the studies conducted by Temsavari et al on the effects of dexamethasone as a brain protector and enhancer of resistance against hypoxia-induced by pneumothorax and reduction of cerebral edema in the neonatal animals following dexamethasone administration were inconsistent with our

research [35]. NMDA activity of calcium channels stimulates neural pathways and stimulates NO synthesis. NMDA receptors are essential regulators of NOS activity in the central nervous system [34, 36-39]. In the central nervous system, nitric oxide acts as a precursor for intracellular signals [11]. Tior et al investigated the acute hypoxia status in adult mice and injected premedication dexamethasone for mother mice with a dose of 0.1mg/kg 2 hours before surgery. Then, by closing the carotid artery and giving 8% oxygen, hypoxia was induced and they concluded that dexamethasone prevented cerebral ischemia without association with cerebral blood flow [40]. In another study, they investigated the premedication dexamethasone injection at a dose of 0.1mg/kg assuming that it prevents cerebral lesions by closing the carotid artery and inducing hypoxia [41]. The results of our studies were different from those of these two studies. In this study, we evaluated the effects of pretreatment dexamethasone in NOS inhibitors or a NO system prerequisite such as L-arginine against acute hypoxia in mice and concluded that single-dose or low-dose NOS inhibitors or The NO system prerequisite does not have a significant effect compared to the control group, but when used in combination with dexamethasone, it has a significant effect on reducing the mice response to acute hypoxia.

The change in response to dexamethasone by inhibiting the pituitary-hypothalamic axis is so clear that N.O.S signal blocking drugs cannot compensate for the adverse effects of corticosteroid. Donna Sapco et al investigated the effects of dexamethasone on NMDA receptors at the time of neurological complications in neonates born from adult mice. They found that pretreatment dexamethasone at a dose of 0.7mg/kg one hour before NMDA injection-induced NMDA bonding, which was in contrast with our clinical results [42, 43]. In our study, patients who received corticosteroids showed lower resistance to acute hypoxia and were immediately immobilized and experienced respiratory arrest and reduced consciousness sooner than others. Compared to the control group, with increasing the dose of dexamethasone and the injection interval close to 3 days before the injury, it seems that the pituitary-hypothalamic axis was impaired and the animal response to hypoxia decreases. In vitro results show that premedication dexamethasone before surgery decreases resistance and response against acute hypoxia and after resolving all hypoxic conditions, all psychological tests can be disrupted for hours. Therefore, it is recommended to avoid premedication dexamethasone two days before surgery or use a combination of dexamethasone and NMDA receptor inhibitor to reduce its side effects.

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