

Effects of Glutamate on the Reflex of Circulatory System under Hypoxic Condition

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Abstract: In this study, we investigated the effects of glutamate, the neurotransmitter, on the reflex mechanism of circulatory system to hypoxia. Male Wistar rats were subjected to hypoxic condition (10% O₂ in N₂). Glutamate were injected to cerebrospinal fluid of rats using Hamilton injector connecting with stereotaxic system with a constant velocity of 1 µl/s for 5 mins. Our results showed that glutamate reduced or suppressed the responses of circulatory system under hypoxic condition. A decrease in mean arterial pressure (55 - 66%) and an increase in heart rate (6 - 12%) in comparison with normal levels were observed. These data suggested that glutamate in cerebrospinal fluid may be a regulatory factor of circulatory system in response to hypoxic condition.

Keywords: Glutamate, hypoxia, circulation, heart rate.

1. Introduction

Glutamate is the important excitatory neurotransmitter of neuron system, and it regulates the mechanism of respiratory-circulatory system via changing physical properties of the pressure receptors [1-3]. The reflex mechanism of circulatory system is also regulated by a decline in partial oxygen pressure in inspired air [4], subsequently a reduction of arterial blood oxygen tension (PaO₂). Respiratory – cardiovascular responses is one of the complex mechanical responses of the body to hypoxia. These reflex mechanisms are dependent on various factors [5-9]. Of those, the internal glutamate

concentration in nucleus of the solitary tract is one of those regulated factors [10-11]. Moreover, nucleus of the solitary tract is also the major sensory nucleus in the dorsal medulla receiving cardiovascular and respiratory information [12-19]. Peripheral chemical receptors are stimulated by the lack of oxygen in the blood (hypoxia or ischemia) and are the center converting afferent impulses to efferent impulses in circulatory-respiratory responses. Moreover, a reduction of oxygen level leads to the increase in internal glutamate concentration [20]. So, glutamate in cerebrospinal fluid may effect not only on circulatory regulation but also on chemical mechanisms [10, 15].

The aim of this study is to investigate the effects of glutamate in cerebrospinal fluid on the chemoreflex of circulatory system. To

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archive the scope of the study, we carried out the experiments using non- or glutamate-treated Wistar rats under hypoxic stimuli.

2. Methods

2.1. Animals

Eight-week-old male Wistar rats (weight, 200 -250 g each) were deeply anaesthetised with Urethane (1350 mg/kg). Adequacy of anaesthesia was assessed by absence of nocifensive movement, such as tail flick reflex. Body temperature of rats were stabilized at 36,8 - 37°C.

Femoral artery pressure (FAP) was measured using catheter (polyethylene, 20 cm in length, 0,2 mm out diameter, and 0,1 mm inner diameter) with a perfusion of heparin:saline mixture (2500 ED/ml, heparin:saline ratio = 1:20).

A tracheostomy is conducted using a breathing tube to provide oxygen-poor air to reach rat lung (10% O₂ in N₂) to evaluate the response of circulatory system to chemical stimuli. Moreover, a craniotomy is also carried out to infuse Glutamate.

2.2. Measurement of circulatory functional indexes

Arterial catheter is connected to the pressure sensor and receiver amplifier ML224. Signal of arterial blood pressure is calibrated in units of pressure (mmHg), then were detected by equipment PowerLab 8/35 (ADInstruments, Australia), recorded, and processed by software LabChart 7.0 .

2.3. Assessment of circulatory responses to chemical stimuli

Inspired airs with normoxia or hypoxia (10% O₂ in N₂) were adjusted by valves' system connecting with the inspired air sacs.

Intermittent hypoxic training were set by 2,5 mins hypoxia and 10 mins normoxia (Figure 1).

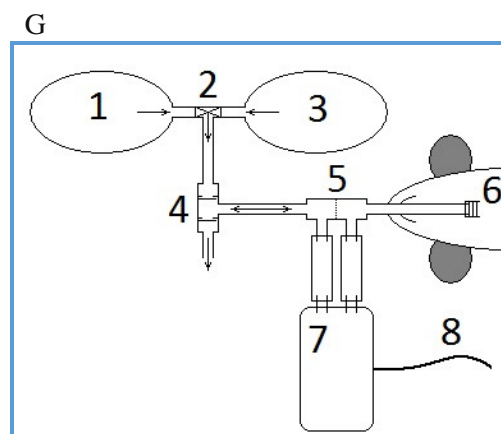


Figure 1. Experimental scheme. 1- air sac, 2, 4 - valve, 3 - mixed air sac (10% O₂ in N₂), 5 - breathing tube, 6 - rat, 7 - spirometer, 8 - capillary fiber.

2.4. Glutamate infusion

We used a stereotaxi injection with Hamilton™ Micro-syringes (150 μm in outer diameter) to infuse 5 μl of 6 μM glutamate into hippocampus with a constant velocity of 1 μl/s for 5 mins. For control group, glutamate containing solution was replaced by saline solution (0.9 %).

After stabilizing, the circulatory indexes were recorded for 90 - 100 mins of experimental periods. A baseline values were counted for the first 40 mins, then the rats were subjected to the interval training of 2,5 mins hypoxia and then 10 mins of normoxia.

After 40 mins of normaline, glutamate were infused into hippocampus. Also, along with this period, rats were subjected to a mixed air (hypoxia) at serious time points 43, 45, 50, and further 10 mins interval until the end of experimental periods. The sensitivity of circulatory reflex to hypoxia was calculated as percentage (%) of mean arterial pressure (MAP), heart rate (HR) during the hypoxic treatment.

2.5. Statistics

Data are analyzed using MS Excel and Univariate analysis. Differences with a *P*-value ≤ 0.05 were considered significant.

3. Results and discussions

3.1. Effects of hypoxia on rat circulatory function

The results of MAP and HR showed that our set experimental conditions were archived normal physiological condition of cardiovascular function [1]. The values of MAP and HR were 92 ± 3 mmHg and 384 ± 7 beats per min (bpm), respectively.

In this study, hypoxia leads to the reduction of MAP to 55 - 65% compared to normal condition (Figure 2, left). In contrast, HR values were elevated 6 - 2% in comparison to normal baseline (Figure 2, right panel; Figure 3 - 1).

As hypoxia induces a decline in O₂ pressure in arterial blood and subsequently leads to

compensatory responses to hypoxia of circulation system. Of those, vasodilation is one of the most important response of circulation system to hypoxia. Vasodilation results a decrease in blood pressure, especially MAP. However, vasodilation response only occurs when PaO₂ in arterial blood pressure less than 40 mmHg, corresponding with the percentage of oxygen in inspiratory air not excessive the value 5 - 6% [21]. In contrast, percentage of oxygen in inspiratory air in our study was about double than the previous study [21]. Thus, the second reason inducing MAP reduction might involve to the sympathetic stimulation to blood vessels, including vessel tension and vessel resistance.

The MAP reduction accompanied with the HR increment might demonstrate the sympathetic elevation. Arterial chemoreceptor's stimuli suppressed the reduction of blood pressure induced by vasodilation [22-23]. Thus, the reduction of hypoxia-induced MAP might be a result of upregulation of autonomic nervous system, especially sympathetic nervous.

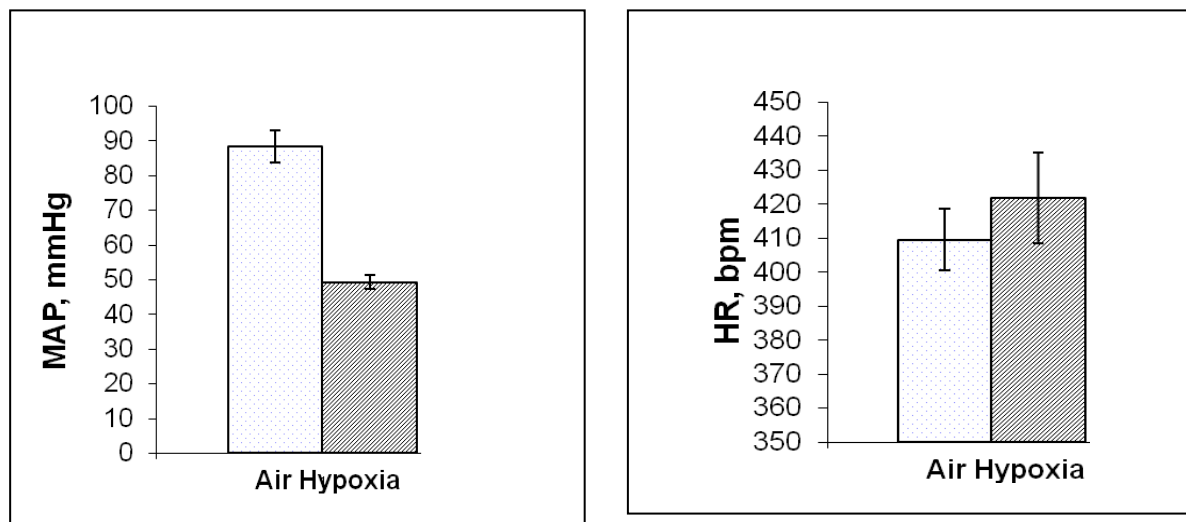


Figure 2. The effects of hypoxia to circulatory indexes.
HR: Heart rate, MAP: Mean arterial pressure.

3.2. Effect of glutamate infusion on hypoxia-stimulated circulation function

Experimental data showed that infusion of glutamate into hippocampus reduced or

eliminated the reflex of circulation system to hypoxic conditions, including the declined MAP and the elevated HR (Figure 3, Figure 4). The hypoxia-induced changes of these values with a supplementation of glutamate are significant different.

Obviously, after 3 mins of glutamate infusion into hippocampus, the values of MAP under hypoxic condition were significantly reduced. The MAP values were then completely eliminated at 5 mins of glutamate infusion. Interestingly, the MAP values were recovered after 10-20 mins of glutamate treatment. In contrast, HR response was eliminated after 3 mins of glutamate infusion into hippocampus. By the 5 mins of glutamate infusion, the elevated reflex to hypoxia of HR was started and prolonged until the end of experimental periods (Figure 3 - 3). However, the recovery of HR values was lower than those values in normal baseline conditions (Figure 3 - 5).

Thus, infusion of glutamate into cerebrospinal fluid immediately suppressed the responses of HR and MAP to hypoxia (Figure 4). However, by the end of experimental periods, the recovery of HR is still lower than that in baseline level. We speculated that glutamate might effect on responses of hypoxia-induced circulation system via two

phases: reduction or elimination phase, and recovery phase.

The increment of glutamate level in cerebrospinal fluid induced the reduction of circulatory response to hypoxia, that could be explained by the way glutamate induce an elevation of arterial blood pressure and tidal volume [1-3]. These increased factors could reduced the reduction of blood oxygen level, increased the blood flow, and declined the hypoxia-related vasodilation. Glutamate also suppressed the increment of HR in response to hypoxia (Figure 4, left panel). Increments of arterial pressure and tidal volume lead to elevation of afferent of aortic baroreceptors and receptors in the lung, finally resulting in a reduction of circulation response to chemical stimuli [24-25]. An increase in glutamate level in cerebrospinal fluid resulted in a reduction of baroreflex and an elevation of Hering- Breuer reponse [1, 3, 26]. Thus, glutamate is potential player regulating the chemical and physical responses of circulation system. The effects of glutamate on circulatory response included two phases and are dependent on the different receptors stimulated by glutamate levels. Of those, the N-methyl-D-aspartate receptor leads to a quick response of circulatory system in the first 3 - 5 mins of glutamate infusion into cerebrospinal fluid.

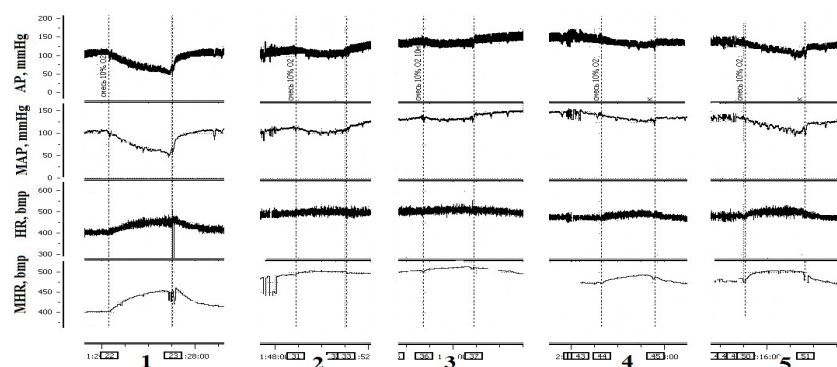
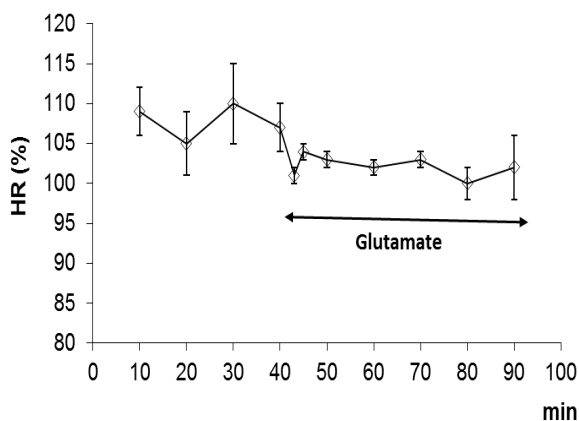


Figure 3. The effects of glutamate on MAP, HR: hypoxic condition, 1: hypoxia, 2-5: hypoxia accompanied with glutamate recorded after 3, 5, 20, 40 mins of glutamate treatment. MAP: mean arterial pressure, AP: arterial pressure, HR: heart rate, MHR: mean heart rate.

Otherwise, a stimulation of N-methyl-D-aspartate receptor effects directly and indirectly

on reflex regulation of circulation system [25, 27]. The recovery phase may be a result of N-

methyl-D-aspartate receptor or other receptors which is triggered by glutamate. Thus, our results showed that in the anesthetized rats, an increment of glutamate level in cerebrospinal fluid can effect on chemoreflex sensitivity of circulatory system or cardiovascular system. This reflex could be divided into two phases: the first phase is a reduction of sensitivity of chemoreflex; the second phase is the glutamate-



treated recovery response of circulation system under hypoxic condition. Effect of glutamate is conducted by stimulating different receptor groups of glutamate in responding to chemical stimuli. In the same way, we speculated that the internal cerebrospinal fluid could lead to reduction of pressure protecting body under hypoxic condition.

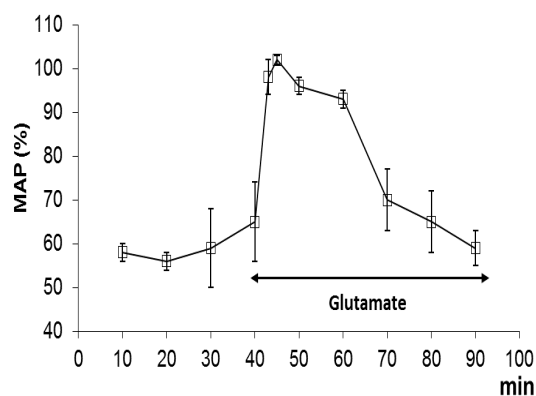


Figure 4. Effects of infusion of glutamate into cerebrospinal fluid on chemoreflex of circulation system.

4. Conclusion

Our data showed that an increment of glutamate level in rat cerebrospinal fluid may effect on the chemoreflex of the circulation system under hypoxic condition, resulting in a reduction of cardiovascular function.

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Ảnh hưởng của Glutamate lên các phản ứng của hệ tuần hoàn trong điều kiện thiếu oxy

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Tóm tắt: Trong nghiên cứu này, chúng tôi tiến hành đánh giá ảnh hưởng của chất trung gian thần kinh quan trọng là Glutamate đến phản xạ của hệ tuần hoàn với sự thiếu oxy trong không khí thở vào (10% O₂ trong N₂). Glutamate được dẫn truyền vào dịch não tủy chuột bạch đực dòng Vista bằng bơm tiêm Hamilton gắn với hệ thống định vị stereotaxic với vận tốc 1 µl/s trong 5 phút. Kết quả nghiên cứu cho thấy Glutamate làm giảm hoặc triệt tiêu những phản ứng của hệ tuần hoàn trong điều kiện thiếu oxy trong không khí vào như giảm huyết áp động mạch (55 - 66%), tăng nhịp tim (6 - 12%). Như vậy, Glutamate trong dịch não tủy có thể là một yếu tố điều hòa các phản xạ với các kích thích hóa học của hệ tuần hoàn.

Từ khóa: Glutamate, hypoxia, tuần hoàn, nhịp tim.